Microbial Modification of Egg Albumen to Improve Its Functional and Physicochemical Properties

by

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A Practicum submitted to the Faculty of Graduate Studies of

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ABSTRACT

The functional and physicochemical properties of egg albumen fulfill important uses in the food processing industry. During processing of eggs, albumen is fermented to remove free glucose, which may cause a browning reaction during storage. The objectives of this study were to assess five microorganisms with respect to their ability to deplete glucose from albumen, while improving its functional and physicochemical properties. Four bacteria were chosen, in addition to a yeast, which served as a control. Viscosity, pH, residual glucose and microbiological growth were analyzed. Fermentations were also conducted in albumen fortified with yeast extract, glucose and sucrose in order to stimulate production of bacterial exopolysaccharide. Scaled-up fermentations were performed after which albumen was spray-dried, pasteurized and subjected to functional testing. In this study, all of the microorganisms completely desugared albumen within 24 hours, however, no improvements in viscosity were observed. Nevertheless, some functional properties did improve upon fermentation of albumen. In general, functionality improved upon pasteurization. Albumen fermented by *Lactococcus lactis* and *Leuconostoc mesenteroides* subsp. *mesenteroides* showed significant increases in whip height, angel cake height and gel strength; albumen fermented with *L. lactis* had significantly higher surface hydrophobicity. Due to Canada's increasing role in the export of processed eggs, a further study is necessary to optimize fermentation practices such that functionality of albumen can be enhanced.

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LIST OF ABBREVIATIONS

ANS	anilinonapthalene-8-sulphonate
ATCC	American Type Culture Collection
CFU	Colony Forming Units
DSC	Differential Scanning Calorimetry
EPS	Extracellular Polysaccharide
MRS	DeMan, Rogosa and Sharpe
PDA	Potato Dextrose Agar
r^2	Correlation Coefficient
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
T_d	Denaturation Temperature
ΔH	Enthalpy of Denaturation

INTRODUCTION

Egg processing is a thriving industry today. Many egg products are processed world-wide, including liquid whole egg, reduced cholesterol egg products, and dried egg albumen and yolk. The functional properties of egg products, in particular dried egg albumen, are of great importance to consumers, and efforts are continually being made to improve these properties. Properties of specific interest include gel strength and foaming characteristics because of their impact on the functionality of egg albumen for the baking and confectionery industries. Physical characteristics such as thermal denaturation and surface hydrophobicity of egg proteins are also important because they describe structural and conformational changes that occur during unfolding of protein molecules. Assessing the physical changes that occur during protein denaturation can help explain parallel changes that are often observed in functionality. When proteins are denatured they unfold, aggregate and expose hydrophobic residues; this often leads to improved foaming, gelation and emulsification properties in egg products (Kinsella, 1976; Yang and Baldwin, 1995).

The overall objective of this research was to determine whether a current industrial processing protocol could be manipulated to improve the functional properties of powdered egg albumen. Since egg albumen is fermented during processing to remove excess glucose that causes undesirable browning reactions upon drying and storage for long periods of time, it was thought that during fermentation, this process could also be used to improve functionality of the albumen. Four bacteria, in addition to a yeast (control) were evaluated as desugaring microorganisms. Many bacteria are able to produce bacterial extracellular polysaccharide or capsular material in selective media (Cerning *et al.*, 1992; Marshall *et al.*, 1995; Ludbrook *et al.*, 1997) and these principles may be applied to albumen. Studies have shown that the increased viscosity observed upon exopolysaccharide production results in enhanced protein functionality (Bejar *et al.*, 1996). Therefore, the bacteria chosen for this

study were grown at their optimum temperature and pH level, in order to facilitate production of bacterial exopolysaccharide.

Following fermentation, the liquid albumen was spray dried and pasteurized. Samples of albumen were then subjected to functional testing, with the results being compared to albumen fermented by the control organism. The three functional tests were whip height, angel cake height and gel strength. In addition, thermal denaturation characteristics and surface hydrophobicity of the albumens were determined using differential scanning calorimetry and fluorescence spectroscopy. These tests were carried out to determine whether structural and conformational changes occurred during pasteurization of egg albumen. Conclusions were drawn to establish whether any of the bacteria chosen for fermentation yielded an improvement in functional properties and physicochemical characteristics, as compared to the control (yeast). Given the current increasingly competitive egg processing market, companies must continually search for ways to improve their products to meet consumers' needs.

REVIEW OF LITERATURE

A. Introduction

Eggs are very important to the food industry due to their nutritional benefits and functionality. Processed eggs are used for production of food products like mayonnaise, pasta and baked goods. Their polyfunctionality makes eggs desirable as a food ingredient. The functional attributes of eggs most pertinent to the food processing industry include coagulation or gelation, foam formation, emulsification and solubility. Eggs are becoming increasingly important to food processors because their functionality can be manipulated to meet consumers' needs.

Egg albumen consists of a mixture of several protein fractions, many of which occur in small amounts. The major protein fraction is ovalbumin, which constitutes over half the protein composition of albumen. Conalbumin is a glycoprotein chelator, which makes up 13% of albumen (Chrusch, 1981). Other smaller protein fractions include ovomucoid (11%), ovomucin (3.5%), lysozyme (3.4%), ovoglycoprotein (1%), ovoflavoprotein (0.8%), ovomacroglobulin (0.5%) and avidin (0.05%) (Li-Chan *et al.*, 1995). Egg albumen contains over half of the protein and 78% of the water contained in whole egg. Albumen contains more than half of the egg's carbohydrate content, consisting of mainly glucose. Albumen is also rich in vitamins, including vitamin B_{12}, biotin, folic acid, niacin and riboflavin. In addition, albumen contains all of the 20 amino acids required for growth and development (Agriculture and Agri-Food Canada, 1999).

The primary egg producers have always been found in the Northern Hemisphere. Egg production is, however, rapidly expanding to developing countries in order to meet the nutrient requirements of the population, but availability of adequate chicken feed impedes this process. Production of eggs in China, for example, increased by 40,000 million between the years of

1987-1990 (Stadelman, 1995). The sites of production have also changed over the last 50 years. Previous production was once concentrated in small farm-flock businesses of no more than approximately 400 layers. However, the trend has moved to commercial size egg production flocks, where up to 15 million layers may be raised by the same owner, distributed over several locations. One of the reasons for the move to the large-scale operations is mechanization of feeding and watering equipment, egg collection, and litter removal (Stadelman, 1995).

In 1997, the world egg processing industry was estimated at $83.6 million, excluding the biochemicals extracted from eggs for use in pharmaceuticals. There are approximately half a billion eggs produced by 22 million laying hens every year in Canada; 82% are sold shelled, while the remaining 18% are processed into liquid, dried and frozen products (Agriculture and Agri-Food Canada, 1999).

B. Fermentation of Egg Albumen

Eggs contain 0.49% carbohydrate, 0.6% of which exists in the free form with glucose being the major component (Kilara and Shahani, 1973). Fermentation is often used in the egg processing industry to remove this free glucose from eggs, in order to prevent the undesirable changes that occur during storage of dehydrated eggs, caused by a browning reaction. Such changes may include loss of solubility, decreased functionality and development of off-odours and flavours. The detrimental effect of glucose on the storage stability of eggs has been linked to glucose-protein interactions, commonly known as the Maillard Browning Reaction. Bonding between glucosidic hydroxyl and amine groups of peptides and proteins results in the development of brown pigmented products. Several biochemical reactions are involved in this phenomenon. The initial reactions occur between glucose and proteins, followed by additional

biochemical reactions that result in fluorescence, colour alterations and solubility deterioration (Sebring, 1995). The reaction causing major deterioration of phospholipid during storage of powdered albumen is a lipid amine-aldehyde reaction, where glucose is the active molecule involved (Kilara and Shahani, 1973).

The practice of desugarizing began in the 1930's when importing of dried egg products from China to the United States decreased due to the outbreak of war in China. American scientists were forced to discover their own methods because the Chinese were no longer sharing information. It was a well-known fact that fermentation was used; however, its sole purpose was thought to be for improving the solubility and whipping characteristics of albumen. The detrimental role of glucose in stored egg products was not discovered until the Second World War, when researchers fought to develop egg products that could withstand long storage conditions, while remaining palatable for soldiers (Sebring, 1995).

Fermentation is considered to be the most efficient, cost-effective and easiest method for removal of glucose from eggs. Several fermentation methods exist, including yeast, enzymatic, and spontaneous and controlled bacterial fermentations.

1. Yeast Fermentation

Fermentation of egg albumen using yeast began in the 1940's. When first developed, *Saccharomyces acipulatus* was used to ferment albumen and whole egg (Sebring, 1995). Upon inoculation of a yeast culture into albumen and incubation at 37^0C, the glucose level decreased from 0.5% to 0.05% within three hours. However, the high concentration of yeast cells resulted in objectionable odours; as a result, other organisms were investigated. *Saccharomyces cerevisiae* was able to perform an equivalent fermentation without development of undesirable

odours and flavours, especially when yeast extract (1%) was added to the albumen (Kilara and Shahani, 1973; Sebring, 1995). Incubation of egg albumen with 0.2-0.4% (w/w) *S. cerevisiae* at 22-23^{0}C resulted in complete conversion of glucose to acid within two to four hours (Sebring, 1995). The resultant desugared egg albumen had a solubility of 95-98% and could be stored at 50^0 C for up to six weeks. It was also found that pH affected the rate of glucose conversion. Fermentation of yeast is possible at neutral pH levels, however, the fermentation rate is faster at acidic pH levels of 6.0-6.5 (Kilara and Shahani, 1973).

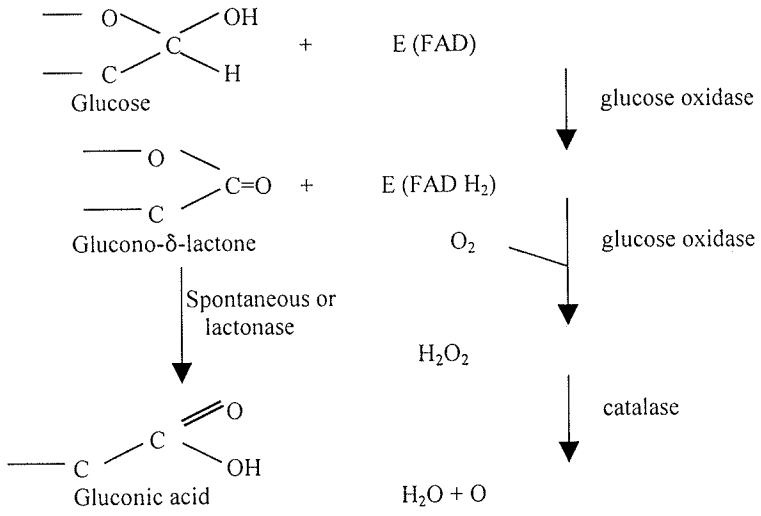
Yeast are considered to be more suitable than bacteria such as streptococci, *Aerobacter* and *Escherichia* because fermentations with these bacteria are likely to become wild fermentations. The growth rate cannot be controlled and mutagenesis of the fermentative culture is possible (Kilara and Shahani, 1973); therefore, yeast fermentations are easier to control. However, the objectionable odours often obtained with yeast fermentation are a distinct disadvantage. In contrast, advantages of yeast fermentation include little changes in acidity, eliminating the need for neutralisation, reduced growth of contaminant microorganisms due to the short incubation time, and ready availability of most species. In addition, fermentation without artificial aeration favours the conversion of glucose to acid rather than growth of yeast. Finally, yeast fermentation greatly reduces the development of objectionable odours and flavours sometimes associated with bacterial fermentations (Sebring, 1995).

2. Enzymatic Desugaring

Enzymatic desugaring involves glucose oxidase which is commonly produced by *Aspergillus niger* and *Penicillium glaucum* (Baldwin *et al.*, 1953). In the presence of molecular oxygen, glucose oxidase catalyzes the conversion of glucose to gluconic acid and hydrogen

peroxide, as shown in Figure 1. The product of the first reaction in the sequence is glucono-δ-lactone, which is subsequently converted to gluconic acid via non-enzymatic hydrolysis (Baldwin *et al.*, 1953). This method is easy to control, reproducible and is capable of yielding a final product that lacks the objectionable odours often obtained with microbiological fermentations (Sebring, 1995). However, one disadvantage is the high cost of the enzyme required for large-scale fermentation operations. Therefore, bacterial and yeast fermentations are often preferred due to economical restraints.

Figure 1. Mechanism of glucose oxidase-catalase system



Adapted from Reed (1966)

3. Spontaneous Bacterial Fermentation

Spontaneous bacterial fermentation involves the natural fermentation of albumen at 24-30^{0}C. Bacteriological anaylsis indicated that species such as *Enterobacter aerogenes* and *Citrobacter freundii* are predominant (Sebring, 1995). During fermentation, the pH gradually decreases and thick albumen begins to develop at the surface. After six to seven days, virtually no thin albumen remains, and a cloudy film appears on the surface. Fermentation results in a

stable, storage egg product; however, heat treatment is required to kill pathogens such as *Salmonella*, which are able to grow in the fermenting medium (Sebring, 1995).

4. Controlled Bacterial Fermentation

Bacterial species of *Streptococcus* and *Lactobacillus* have been used to desugar whole egg. Complete conversion of glucose to acid was achieved within 24 hours (Sebring, 1995). Similar results were reported with *Enterobacter aerogenes* (Sebring, 1995). Further investigations showed that for some microorganisms such as *Lactococcus lactis* desugaring was incomplete unless 1% yeast extract was added to speed the process (Sebring, 1995).

Most industrial fermentations use a batch process, where desugarization of albumen is initiated using a starter culture such as *Saccharomyces cerevisiae*. In this respect, once acidified to pH 7.0-7.5 with citric or lactic acid, the albumen is inoculated with a starter culture and incubated at an appropriate temperature for the duration of the fermentation. The fermentation is normally stopped before the glucose has been completely converted to acid by decreasing the incubation temperature. In a batch process, this culture can be used as the inoculum for future large-scale fermentations (Sebring, 1995). Most industrial desugarization processes use a controlled bacterial fermentation as the method of choice because of financial and functional advantages. The desugarized egg white product has excellent whipping characteristics, and results in a product with high solubility and is devoid of objectionable flavours (Sebring, 1995).

C. Growth Characteristics of Fermentative Microorganisms

For effective fermentation of egg albumen, the microorganisms must meet certain growth characteristics. The optimum growth temperature should be in the range of 25-37^{0}C, and the

optimum pH level for growth should be between 6.0 and 7.0. Production of extracellular polysaccharide (EPS) and/or a bacterial capsule are other criteria for the choice of fermentative microorganisms. These traits were considered to be fundamental because it has been shown that production of EPS/capsule improves functionality of proteins (Sanderson, 1996). Production of EPS has also been shown to be species-specific. For exampe, *Lactococcus lactis* subsp. *cremoris* is more likely to produce significant amounts of EPS than *L. lactis* subsp. *lactis* (DeVuyst and Degeest, 1999).

In this study, four microorganisms were chosen for investigation. The choice was based on their optimum growth conditions and potential for production of a slime layer or capsule.

1. *Saccharomyces cerevisiae* (control)

Yeast are unicellular microorganisms and can be differentiated from bacteria by their larger-sized cells and by the fact that they are eukaryotic with a nucleus. They have oval, elongate, elliptical or spherical-shaped cells and their typical cell size is 5-8 μm diameter. *S. cerevisiae* is a yeast which is able to grow in albumen, rarely causes food spoilage and cannot ferment lactose. Its optimum growth temperature is 37^0C. Yeast are known as a source for bakers' and brewers' yeast, as well as for wine and champagne (Jay, 1992).

2. *Leuconostoc mesenteroides*

The lactic acid bacteria classically include *Lactobacillus, Leuconostoc, Pediococcus, Bifidobacteria, Streptococcus, Enterococcus, Lactococcus* and *Vagococcus* (Jay, 1992). Lactobacilli are gram-positive, non-spore forming rods; they are catalase negative and usually non-motile (Sharpe, 1981). *Leuconostoc* spps. are heterofermentative lactic acid bacteria

(produce lactic acid, CO_2, ethanol and acetic acid from glucose), and their metabolism is not as efficient as homofermentative bacteria, which produce lactic acid from glucose (Jay, 1992). Some *Leuconostoc* organisms are also capable of producing extracellular polysaccharide under optimum conditions however, this ability is often strain-specific. *Leuconostoc mesenteroides* in particular, hydrolyzes sucrose, synthesizing a glucose polymer known as dextran and is typically produced when growth temperatures are in the range of 20-25^{0}C with pH levels between 6.5 and 7.0 (Jay, 1992).

3. *Lactococcus lactis*

The *Lactococcus* group of organisms was once classified as *Streptococcus* but has recently been elevated to generic status. These non-motile Lancefield serologic group N cocci are gram-positive, catalase-negative spherical or ovoid cells that may occur singly, in pairs, or in chains. They are able to grow at 10^{0}C, but not at 45^{0}C, are homofermentative and their optimum pH range is 5.5-6.0. Their advantage as a homofermentative organism is that they are able to extract double the energy from the same concentration of glucose as a heterofermentative organism such as *Leuconostoc mesenteroides*. *Lactococcus lactis* has been classified into 3 subspecies, *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* and *L. lactis* subsp. *hordniae* (Jay, 1992). *L. lactis* are typically ovoid cells, range from 0.5-1.0 μm in diameter, and usually occur in pairs or short chains. They are microaerophilic, but aerobic growth is often observed on media containing fermentable carbohydrate, supplemented with yeast extract as a growth stimulant (Jay, 1992; Kilara and Shahani, 1973).

4. *Klebsiella pneumoniae*

Klebsiella spps. are classified as coliforms. They are gram-negative asporogenous rods that are able to ferment lactose. Coliforms are classified under four genera: *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella.* Some *Klebsiella* spps. are often mistaken for faecal coliforms due to similar traits with *E. coli. K. pneumoniae* can be found in the intestinal tract of man and animals, but is usually observed in much lower numbers than *E. coli. K. pneumoniae* is widely known as an opportunistic pathogen, an agent of bacterial pneumonia, and has been known to cause gastroenteritis in immunologically compromised patients. Its enteropathogenicity was elucidated in 1976 when toxigenic strains were isolated from patients with diarrhea (Singh and Kulshreshtha, 1992). *K. pneumoniae* has also been isolated from many food environments as an integral part of the natural microflora, and is often found in foods with high carbohydrate content (Massa *et al.*, 1992). Its optimum growth temperature is 37^0C and grows best at pH levels of 6.5 - 7.0. *Klebsiella* species are capable of producing an exopolysaccharide consisting mainly of repeating rhamnose-galactose carbohydrate residues. To date, these microorganisms have not been used for purposes of fermenting albumen.

D. Functional Properties of Proteins

Functional properties are the physicochemical attributes, determined by the quality of the final product, that affect processing and behaviour of proteins in food systems (Kinsella, 1976). Functionality describes the interaction between the molecular composition, chemical structure, structural conformation and physicochemical properties of proteins. Various factors affect the functionality of proteins, including precipitation; concentration; dehydration; enzymatic, hydrolytic and chemical modifications; and environmental conditions such as temperature, pH

and ionic strength (Kinsella, 1976). The functional properties of a food ingredient are imperative for development of new products and technology for the food industry (Kinsella, 1976). Food proteins should possess favourable intrinsic properties such as colour, flavour and texture. They must also have good solubility, gelling, emulsifying and foaming properties. In addition, they should be thermally stable and possess the ability to be incorporated into complex food systems (Kinsella, 1976). Functional properties most commonly associated with eggs include gelation, foaming, emulsification and solubility. In fact, eggs are polyfunctional; they have high nutritional value, have excellent functional properties and therefore, are used as an ingredient in a variety of food products. No other food substitute has been successful in replicating all of eggs' functional attributes (Yang and Baldwin, 1995).

1. Gelation

Gelation is the process by which a liquid changes from a fluid (sol) to a semi-solid (gel) state. Of particular importance to processed foods is the irreversible heat coagulation of proteins. Protein gels are three-dimensional matrices where ordered polymer-polymer and polymer-solvent interactions cause immobilization of small amounts of protein by liquid. This immobilization of liquid in protein gels induces an elastic-like characteristic to gel systems (Kato *et al.*, 1990a). Egg albumen is considered to be thermotropic because it is able to form gels upon heating.

i. Mechanism of Gelation

The mechanism of gelation is not completely understood (Yang and Baldwin, 1995). In general, gelation is regarded as the phenomenon whereby proteins unfold and form an aggregated substance. Upon denaturation, conformational changes occur, including exposure of

hydrophobic amino acid residues at the surface. These changes enable the denatured protein molecules to orient themselves so that a stable three-dimensional matrix can be formed. The aggregation stage of gelation occurs more slowly than the denaturation step to allow the protein molecules to orient themselves in a stable conformation (Kato *et al.*, 1990a). Hsieh and Regenstein (1992) offer another explanation for the gelation mechanism. During heating, changes in protein conformation cause formation of aggregated clusters, which interact as sticky molecules, rather than random coils. The unstable intermediate aggregates then precipitate from solution by aggregation, through molecular interactions such as hydrogen bonding and disulphide linkages (Hsieh and Regenstein, 1992). When urea, a common denaturant, was studied with egg white proteins, a three-step gelation mechanism was revealed. The denaturation and subsequent aggregation of protein molecules was followed by further protein denaturation (Yang and Baldwin, 1995). Research has shown that this three-step mechanism is the most accurate explanation for gelation of egg albumen. Protein molecules unfold and aggregate together, and these aggregates interact to form a gel network (Yang and Baldwin, 1995).

The quality of the gel formed is dependent on the composition of the proteins and the rate of protein unfolding. At low denaturation rates, aggregation occurs slowly, resulting in a fine gel network. Alternatively, gelation may occur before the small molecular weight chains have had a chance to form, resulting in a coarse, less stable gel network (Yang and Baldwin, 1995).

ii. Gel Strength

Gel strength is a parameter often used to describe the effectiveness of three-dimensional gel networks. Covalent disulphide cross-linking, polar and hydrophobic interactions, plus hydrogen bonding stabilize the structure of protein gels. Disulphide cross-linkages in particular, have been attributed to gel strength (Hsieh and Regenstein, 1992). Many researchers have

studied the effect of heating in the dry state on gelation characteristics of protein gels. Kato *et al.* (1989) found that gel intensity increased over a 10-day period when albumen was heated at 80^0C. This can be explained by exposure of hydrophobic amino acid residues at the surface, with subsequent orientation of the denatured proteins in such a way that a stable gel network is formed (Kato *et al.*, 1990a).

The texture profile of a gel can be a very effective tool for monitoring gel strength. Compression testing involves a small round plunger that is lowered with constant speed toward a cylindrical piece of gel. A rheometer measures the time and force required for the plunger to break the gel. The force required to achieve compression of a gel is measured over time with the gel breaking point being referred to as gel strength (Yang and Baldwin, 1995).

iii. Measurement of Gels

The measurement of gels can be undertaken in many ways, including crossover of the storage modulus (G') and the loss modulus (G") determined by rheology, light scattering, polyacrylamide gel electrophoresis, viscosity and spectroscopy (Hsieh *et al.*, 1993). The stress required to break apart a sample of standard size and shape, and the depth of penetration by a steel ball can also be used to measure gel strength. In addition, the force required to penetrate a coagulum can be measured by placing a plunger into the gel (Yang and Baldwin, 1995).

2. Foaming

The ability of egg whites to produce foam is especially important to the baking and confectionery industries. Foam improves the texture, consistency and physical appearance of many food products (Vani and Zayas, 1995). Eggs are one of the food products with the highest capacity for foaming. They can produce stable, high volume foams that coagulate upon heating.

The lightness of foods such as meringue, omelettes and soufflés is attributed mostly to beaten egg whites (Yang and Baldwin, 1995). Foam is a two-phase colloidal dispersion where a distinct gas bubble is surrounded by a continuous liquid lamellar phase (Phillips *et al.*, 1987). Pure liquids cannot form foams independently; therefore, surface-active substances such as proteins are used for their film-forming capabilities.

Various molecular factors affect the ability of egg white proteins to form stable foams, including amino acid sequence and disposition, molecular size, shape, conformation and stability, plus surface charge, polarity and hydrophobicity (Vani and Zayas, 1995). Physical factors that contribute to good foaming properties of egg whites include low surface tension, high viscosity and low vapour pressure (Phillips *et al.*, 1987). Egg albumen is an effective foaming agent because it is a mixture of various protein components, each component performing a specific function in the foaming process. Globulins facilitate foam formation by lowering surface tension and contribute to the high viscosity that prevents liquid drainage from air bubbles. Ovalbumin and conalbumin are responsible for heat –setting properties and ovomucin forms an insoluble film that stabilizes the foam (Yang and Baldwin, 1995).

i. Mechanism of Foam Formation

When egg whites are beaten or whipped, air bubbles become entrapped within the albumen, forming foam. As the beating progresses, the air bubbles decrease in size and number, resulting in a moist, translucent albumen film. As more air is incorporated, the foam loses the ability to flow as a liquid (Yang and Baldwin, 1995). The successful formation of foam is determined by the ability of proteins to form a cohesive film that retains and entraps air. The outer film must be able to withstand external stress such as mechanical shock and shearing (Phillips *et al.*, 1990). Formation of foams is thought to involve unfolding of protein molecules,

exposing long polypeptide chains with long axes oriented parallel to the surface. This unfolding and orientation causes loss of solubility and precipitation of protein at the air-liquid interface (Yang and Baldwin, 1995). Foams are normally described by two parameters, foaming power/capacity and foam stability.

ii. Foaming Power/Capacity

Foaming power measures the capacity for incorporation of air into a protein film. Globulin proteins aid in the foaming process because they increase the viscosity of the albumen and diminish the tendency for liquid drainage. Globulins also lower the surface tension of albumen, which is important to initial foam formation (Phillips *et al.*, 1990).

Various methods can be used to measure the foaming capacity of proteins. Among these include the seed method, whipping, sparging, shaking and specific gravity measurements. Whipping is a common method for measuring protein foaming power and is usually the method of choice because it most closely mimics industrial processing (Phillips *et al.*, 1990). The protein dispersion is whipped using an electric mixer for a specific length of time, and the density of the resultant foam is measured (Phillips *et al.*, 1990). Angel cakes are also commonly used to measure the foaming power of egg white foams. Measuring cake height involves baking an angel cake in a pan of predetermined volume. If the pan is slightly deeper than the baked cake, the difference between the volume held by the pan, with and without the cake, determines the volume of the cake (Yang and Baldwin, 1995).

iii. Foam Stability

Foam stability measures the ability of protein foams to resist drainage and collapse. It is dependent on the ability of proteins to form strong, cohesive viscoelastic foams that reduce gas permeability and prevent coalescence (Kato *et al.*, 1990b; Vani and Zayas, 1995). In

homogeneous protein systems, short-range hydrogen bonds, hydrophobic and electrostatic interactions, plus van der Waals' forces determine foam stability. However, in heterogeneous systems like egg whites where various protein fractions are involved, the isoelectric point and molecular charge of the individual protein fractions also affect foam stability (Vani and Zayas, 1995).

Foaming capacity and stability are partially governed by the exposure of hydrophobic amino acid residues at the surface. Protein films adsorbed at the air-liquid interface reduce surface tension. This adsorption is due to the amphiphilic nature of the amino acid residues, indicating a close relationship between foaming capacity and hydrophobicity (Townsend and Nakai, 1983). Further study revealed that a significant ($P< 0.01$, $r^2=0.823$) relationship existed between foaming capacity and surface hydrophobicity of proteins. However due to extensive unfolding of proteins at the air-liquid interface, a greater correlation was found between total hydrophobicity and foam capacity (Townsend and Nakai, 1983).

An indirect method for the determination of foam stability is the use of specific gravity measurements. The specific gravity is proportional to foam stability and viscosity of protein foams. Another method for measuring foam stability is to determine the amount of liquid drainage from foam over a pre-determined length of time (Yang and Baldwin, 1995).

3. Emulsification

An emulsion is a colloidal dispersion of liquid droplets in a continuous medium. Emulsions are described either as oil-in-water or water-in-oil. Mayonnaise and homogenized milk are oil-in-water, while margarine is a water-in-oil emulsion. Many foods, such as ice cream and whipped cream, can be described as emulsions and foams (Dickinson, 1992). Proteins are

effective emulsion stabilizers due to their surface-active properties. As a result of their amphiphilic nature, proteins reduce the interfacial surface tension by adsorption at the oil-liquid interface. Although all soluble proteins exhibit emulsifying properties, some foods such as eggs are especially effective. Good emulsification properties are governed by the ability of proteins to adsorb, resulting in thick, strong, flexible coherent films surrounding oils droplets and air cells (Dickinson, 1992). Hydrophobicity has a large effect on emulsification; a balance between hydrophobic and hydrophilic amino acid residues is imperative for emulsification, and high surface hydrophobicity (So) of proteins is often desired to achieve stable emulsions (Nakai, 1983; Mine *et al.*, 1991). Voutsinas *et al.* (1983) also reported that although solubility correlates well with emulsification, surface hydrophobicity of proteins is the best indication of emulsion stability. A fluorometric study by Nakai (1983) confirmed the relationship between surface hydrophobicity and emulsifying activity.

i. Emulsion Activity

Emulsion activity is the ability of proteins to adsorb to the oil-water interface, resulting in a stable emulsion. The pH level of the emulsion primarily affects emulsifying activity. Comparison of emulsion activity at acidic and neutral pH levels revealed greater emulsification at lower pH values. This was attributed to increased molecular flexibility and exposure of hydrophobic amino acid residues at the surface, facilitated by changes in structural conformation (Mine *et al.*, 1991).

ii. Emulsion Stability

Emulsions are very unstable due to the disappearance of oil droplets or air bubbles. Three phenomena can destroy emulsions; these include creaming, flocculation and coalescence. Creaming occurs when droplets are affected by gravitational pull, and form a concentrated layer

at the surface, while serum forms at the bottom. Flocculation results in oil droplets sticking together, while each individual droplet remains intact. Finally, coalescence is the irreversible attachment of two or more droplets to form a larger single droplet (Dickinson, 1992). In addition, emulsion stability is affected by protein concentration. A stable emulsion cannot be formed unless there is greater than 0.5% protein concentration. Also, when the oil: water ratio for oil-in-water emulsions is increased greater emulsion stability is observed, especially with high protein concentrations. This improved stability was explained by the dramatic increase in viscosity of the emulsion and the amount of protein adsorbed to surface fat globules (Mine *et al.*, 1991).

iii. Measurement of Emulsions

Emulsion capacity can be determined by several methods. Dye binding involves the addition of an oil-soluble dye to an emulsion. When the emulsifying capacity of proteins is exceeded, a phase inversion occurs, facilitating visual monitoring due to presence of the coloured dye. Emulsions can also be evaluated by determining the point at which the emulsion breaks, indicated by a decrease in viscosity. Another method involves measuring the amount of oil released upon centrifugation of a heated emulsion (Kato *et al.*, 1985). The most common measure of emulsion stability involves determining the amount of oil/cream that separates from the emulsion in a given amount of time, with a specific gravitational field. (Kato *et al.*, 1985).

E. Physicochemical Properties of Proteins

1. Thermal Denaturation

Denaturation is the process by which proteins change from an ordered to a disordered state without disrupting covalent bonds. Denaturation involves protein-solvent interactions that

disrupt the three-dimensional structure of proteins, often leading to decreased solubility (Gossett *et al.*, 1984). Denaturation of proteins can be achieved by treatment with heat, acid or alkali, specific chemicals and enzymes. The context of this discussion will deal only with heat denaturation because it is the method of denaturation examined during this study.

Heat denaturation occurs when the thermal energy exceeds the binding energy of hydrogen bonds, and the hydrophobic interactions that stabilize the secondary and tertiary protein structure are interrupted (Hayakawa *et al.*, 1992). Denaturation causes unfolding of protein structure, and aggregation of denatured molecules often ensues. Heat treatment insolubilizes the protein, leading to thickening and gelation (Wu and Ingles, 1974). Because egg white is a complex mixture of proteins, it is often difficult to ascertain the effect of heat on albumen. The varying heat sensitivities of individual protein fractions found in egg white cause heat treatment such as pasteurization to affect some protein fractions, but not others. For example, ovomucin has high heat stability and is not denatured by temperatures reached during pasteurization (Donovan *et al.*, 1975).

Thermal denaturation is often measured by Differential Scanning Calorimetry (DSC). This analytical method measures the denaturation temperature and amount of energy required to cause unfolding of protein molecules. A typical DSC thermogram of native egg white shows two distinct endothermic peaks. The smaller peak is due to denaturation of conalbumin with a denaturation temperature of 67^0C. The larger peak belongs to ovalbumin, the most abundant protein fraction in egg white, with a denaturation temperature of 84.5^0C. Conalbumin has been found to be more heat-stable in egg white than alone in solution, probably due to interactions with other protein fractions (Raeker and Johnson, 1995). The enthalpy of denaturation (ΔH) reflects stability of proteins to heat treatment. A decrease in ΔH usually occurs after heat

treatment such as pasteurization and is often accompanied by a slight downward shift in denaturation temperature, suggesting changes in the molecular conformation caused by unfolding of tertiary protein structure (Kato *et al.*, 1990b).

Thermal denaturation of albumen has an impact on its functionality. The denaturation of ovalbumin coincides with starch gelatinization during the baking process of angel cakes and is important to cake volume and texture. Due to the sensitivity of albumen proteins to heat, denaturation of albumen occurs during cake baking. This protein unfolding causes adsorption of air, resulting in increased cake volume (Raeker and Johnson, 1995). Strong negative correlation has been observed among the enthalpy of denaturation (ΔH) and foaming properties of heat denatured albumen. Both foam capacity and stability increased with heat treatment, and dried egg white had the greatest foaming power compared with a variety of plant proteins. This suggests that protein-protein interactions facilitate the formation of strong foams (Kato *et al.*, 1990b).

2. Surface Hydrophobicity

Hydrophobicity and hydrophobic interactions affect all facets of protein functionality. Surface properties of proteins are extremely important because they dictate how proteins behave in specific environments. When proteins are in their native conformation, hydrophobic amino acid residues are mainly hidden in the interior of protein molecules; however, when denaturation occurs, these residues are exposed at the air-liquid interface. Due to the significance of hydrophobic interactions in the aggregation of proteins, surface hydrophobicity (So) is often used as a determinant of surface functional properties.

i. Measurement of Surface Hydrophobicity

There are many methods by which So may be measured. Among these include fluorescence spectroscopy using a fluorescent probe, reverse-phase chromatography, binding hydrocarbons to protein, hydrophobic partition techniques, and salting-out and surface tension measurements (Nakai, 1983). Earlier studies measured the total hydrophobicity of proteins, however, the 'effective' or 'true' surface hydrophobicity has been shown to have more significance in determining the activity of biological molecules.

Fluorescence spectroscopy was the method chosen for determination of surface hydrophobicity of egg proteins in this study. Fluorescence methods have been divided into two categories; fluorescence quenching and fluorescent probe methods. The fluorescent probe is the more common, using *cis*-parinaric acid (CPA) and 1-anilinonapthalene-8 sulphonate (ANS) as probes. CPA is used because of its interaction with aliphatic hydrophobic residues, while ANS is used because it fluoresces upon binding with aromatic hydrophobic residues (Kato and Nakai, 1980). For both methods, the resulting So is the slope of the relative fluorescence intensity determined at various protein concentrations (Kato and Nakai, 1980).

ii. Effect of Surface Hydrophobicity on Solubility

Hydrophobicity impacts various functional properties of proteins, including solubility, foaming, emulsification and aggregation. Solubility is an important property pertaining to protein functionality because denaturation often implies a loss of solubility. Solubility of proteins is especially important to the food processing industry, as soluble proteins generally have greater functionality for use in food processing (Nakai, 1983). Protein insolubility begins with a reversible aggregation of protein monomers, which is followed by the irreversible formation of insoluble protein (Nakai, 1983).

F. Factors Affecting Protein Functionality

Many factors, such as protein concentration, pH, temperature, and processing conditions, affect the way that proteins behave in food systems. Some of these factors have detrimental effects, while others may improve the functionality of the proteins.

1. Protein Concentration

Gosset *et al.* (1984) reported that all protein aggregates at 80^0C, regardless of concentration. However, at 70^0C, a protein concentration of at least 1% is required for adequate gelation. Higher protein concentrations ensure a closer-knit network of aggregates (Gosset *et al.*, 1984). The effect of protein concentration on gel strength when proteins are heated in the dry state was studied by Kato *et al.* (1990c). They observed greater improvements in gel strength at protein concentrations of 10% or more. They also found that the dependency of gel strength on protein concentration was greater with heat-treated than native dried egg white (Kato *et al.*, 1990c). Similarly, Kato *et al.* (1990a) observed an increase in the molecular weight of dried egg white aggregates with higher protein concentrations, indicating formation of a gel network. Hsieh *et al.* (1993) studied the elastic modulus (G') and storage modulus (G") values of various protein fractions. They found that the gel point temperature was concentration-dependent for ovalbumin and ovotransferrin. Similarly, Hsieh and Regenstein (1993) studied the true stress-strain relationships of egg white gels and found that stress values increased significantly with increasing protein concentration.

Vani and Zayas (1995) examined the effect of varying protein concentrations on the foaming properties of several plant and animal proteins, including wheat germ protein flour, corn germ protein flour, soy flour, non-fat dried milk and dried egg white. Results showed that

increasing concentrations (up to 8%) of egg white protein had the greatest capacity for foam expansion, compared with the other proteins. This was explained by changes in viscosity; high viscosity often prevents incorporation of air into a liquid. As the protein concentration increased, unfolding and looping of the protein was apparent, resulting in more rapid adsorption at the air-liquid interface (Vani and Zayas, 1995). Protein concentration also affected the foam stability of protein dispersions. Increased foam stability was observed at higher protein concentrations because serum flow was reduced from the lamellar structure. Of the proteins tested, egg white protein had the greatest foam stability (Vani and Zayas, 1995).

2. pH

The pH of a protein system may have either a detrimental or positive effect on protein functionality, depending on the isoelectric points of the protein fractions involved. For example, ovalbumin, ovotransferrin and ovomucoid have isoelectric points of 4.5, 6.1 and 4.1, respectively (Sebring, 1995). With increasing protein concentration, the pH range of gelation also increases. The hydrophobic interactions and disulphide bonds formed at high protein concentrations counteract the electrostatic repulsion associated with pH values that fall far from the isoelectric points of individual protein fractions (Mulvihill and Kinsella, 1987). Mine (1996) studied the effect of pH on the gelling properties of dried egg albumen. He found that at all pH levels tested (6.88, 8.55 and 9.40), the gel strength increased over a 10-day heating period. The rate of increase in gel strength was greater at pH 9.40 than at 6.88. This can be attributed to the fact that thicker and stronger foams are likely to form when the pH is closer to the isoelectric point. At the isoelectric point the net charge of proteins is zero, minimizing electrostatic interactions among protein molecules (Hammershøj et al., 1999). The proteins were likely to retain their

solubility, resulting in a stronger gel (Mine, 1996). Differential scanning calorimetric (DSC) results confirmed that partial unfolding had taken place, and that this degree of protein denaturation played a role in the improvement of gelation properties. Heating egg white proteins at alkaline pH levels resulted in the formation of low-molecular weight aggregates that contributed to the development of a strong gel network (Mine, 1996).

Vani and Zayas (1995) studied the effect of pH on the foaming properties of wheat germ protein flour, in particular. They found that foam expansion and stability were highest at pH 8.0 and lowest at pH 7.0. The net charge and conformation of the proteins at different pH levels were the most obvious explanations for the changes in foaming properties. Physical forces such as electrostatic attraction, hydrogen bonding and hydrophobic interactions were also affected by variations in pH. In addition, egg white showed optimal foaming characteristics when the pH ranged from 5.0 to 10.0 (Vani and Zayas, 1995).

3. Temperature

Proteins can undergo denaturation by forces such as high pressure, heat, or chemical denaturants. The heat denaturation of egg albumen is difficult to quantify because it contains a complex combination of protein fractions, each of which has a characteristic heat sensitivity (Donovan *et al.*, 1975).

The best indication of heat denaturation is the coagulation or gelation of proteins. Albumen begins to coagulate at a temperature of 62^0C and the coagulum is no longer liquid at 65^0C. As the temperature continues to increase, the coagulum becomes firmer and more rigid (Yang and Baldwin, 1995). Gel firmness varies, depending on heating time and temperature. Increased time and temperature of coagulation also increases the water-holding capacity,

resulting in a gel with a high degree of cross-linkages (Yang and Baldwin, 1995). Temperature is the most influential factor affecting gel rheology. Gel rigidity is determined at temperatures of 71^0 - 83^0C, while the elasticity development occurs at 70^0 - 74^0C. The rigidity development of gels observed at elevated temperatures can be attributed to decreasing protein concentration and denaturation of ovalbumin at 79^0 to 84^0C (Yang and Baldwin, 1995).

Partial denaturation may, in some cases, improve functional properties such as formation of strong gel networks. Hayakawa *et al.* (1992) studied the effect of heat denaturation on the gelling properties of bovine serum albumin (BSA) and ovalbumin. Fluorescent spectroscopy revealed that BSA started to denature at a temperature of 40^0C, while ovalbumin remained stable until 60^0C. DSC confirmed the denaturation properties; native ovalbumin had a denaturation temperature (T_d) of 77 ^{0}C, while ovalbumin heated to 100^0C had a T_d of 80.6^0C (Hayakawa *et al.*, 1992).

Kato *et al.* (1990b) studied the effects of heating in the dry state, on the gelling, foaming and emulsifying properties of egg white proteins. Proteins were heated in the range of 65^0 - 100^0C, and results showed that optimal functionality was observed at 80^0C. It should also be pointed out that heating at 80^0C is sufficient for inactivation of most spoilage and all pathogenic microorganisms (Kato *et al.*, 1990b). In addition to the temperature of heating, the length of heating time affected gel strength. Proteins were heated for up to 10 days. As heating time increased, the gel strength also increased. Similarly, the foaming and emulsifying properties increased with increased heating time, up to 10 days (Kato *et al.*, 1990b).

G. Extracellular Polysaccharide Production

Polysaccharides, or gums, are present in most plant and animal tissues. The specific role of these polysaccharides is not certain, however, many act as structural components and/or energy sources. Commercially, gums are useful for their ability to alter the properties of water. Gums are often used as thickening and suspending agents, as well as for water retention and film formation (Sandford, 1972).

Bacterial growth is often accompanied by the production of extracellular or exo-polysaccharides. These extracellular polysaccharides (EPS) may encapsulate the bacterial cell or may be excreted as slime into the cellular environment. In addition, production of EPS is favoured under conditions of excess carbohydrate availability (Bryan *et al.*, 1986). Production of EPS is generally favoured at temperatures of 25^0C and a pH of 7.0 (Bryan *et al.*, 1986). EPS can be either homo- or hetero-polymers, and may also possess other constituents such as ester-linked acetate or pyruvate. Production of EPS by bacterial cultures is of importance to the food industry because EPS is suitable both as a digestible and non-digestible food ingredient. Microorganisms such as *Leuconostoc mesenteroides* and *Xanthomonas campestris* have been found to produce EPS gum by utilization of glucose and sucrose (Stauffer and Leeder, 1978).

EPS is economical, can be used to supplement naturally occurring gums, improves protein functionality and synergistic reactions are often observed when EPS is used in combination with other gums and food ingredients (Stauffer and Leeder, 1978). Bacterial EPS is a favourable alternative to natural and synthetic gums because it is not susceptible to crop pollutants, has a wide range of physical attributes, can be used as a suspending agent and has been found to possess favourable functional properties, including gelation and emulsification (Bejar *et al.*, 1996).

Several gums have received attention in the last few years regarding their possible uses as a food ingredient, including gellan, xanthan, dextran and a cell wall material known as baker's yeast glycan.

1. Gellan Gum

Gellan is a linear anionic heteropolysaccharide with a tetrasaccharide repeating unit, and its native form is esterified (Morris, 1991). It is produced by *Pseudomonas elodea*. Its production is favoured at temperatures of 25-30^{0}C and neutral pH (Milas *et al.*, 1990), with the preferred substrate being glucose. *Sphingomonas* is able to produce a gellan yield similar to xanthan and has been suggested as a substitute to agar for microbiological purposes (Lobas *et al.*, 1992). It has been approved in Japan for use in food and is generally regarded safe as a food ingredient by the Food and Drug Administration (Morris, 1991; www.frwebgate4.access.gpo.gov, August 12, 2000).

2. Xanthan Gum

Xanthan gum is a bacterial EPS produced upon growth of *Xanthomonas* species. It is presently the only microbial polysaccharide permitted for food use in the United States and Canada. The structure of xanthan gum consists of repeating units containing two glucose units, two mannose units and one unit of glucuronic acid. The backbone of the main chain consists of B-D-glucose attached through 1→4 linkages (Cottrell and Kang, 1978). Production of xanthan is favoured at 30^{0}C at pH levels of 7.0. The preferred substrate is glucose and the increased viscosity observed upon its production is independent of pH (Sanderson, 1981). Xanthan is rendered soluble when short chains are attached at alternating glucose residues. This solubility

imparts several functional attributes to xanthan, including high viscosity at low-shear values, highly pseudoplastic behaviour, increased viscosity in the presence of salts, stability of viscosity at high temperatures, and synergistic interactions with other gums. The high viscosity at low shear values enables stability of suspensions, emulsions and foams (Sanderson, 1981).

3. Dextran

Dextran was the first gum to be produced commercially; however, it is not currently as popular as xanthan gum. Dextran describes a class of extracellular bacterial β-glucans and is produced by several bacterial species, including *Leuconostoc mesenteroides*. Each bacterial species produces a unique dextran, but the common attribute of all dextrans is the large proportion of 1→ 6 linkages. Dextran is produced by slime or capsule-producing bacteria, is favoured at 30^0C at pH levels of 7.5, and the preferential substrate is sucrose. Dextran is used mostly in the pharmaceutical and fine chemical industries, however, is can also be used to treat anaemia and hypoglycaemia (Sandford, 1972).

Dextran is formed when *L. mesenteroides* bacterial cells secrete an enzyme, which catalyses the conversion of sucrose to glucose and fructose (Sanderson, 1996). Dextran has a very high molecular mass and as a result, causes increased viscosity of fermentation media. Production of bacterial exopolysaccharide is desirable in fermented egg albumen because it has been demonstrated that exopolysaccharide production is linked to improved albumen functionality (Sanderson, 1996).

4. Baker's Yeast Glycan

Baker's yeast glycan is a cell wall material produced by *Saccharomyces cerevisiae* that is composed of sugar in a 3:2 glucose to mannose ratio. Although not a true extracellular polysaccharide, baker's yeast glycan exhibits similar functional characteristics to its EPS relatives. Advantages include its fat or oily mouthfeel, making it useful as a fat replacer. It can be used as a thickener and emulsifier and undergoes gelation upon heating and cooling (Sandford, 1972).

Many polysaccharide gums are currently being re-evaluated for food use because of their functionality and usefulness as sources of dietary fibre.

H. Effect of Exopolysaccharide Production on Protein Functionality

One of the great benefits of using EPS and gums in food systems is their improvement of protein functionality. When proteins and polysaccharides interact, they increase the gelling and emulsifying properties of a food product, and act as thickening agents. When gums are used as thickeners, they inhibit separation of dispersed materials. EPS stabilizes emulsions and foams, and promotes gelation (Sanderson, 1996).

Protein-polysaccharide mixtures are favourable for the formation of strong gels, due to hydrophobic interactions and hydrostatic attractions. For example, gellan gum, a bacterial EPS produced by *Pseudomonas*, was found to have excellent gelling properties. The gel setting point was in the range of 35-50^{0}C, and it was thermally stable up to 90-100^{0}C (Kang *et al.*, 1982). Miyoshi *et al.*, (1994a) found that more stable gels were formed in the presence of cationic salts. Salt promoted aggregation at lower temperatures, while it reduced helical coil structure at higher temperatures (Miyoshi *et al.*, 1994b). Proteins are effective emulsifiers because they are able to

decrease the surface tension at the oil-liquid interface, stabilizing the emulsion. In this capacity, protein-polysaccharide interactions facilitate emulsion stability by reducing interfacial surface tension (Sanderson, 1981).

I. Relation of EPS Production to Fermentation of Egg Albumen

Studies have shown that production of bacterial exopolysaccharide provides favourable improvements in the functionality of protein-polysaccharide solutions (Bejar *et al.*, 1992). It is therefore hypothesized that similar improvements in functionality of egg albumen are likely during fermentation with exopolysaccharide-producing bacteria. The development of an industrial fermentation process that incorporates bacterial depletion of glucose, extracellular polysaccharide production, as well as improvement of functional properties such as gelling, foaming, solubility and emulsification is a definite asset to the egg processing industry. The desired functional properties can be manipulated to fit individual customer's requirements. This innovation would not only improve product quality, but would also have the potential for gross economic benefits for the entire food industry.

MATERIALS AND METHODS

A. PRELIMINARY EXPERIMENTS

1. Fermentation of Albumen

i. *Saccharomyces cerevisiae* (control) Fermentation

Fresh lysozyme-extracted egg albumen (20 L) was obtained from Canadian Inovatech (Winnipeg, MB), distributed into 500 ml plastic containers and immediately frozen at -20^0 C until needed. Prior to fermentation, the frozen egg albumen was thawed rapidly in a 45^0 C water bath (Blue M, Blue Coast, IL), to minimize protein denaturation. Albumen fermentation (300 mL) was carried out in 1000 ml Erlenmeyer flasks. The inoculum consisted of 0.0132g of *Saccharomyces cerevisiae* culture (Lallemand Eagle Brand Wet Yeast Cake) suspended in albumen to obtain an inoculum level of 10^5 CFU/ml. The pH of the albumen was adjusted to the desired level (approximately 6.5 and 7.0) using 1.0 M citric acid. After inoculation, the flasks were incubated at 37^{0}C on a shake-flask incubator rotating at 25 rpm, for 72 h. Samples (20 ml) were removed at 30 minutes, and after 24, 48 and 72h of fermentation, and were placed immediately on ice in order to retard microbiological growth. In addition, 0.2% sodium azide (1ml) was added to minimize further microbial growth. All samples were subsequently analyzed for viscosity, pH, residual glucose and yeast growth. All experiments were performed in duplicate.

a. Viscosity Determination:

Viscosity of the albumen was measured using a Ubbelohde capillary viscometer (Canon Instruments, State College, PA). Egg albumen samples were vacuum-filtered prior to viscosity determination to remove particulate matter. In this respect, a vacuum-operated milk filter (Schwartz Blue Streak filter, Schwartz Manufacturing Co., WI) equipped with a Sartorius funnel was used. Viscosity was measured immediately following filtration. The filtrate was poured through tube G into the lower reservoir of the viscometer (see Fig. 2),

until the volume reached any point between lines J and K. An index finger was placed over tube B and suction applied to tube A, until the liquid reached the centre of tube C. Upon removal of suction from tube A, the index finger was removed from tube B and immediately replaced over tube A when the sample dropped into the lower end of the capillary, into bulb I. To measure the efflux time, egg albumen was allowed to flow through the capillary and the time elapsed for the albumen to move from line D to line F was measured. To determine the kinematic viscosity, the efflux time was multiplied by a viscometer constant, as determined by calibration of the instrument. One viscosity determination per sample was done at each time interval, and viscosity was expressed as centipoise/s.

b. pH Determination:

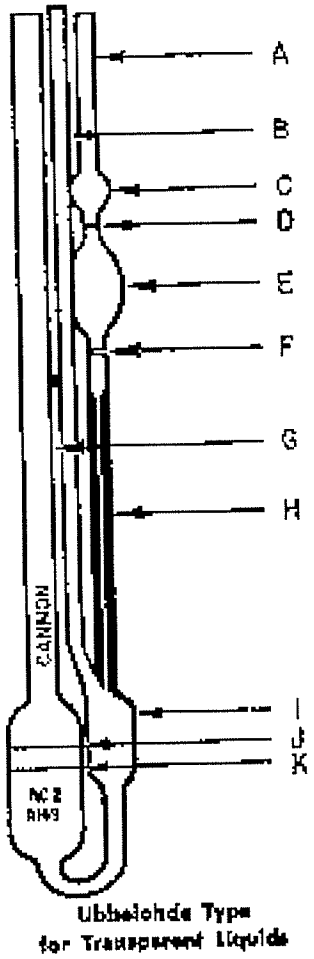
The pH values of the samples were assessed at 4-5^0 C (maintenance temperature of samples). Sample temperatures were maintained with ice water. The pH of samples was evaluated using a standardized Accumet 910 pH meter (Fisher Scientific Co., Nepean, ON). One pH determination was done per sample at each time interval.

c. Glucose Determination:

Residual glucose in samples at each time interval was determined using a colourimetric method adapted from AOAC (1975). Samples (~ 5ml) were taken at each of the four time intervals, placed in test tubes and immediately frozen at -20^0 C for subsequent determination.

To determine glucose, egg albumen (2 ml) was added to distilled water (10 ml) in a test tube and shaken; 20% sodium tungstate (2 ml) and 2N H_2SO_4 (2 mL) were then added to the solution and mixed. After 5 min the solution was filtered through a #2V fluted filter (Whatman) and the filtrate (2 ml) transferred to a clean dry Folin-Wu sugar tube. A standard

Figure 2: Diagram of Ubbelohde Capillary Viscometer



(Canon Instruments, State College, PA)

Letters indicate portions of viscometer that a liquid passes through during viscosity determinations

alkaline cupric sulphate solution (5 ml) was added; the tube was then placed in a boiling water bath for exactly 6 min and cooled for 2-3 min in a cold water bath. Upon cooling, 5ml of phosphomolybdic acid (0.5M) was added and once the cupric sulphate (0.02M) dissolved, the solution was diluted to the 25ml mark with distilled water and shaken. After 10-15 min. the absorbance of the prepared solution was determined at 420 nm using a UV/VIS spectrophotometer (Pharmacia Biotech Ltd., Cambridge, England) with distilled water as a blank. A standard curve of known glucose concentrations was constructed. A 1.0% standard solution was prepared form 10% glucose stock solution and subsequent serial dilutions ranging from 0 to 0.5% glucose were prepared in distilled water. A linear regression analysis was used to assess the unknown glucose concentrations (Appendix 9). A new standard curve was constructed with each new batch of reagents. One determination per sample was tested for each time interval.

d. Determination of Yeast:

Yeast counts were determined using a spread plate technique employing Potato Dextrose Agar (Difco Laboratories, Detroit, MI) acidified with 10% tartaric acid. Enumeration followed 5d incubation at 22^0C. Serial dilutions were carried out using 0.1% peptone. Plates containing between 30 and 300 colonies were counted using a Quebec Colony Counter (Americal Optical, Buffalo, NY) and were expressed as $\log_{10}$ CFU/ml. Duplicate plate counts were averaged (n = 4).

ii. *Leuconostoc mesenteroides* Fermentation

Cultures of *Leuconostoc mesenteroides* strains #1 and 11 were isolated from fermented meats and obtained from Dr. Richard Holley, Dept. of Food Science, University of Manitoba. Frozen cultures were thawed to room temperature (21^0C). After thawing, the cultures (1ml)

were grown in 50 ml of MRS Lactobacillus broth (Difco Laboratories, Detroit, MI) at 25^0C for 24 h.

a. Inoculum Standardization:

Following growth, the culture was centrifuged (20 min., 10 000 g at 5-10^0C) using an RC2B Sorvall centrifuge (Wilmington, DE) and the resulting pellet was re-suspended in physiological saline (10 ml). Centrifugation and washing of the pellets in physiological saline were repeated. The optical density (0.6) of the culture was then determined using a UV/VIS (Pharmacia Biotech Ltd., Cambridge, England) at 600nm. Concurrently, viable numbers were determined using MRS Lactobacillus agar (Difco Laboratories, Detroit, MI) following incubation at 25^0C for 48 h. Plate count results were correlated to optical density. The desired inoculum level was 10^6 CFU/ml (colony forming units/ml). Both strains of *Leuconostoc mesenteroides* were standardized according to this method.

b. Fermentation of Albumen using Leuconostoc mesenteroides:

L. mesenteroides (3 ml, 10^6 CFU/ml) was inoculated into 300 ml of egg albumen contained in 1000-ml Erlenmeyer flasks and incubated for 72 h at 30^0C on a gyratory incubator operating at 25 rpm. Duplicate trials were carried out with albumen adjusted to approximately pH 6.5 and 7.0 using 1.0 M citric acid.

Viscosity, pH, residual glucose and total plate counts were determined after 30 min, and 24, 48 and 72h, according to methods previously outlined. Total plate counts were determined using MRS Lactobacillus agar (Difco Laboratories, Detroit MI) following incubation for 48 h at 30^0C, and were expressed as $\log_{10}$ CFU/ml. Duplicate plate counts were averaged (n=4).

iii. *Lactococcus lactis* Fermentation

L. lactis (ATCC #1145-4) was obtained from the Culture Collection, Dept of Microbiology, University of Manitoba. One loopful of culture was inoculated into 50 ml trypticase soy broth (BBL, Becton Dickinson and Company, Cockeysville, MD) and incubated at 28^0C for 24 h. Following incubation, the culture was centrifuged (20 min., 10 000 g at 5-10^0C). The supernatant was discarded and the pellet was re-suspended in physiological saline (10 ml) to obtain an inoculum level of 10^8 CFU/mL. Optical densities were measured as previously described, at a wavelength of 600 nm and were found to be approximately 0.8. *L. lactis* (3 ml) was transferred into 1000-ml Erlenmeyer flasks, containing 300 ml of albumen to obtain a final inoculum level of 10^6 CFU/ml. Cultures were incubated on a gyratory incubator operating at 25 rpm for 72 h. Duplicate fermentations were carried out with albumen adjusted to approximately pH 6.5 and 7.0 using 1.0 M citric acid, at temperatures of 28^0 and 32^0C.

Viscosity, pH, residual glucose and total plate counts were determined after 30 min, and 24, 48 and 72h, according to methods previously outlined. Total plate counts were enumerated on trypticase soy agar (BBL, Becton Dickinson and Company, Cockeysville, MD) following incubation for 24 h at 28^0C. Duplicate counts (n = 4) were expressed as $\log_{10}$ CFU/ml.

iv. *Klebsiella pneumoniae* Fermentation

K. pneumoniae (ATCC #13883) was obtained from Culture Collection, Dept. of Microbiology, University of Manitoba. One loopful of culture was inoculated into 50 ml sterile trypticase soy broth (BBL, Becton Dickinson and Company, Cockeysville, MD) and incubated at 37^0C for 24 h. As previously described, optical densities were measured at 600 nm to determine inoculum level. Following incubation, the culture was centrifuged (20 min.,

10, 000 g at 5-10^{0}C). The supernatant was discarded and the pellet was re-suspended in sterile physiological saline (10 ml). The inoculum (3 ml) was transferred to 300 ml albumen contained in a 1000 ml Erlenmeyer flask to obtain a final inoculum level of 10^6 CFU/ml. Cultures were incubated on a gyratory incubator operating at 25 rpm for 72 h. Duplicate fermentations were carried out with albumen adjusted to approximately pH 6.5 and 7.0 using 1.0 M citric acid, at temperatures of 37^0 and 32^{0}C.

Viscosity, pH, residual glucose and total plate counts were analyzed after 30 min, and at 24, 48 and 72h, according to methods previously outlined. Duplicate total plate counts (n = 4) were enumerated using trypticase soy agar (BBL, Becton Dickinson and Company, Cockeysville, MD) following incubation for 24 h at 37^{0}C, and were expressed as $\log_{10}$ CFU/ml.

2. Fermentations with the Addition of Yeast Extract, Glucose and Sucrose

Fresh lysozyme extracted egg albumen was obtained from Canadian Inovatech (Winnipeg, MB), distributed into 500 ml plastic containers and frozen at -20^{0}C. When required for fermentation, the albumen was thawed in a ≤45^{0}C water bath for 30 minutes and adjusted to approximately pH 6.5 using 2N HCl. Glucose (1 and 2%) and yeast extract (0.1 and 0.2%) were added to the albumen to initiate exopolysaccharide production. In a separate trial, sucrose was added at 3, 4 and 5%, while yeast extract was added at 0.1%. *Saccharomyces cerevisiae,* originally supplied as a dried yeast cake (0.0132 g), was prepared in physiological saline to yield an inoculum level of 10^5 CFU/ml; the other four test organisms were inoculated (3 ml) as cultures suspended in sterile physiological saline, as previously described. Fermentations were carried out in duplicate using 1000-ml Erlenmeyer flasks on a gyratory incubator operating at 25 rpm, at the optimum temperature for each

organism. Fermentations were carried out for 24 h. Samples were analyzed at 0 and 24 h for viscosity, pH and microbiological growth using methods previously outlined.

Microscopic evaluation of albumen following fermentation was performed to determine if exopolysaccharide production was evident. The Hiss method for staining capsules was used. A loopful of fermented albumen and skim milk were mixed on a glass microscope slide, and were air dried and gently heat-fixed on the slide. The film was then covered with crystal violet stain (0.1 g crystal violet, 100 ml distilled water) and heated until steam rose. The stain was washed off with 20% (w/v) aqueous $CuSO_4 \bullet 5\ H_2O$. The slide was blotted dry and examined using light microscopy. If present, capsules appear faint blue, while the microorganisms are dark purple.

B. PILOT SCALE FERMENTATIONS

L. mesenteroides strains #1 and #11, *L. lactis* and *K. pneumoniae* were inoculated into albumen and fermented in the pilot plant at Canadian Inovatech, Winnipeg, MB. *S. cerevisiae* was used as a control.

Lysozyme-extracted fresh liquid albumen, adjusted to pH 6.5 using 10% citric acid (pH 6.5 was chosen after preliminary experiments and to avoid precipitation of proteins with isoelectric points near pH 6.0) was contained in 9-L stainless steel fermenter containers. A final inoculum level of 10^6 CFU/ml was used in each fermentation.

Albumen inoculated with *S. cerevisiae* (control) and *K. pneumoniae* were fermented at 37^0C; fermentations with *L. mesenteroides* species and *L. lactis* were carried out at 28^0C, (optimum temperatures). Fermentation times ranged from 24-48 h, depending on the organism. A Diastix Urinalysis test strip (Bayer Diastix, Health Care Division, Etobicoke, ON) was used to initially monitor glucose utilization. Residual glucose was also determined as previously outlined (AOAC, 1975).

Following fermentation, the albumen was filtered through a 500μ metal filter to remove any excess chalaze and other impurities, and spray-dried in an industrial dryer (Anhydro, Denmark) at a heater setting of 6 kW, yielding a drying temperature of 180-200^{0}C. The spray dryer air pump speed was 3.5 ml/min and the pH of the liquid albumen was adjusted to pH 7.0 using 10% citric acid. Three 9-L batches of fermented albumen were prepared using each microorganism.

Following spray drying, the powdered albumen was pasteurized in plastic bags in a hot room (at least 65^{0}C). Functionality testing was performed on the various batches of albumen.

C. FUNCTIONAL PROPERTIES OF EGG ALBUMEN

1. Whip Height:

Spray-dried fermented albumen (43 g) was dissolved in distilled water (430 mL) held at room temperature (21^{0}C). The solution was whipped using a Hobart Mixer for 90 s at medium speed, followed by 90 s at high speed. After removing the wire whip, the resultant foam was levelled with a metal spoon and the depth was measured using a ruler. The mean of four replicates was considered to be the whip height (cm).

2. Angel Cake Height:

i. Cake Recipe:

Fermented egg albumen (80 g) was added to 920 g Robin Hood Angel Food Cake mix containing no albumen. The mixture was shaken in a plastic bag for two min. and then added to 560 ml ice water and mixed for one min on low speed using a Hobart mixer. The resultant cake batter was then whipped (Hobart) on high speed for 4 min. Subsequently, 567 g of the cake batter was weighed into an aluminium angel food cake pan and evenly

distributed using a stainless steel spoon. The cake was baked in a conventional oven at $210^0C \pm 15^0C$ for 30 min.

ii. Cake Height Measurements:

After cooling the cake for 1 h at room temperature, cake height was measured with a vernier calliper/depth gauge, calibrated to the nearest mm. Measurements were taken approximately 5 cm from the centre chimney to avoid the area of pan depression near the centre. Four measurements were determined at the highest portion of each cake (avoiding cracks). The mean (cm) of these values was reported as the cake height.

iii. Specific Gravity of Cake Batter:

The specific gravity of the cake batter was measured using a densitometer. The weight of batter held in a round metal container was measured and the specific gravity was determined using a conversion chart.

iv. Viscosity of Cake Batter:

The viscosity of the cake batter was measured with a Brookfield viscometer (Brookfield Engineering, model RVT, Middleboro, MA) using a #6 spindle.

3. Gel Strength:

Gel strength was determined according to the method of Mine *et al.* (1990). A 10% (w/v) solution of spray-dried albumen was prepared in 20 mM phosphate buffer, pH 7.5. The protein solution (200 ml) was then placed in 30 mm diameter plastic sausage casings. The casings were sealed with string and held vertically at room temperature for 1 h. They were then immersed in an 80^0C water bath for 40 min. Resulting gels were subsequently cooled in a cold water bath and refrigerated overnight. Duplicate gels were prepared for each analysis.

To assess the gel strength, each original cylindrical-shaped gel was cut into 30 mm pieces and each gel piece was placed on the detachable table of a Rheometer (model# RT-

2002J, Fudoh, Japan) equipped with a spherical plunger. The sample was compressed with a table speed of 60 mm/min. The standard method for determination of gel strength involved taking five deformation measurements. The mean of the three measurements closest to one another was expressed as the gel strength (g).

D. PHYSICOCHEMICAL CHARACTERISTICS OF EGG ALBUMEN

1. Differential Scanning Calorimetry (DSC)

The method according to Arntfield and Murray (1981) was modified for DSC analysis of egg albumen. Dried albumen samples were dissolved in 0.2M sodium chloride to make 20% (w/w solids) protein slurries. The thermal properties of albumen were measured using a Dupont 990 Thermal Analyzer with a 910 DSC Cell Base (Westech Industrial Ltd., Missisauga, ON). Samples were heated over a 20-120^{0}C temperature range at a linear rate of 2^{0}C/min. Results were expressed in terms of maximum heat flow into the albumen (thermal denaturation temperature, Td in ^{0}C) and enthalpy of denaturation (ΔH in J/g).

2. Surface Hydrophobicity

The surface hydrophobicity (So) of albumen samples was determined using the fluorescent probe method, according to Kato and Nakai (1980). The fluorescent probe used was 1-anilino-8-napthalenesulfonate (ANS), which evaluates aromatic hydrophobicity. Albumen samples were serially diluted with 0.1M phosphate buffer, pH 6.0, to obtain a range of protein concentrations from 0.02 to 0.6 mg/ml. A 10 µL aliquot of ANS (8mM in 0.01M phosphate buffer, pH 7.0) was added to each 2 ml sample and the relative fluorescence intensity was measured using a Perkin-Elmer LS-5 fluorescence spectrophotometer (Coleman Instruments Division, Oak Brook, IL). A slit width of 0.5nm and a fixed scale of 1.0 were used. Excitation and emission wavelengths were 390 and 420 nm, respectively. The

fluorescence intensity of each solution without the probe was subtracted from that with the probe to obtain the net fluorescence intensity at each protein concentration. The initial slope of the plot of fluorescence intensity as a function of protein concentration was expressed as the So.

E. STATISTICAL ANALYSIS

Statistical analyses of viscosity, pH, glucose concentration, microbiological growth plus the functional and physicochemical properties of fermented egg albumen were evaluated using a one-way ANOVA table and the student's t-test ($p \leq 0.05$).

RESULTS

A. FERMENTATION OF EGG ALBUMEN

Time course changes in viscosity, pH residual glucose and microbial growth over the course of a 72-hour fermentation were evaluated in this study, with samples evaluated every 24 h. In preliminary trials, several fermentations of shorter duration (24 h) were also carried out and no net increases in viscosity were observed. The absence of increases in viscosity lead to the conclusion that no EPS was formed during the first portion of fermentation. This negates the possibility of EPS production, followed by enzymatic degradation. The lack of viscosity increases over a 24-h fermentation lead to the adoption of a longer fermentation time.

1. *Saccharomyces cerevisiae* (control)

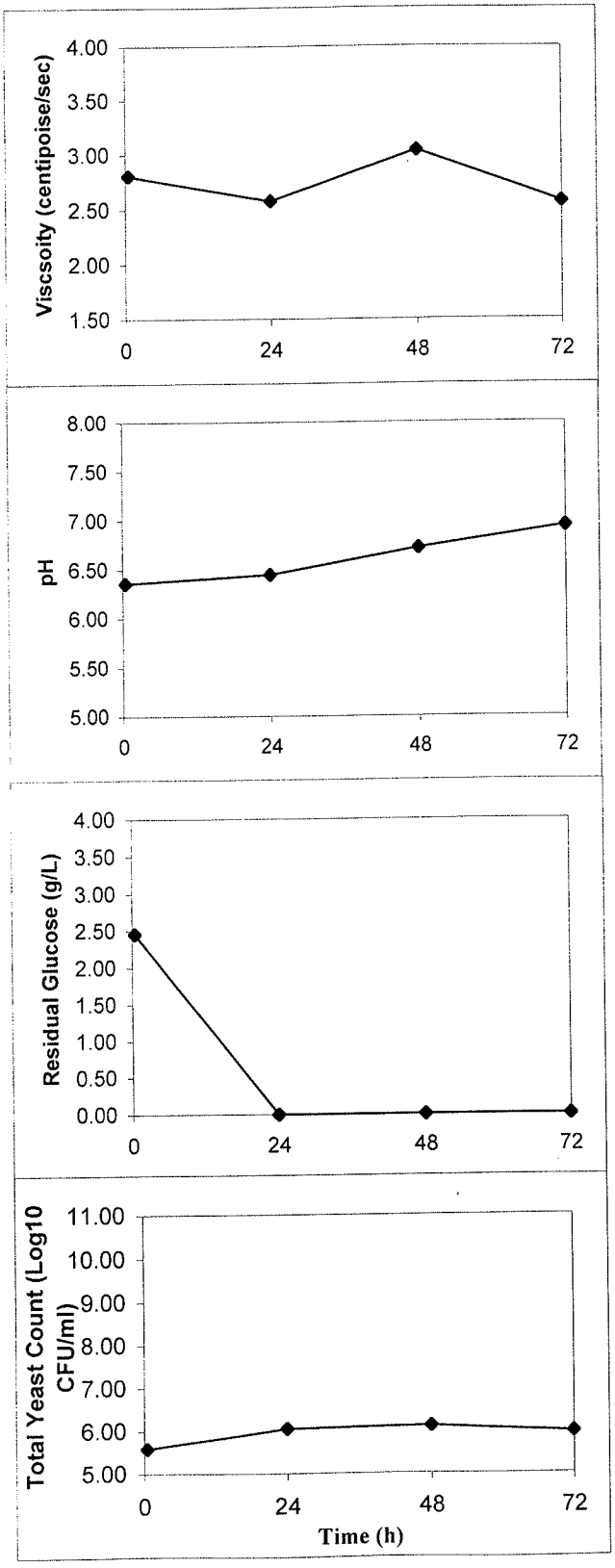
Time course changes in viscosity, pH, glucose concentration, and yeast growth in albumen fermented with *Saccharomyces cerevisiae* at 37^0C are shown in Figs. 3 and 4. In both cases, no increase (over the initial value) was observed in viscosity during the course of fermentation (Appendices 1 and 5). The initial glucose concentration, which ranged from 2.0 to 2.5 g/L, was completely utilized within the first 24 hours of fermentation (Appendix 3 and 7). The microbial population did not appear to increase (less than 1 $\log_{10}$ CFU/ml) throughout both fermentations (Appendices 4 and 8). The pH, initially adjusted to approximately pH 6.5 and 7.0, increased to approximately 6.9 and 7.7, respectively (Appendices 2 and 6).

2. *Leuconostoc mesenteroides*

i. *Leuconostoc mesenteroides* subsp. *mesenteroides* strain #1

Time course changes in the fermentation of albumen adjusted to approximately pH 6.5, with *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C are shown in

Figure 3. Time course changes in viscosity, pH, glucose concentration and yeast growth in albumen fermented by *Saccharomyces cerevisiae* at 37^0C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.



* Initial observation was taken at t = 30 minutes

Figure 4. Time course changes in viscosity, pH, glucose concentration and yeast growth in albumen fermented by *Saccharomyces cerevisiae* at 37^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.

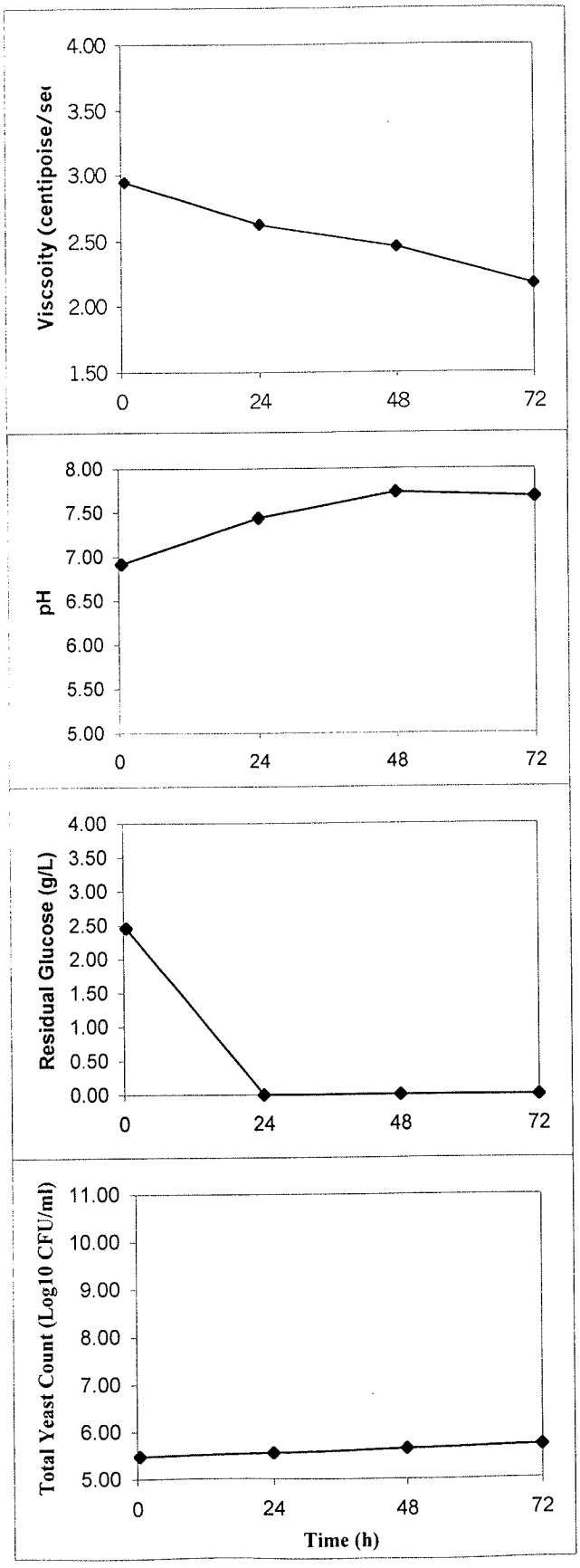


Fig. 5. Following an initial decrease in viscosity, values appeared to remain constant (Appendix 1). The pH decreased to 5.3 after 24 h, and subsequently increased to approximately 5.7 over the final 48 h of fermentation (Appendix 2). Growth of *L. mesenteroides* subsp. *mesenteroides* strain #1 increased by 2.5 $\log_{10}$ over 72 h of fermentation (Appendix 4). By comparison, the population of the control increased by only 0.52 $\log_{10}$ CFU/ml (Fig. 3 and 4).

Similar results were observed with albumen, which was adjusted to approximately pH 7.0 prior to fermentation (Fig. 6). As shown, both the viscosity and pH appeared to decrease during the first 48 h of fermentation (Appendix 5). Total plate counts increased by approximately 2.5 $\log_{10}$ cycles over the course of the fermentation (Appendix 8). This level of growth was higher than that observed for the control (Fig. 3). Glucose levels appeared exhausted by 48 h.

Fermentation with albumen adjusted to approximately pH 7.0 resulted in significantly higher viscosity values, especially at 24 h.

ii. *Leuconostoc mesenteroides* subsp. *mesenteroides* strain #11

Changes in viscosity, pH, glucose concentration and total plate count of albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #11 at 30^0C and approximately pH 6.5, are shown in Fig. 7. As observed, results appeared similar to those of *L. mesenteroides* strain #1. However, significant differences were observed between albumen fermented with *L. mesenteroides* subsp. *mesenteroides* strain #11 and the yeast control (Appendices 10 and 11). Albumen initially adjusted to approximately pH 6.5 decreased to approximately 5.6 during the first 24 h (Fig. 7, Appendix 2) and thereafter increased to approximately 6.0. The initial glucose concentration of egg albumen (1.62 g/L) was nearly depleted within 24 h (Appendix

Figure 5. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *Leuconostoc mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

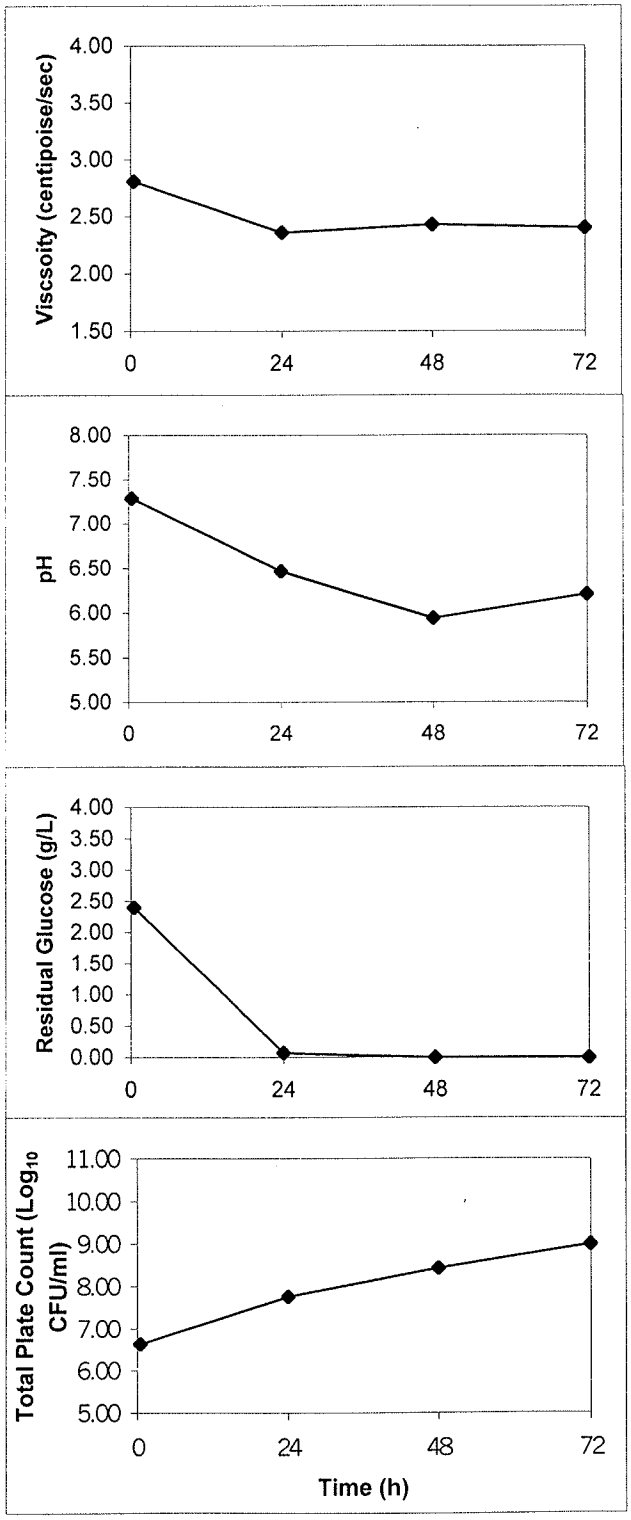


Figure 6. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.

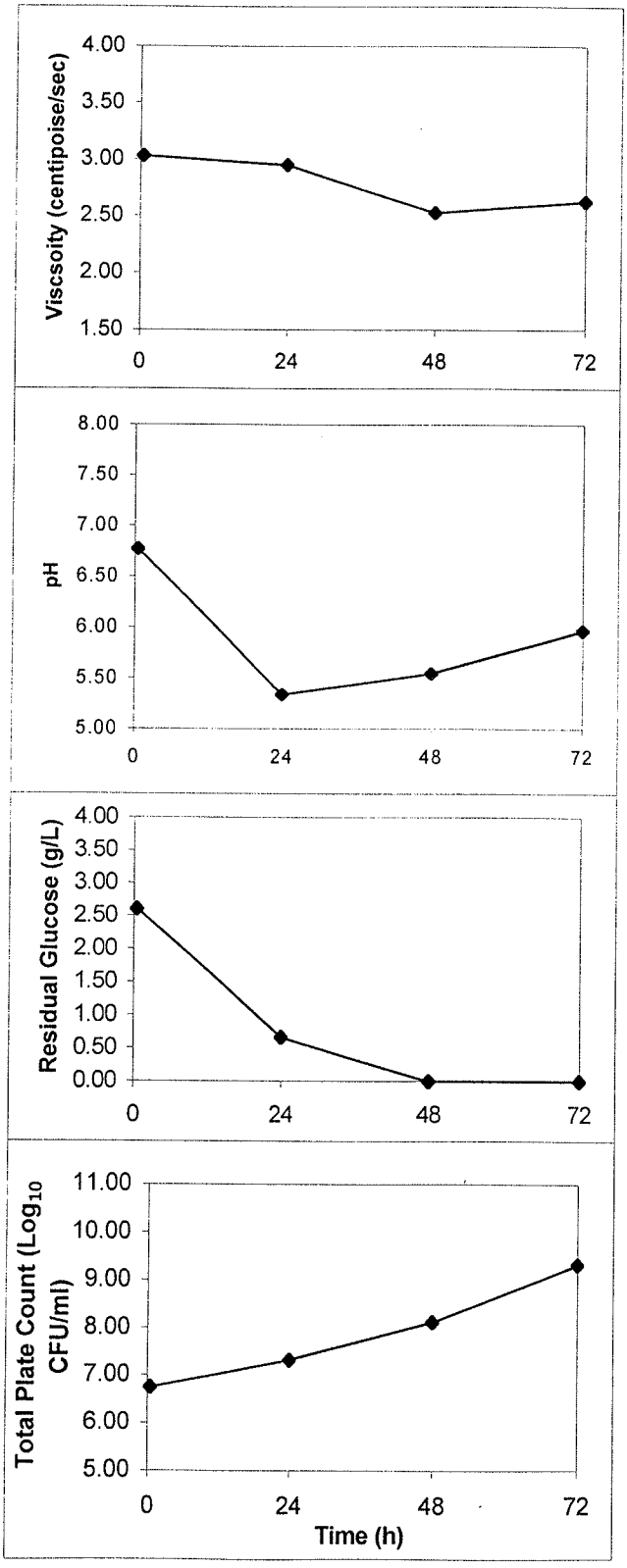


Figure 7. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #11at 30^0C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

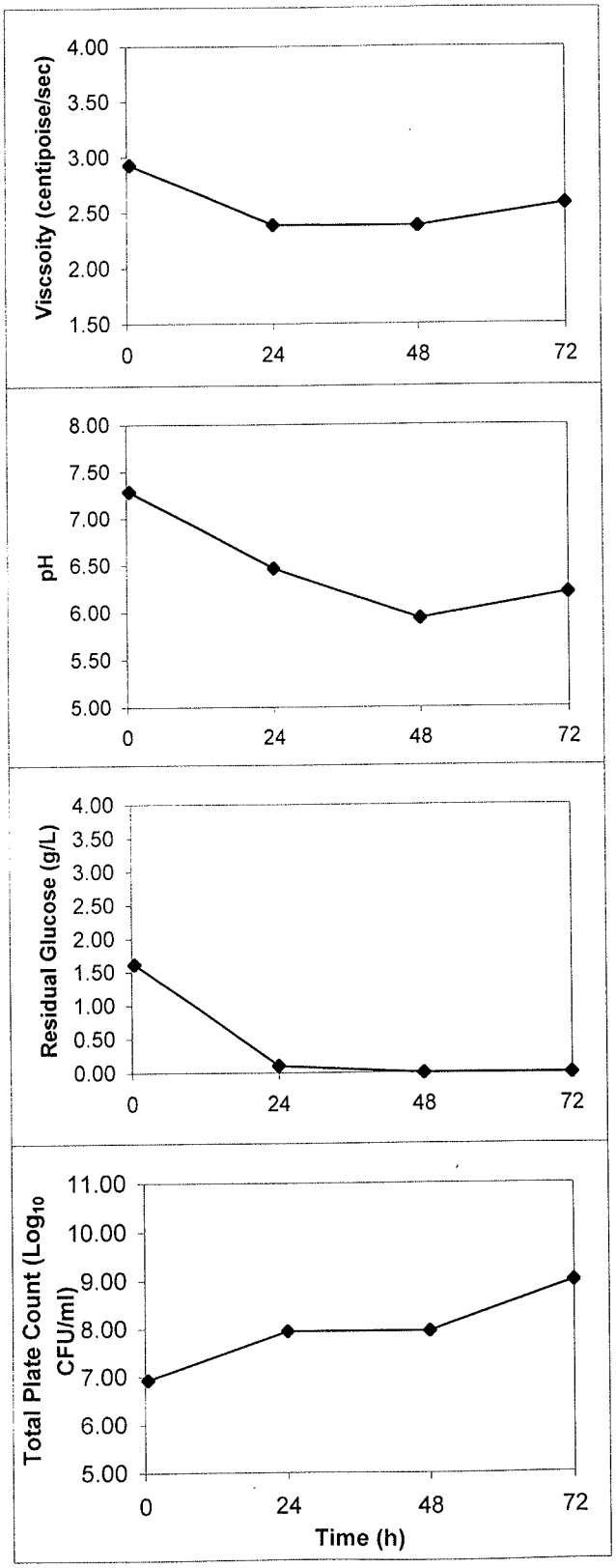
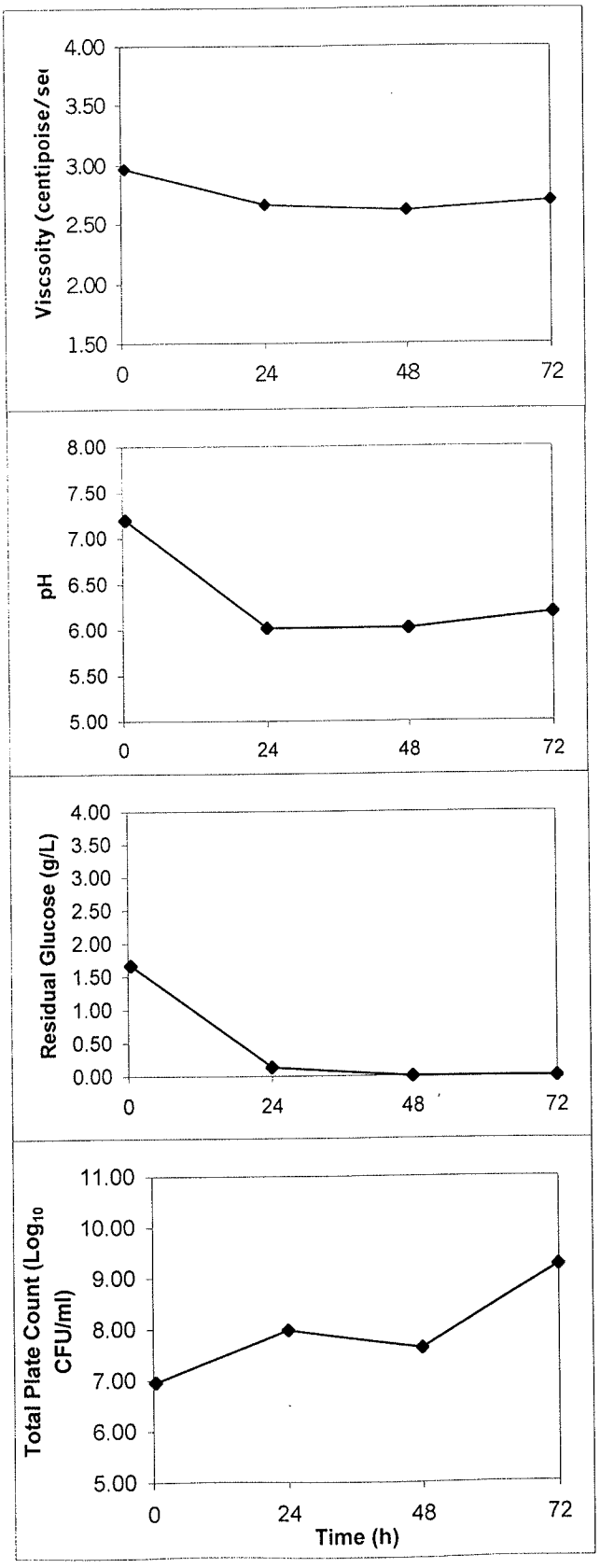


Figure 8. Time course changes in the viscosity, pH, glucose concentration and microbial growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #11 at 30^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.



3). Growth levels increased by at least 2 $\log_{10}$ cycles over the 72 h fermentation period (Fig. 7, Appendix 4).

Similar results were observed with albumen adjusted to approximately pH 7.0 (Fig. 8). The pH decreased from approximately 7.0 to 6.0 within 24 h and thereafter, remained constant (Appendix 6). In addition, glucose was nearly depleted during the first 24 h of fermentation (Appendix 7). The initial total plate count (6.96 $\log_{10}$ CFU/ml) increased 2.5 $\log_{10}$ cycles over the course of the fermentation (Appendix 8).

3. *Lactococcus lactis*

Changes in viscosity, pH, glucose concentration and growth of *L. lactis* at 28^0C in albumen initially adjusted to approximately pH 6.5 and 7.0 are shown in Figs. 9 and 10. No significant differences in viscosity were observed between fermentations (Appendix 12). Glucose levels in both fermentations gradually decreased. At 48 and 72 h glucose levels approached zero for *L. lactis* which was adjusted to approximately pH 6.5 and 7.0, respectively (Appendix 12). The initial glucose levels fluctuated among fermentations, which may be attributed to partial natural fermentation of albumen by microorganisms inherent to the natural microflora. Samples were frozen in 5-ml test tubes so that several samples could be analyzed at once; the gentle thawing may have caused partial fermentation. An increase in total plate count (2 $\log_{10}$ cycles) was observed over the course of the fermentation when the initial pH was approximately 6.5. In contrast, when the pH of the albumen was initially adjusted to pH 7.0, the plate count increased 3.5 $\log_{10}$ cycles over the course of the fermentation. No significant differences were observed between pH 6.5 or 7.0 fermentations for any of the four parameters tested.

Figure 9. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *L. lactis* at 28^0C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

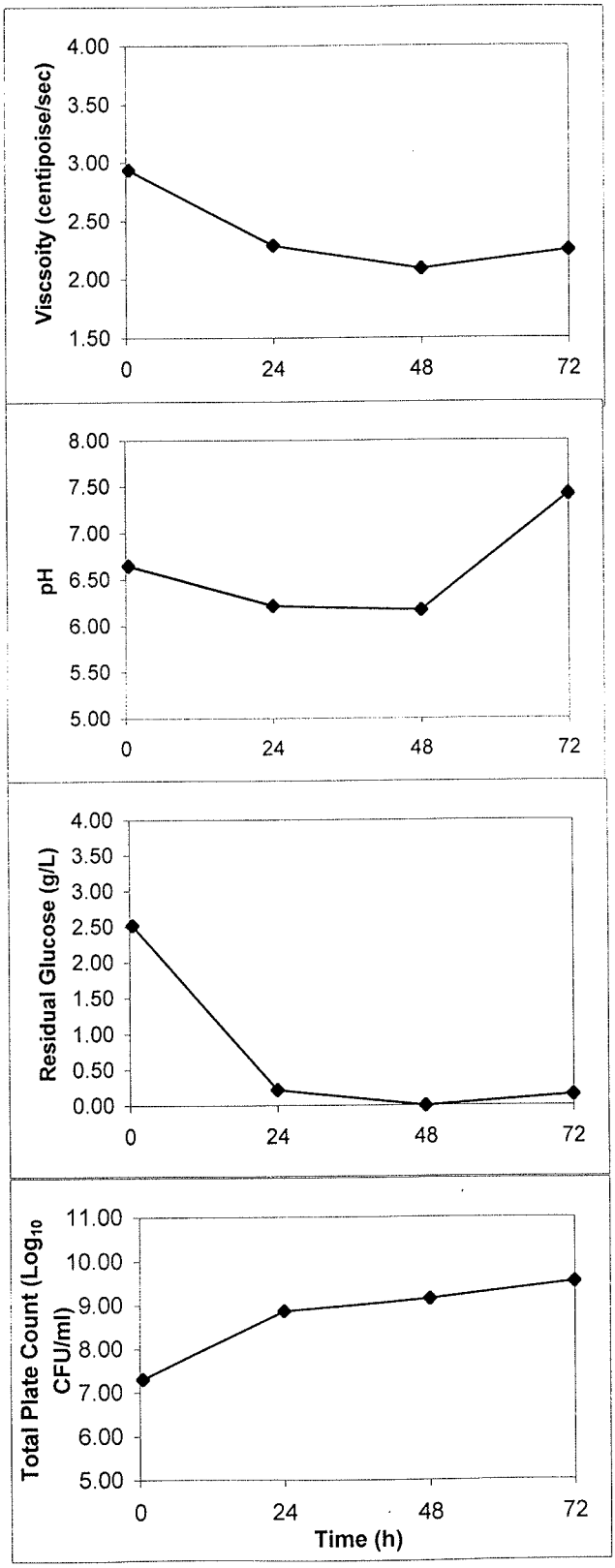
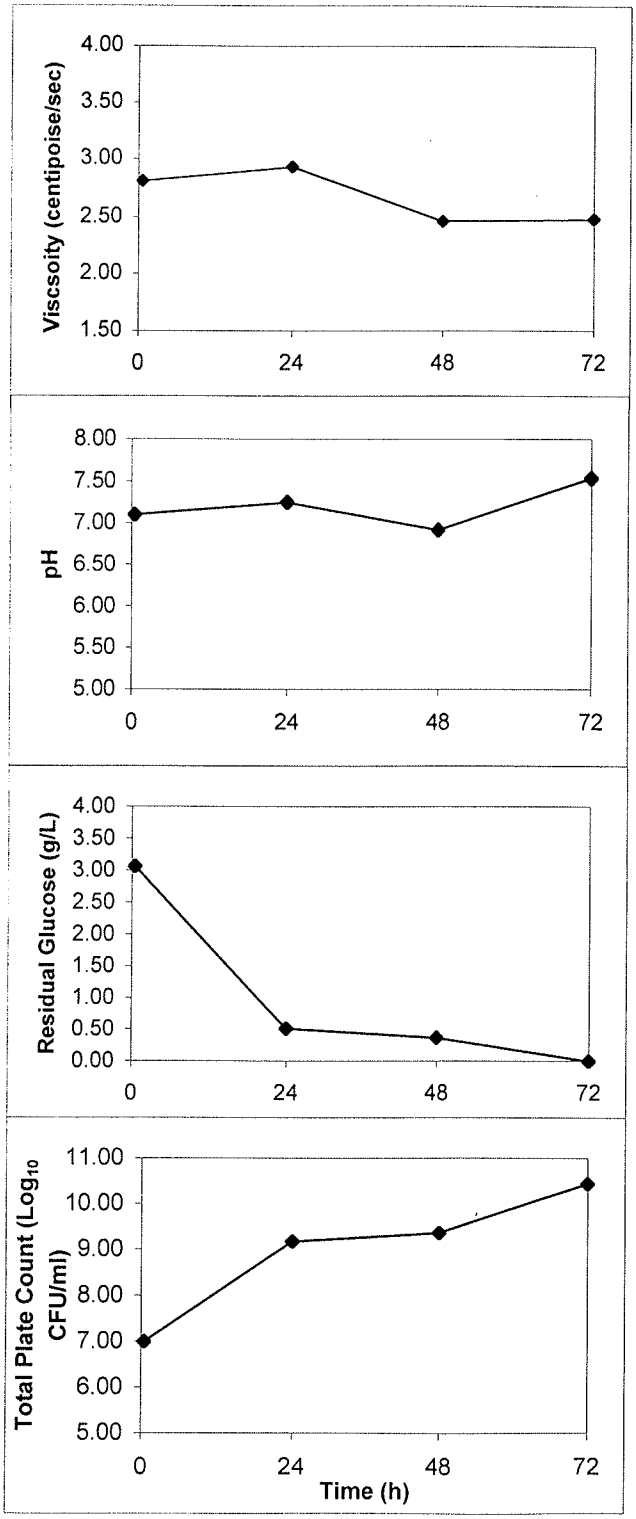


Figure 10. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *L. lactis* at 28^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.



In fermentations using *L. lactis* at 32^{0}C, however, viscosity values remained somewhat constant (2.49-2.58 centipoise/sec) with albumen adjusted to approximately pH 6.5 (Fig. 11). When adjusted to pH 7.0, albumen viscosity decreased continually over the course of the 72-h fermentation (Fig. 12). Significant differences in viscosity were observed between pH 6.5 and 7.0 adjusted fermentations (Appendix 13). Viscosity values were significantly higher at all time intervals when albumen was initially adjusted to approximately pH 6.5 prior to fermentation. Fermentation of albumen adjusted to approximately pH 7.0 resulted in pH values that initially increased and subsequently decreased over the course of the fermentation.

Glucose levels appeared depleted within 48 h (pH 6.5) to 72 h (pH 7.0) of the start of fermentation (Appendix 13). Enhanced depletion of glucose in albumen initially adjusted to pH 6.5 can be attributed to the more rapid growth of *L. lactis* under acidic conditions. Fermentation at both pH levels resulted in at least a 2.5 $\log_{10}$ cycle increase in total plate count; no significant differences in total plate counts were observed between the two fermentations (Figs. 11 and 12, Appendix 13).

Overall, no significant differences were observed between the fermentations operating at two temperatures for any of the parameters tested at pH levels of both 6.5 and 7.0.

4. *Klebsiella pneumoniae*

Changes in viscosity, pH, glucose concentration and total plate count in albumen fermented with *K. pneumoniae* at 37^{0}C, at approximately pH 6.5 and 7.0 are shown in Figs. 13 and 14. In both cases, the pH dropped during the first 24 h of fermentation and subsequently increased. In both fermentations, glucose was

Figure 11. Time course changes in viscosity, pH, glucose concentration and total growth in albumen fermented by *L. lactis* at 32^{0}C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

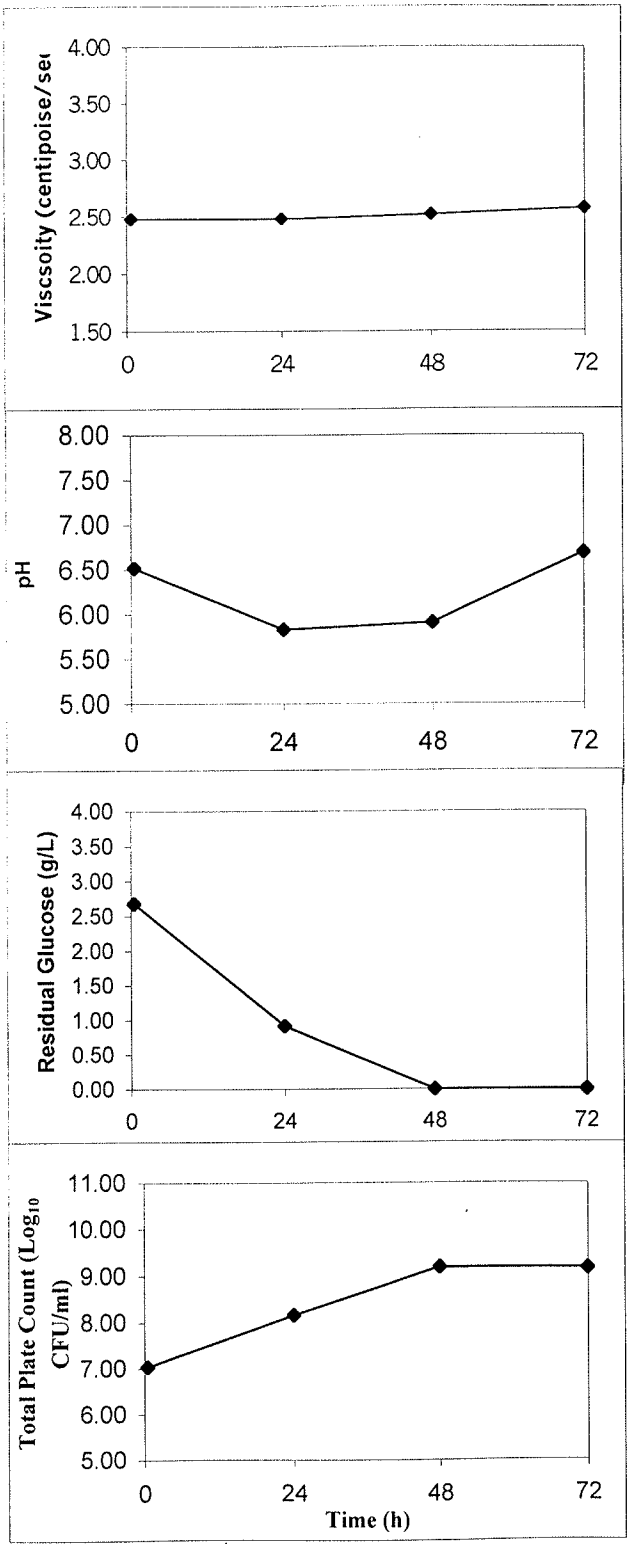
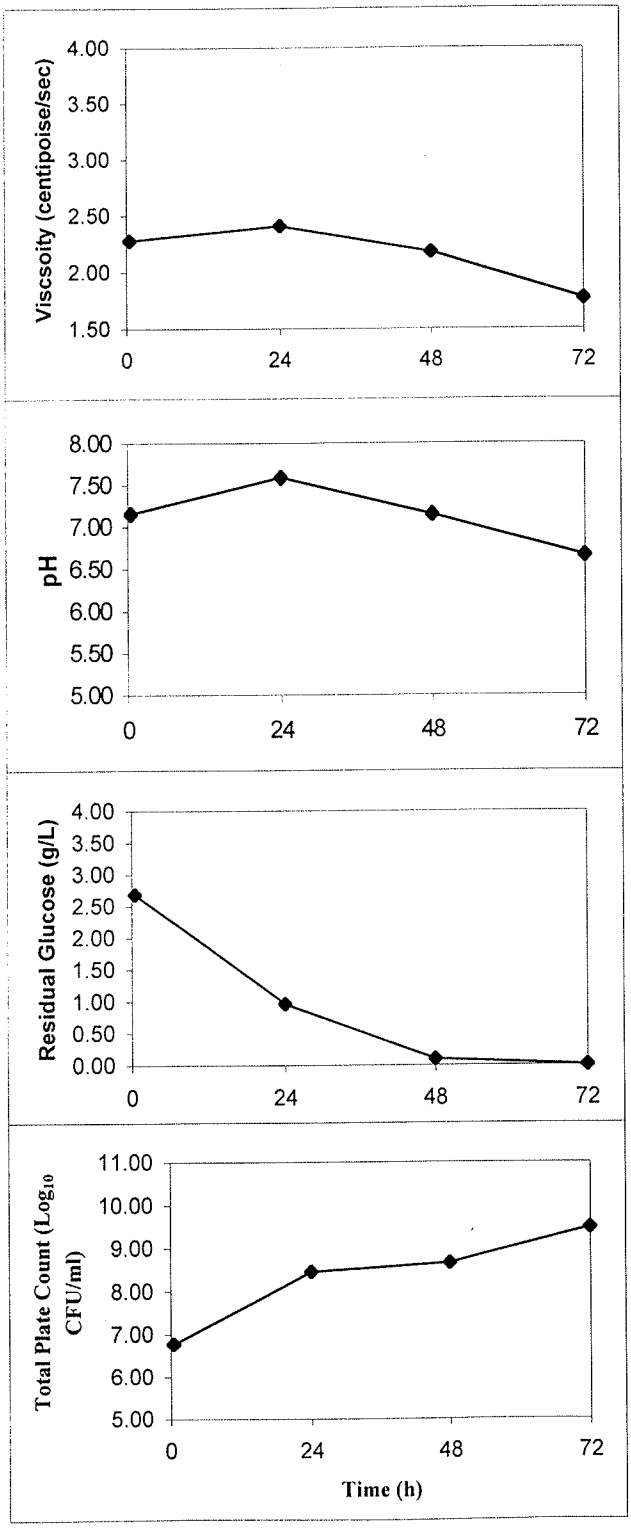


Figure 12. Time course changes in viscosity, pH, glucose concentration and total growth in albumen fermented by *L. lactis* at 32^{0}C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.



completely depleted during the first 48h, although utilization of glucose appeared more rapid at approximately pH 7.0. The total plate count increased 2.5 and 2 $\log_{10}$ cycles in albumen adjusted to pH 6.5 and 7.0, respectively over the course of the 72-hour fermentations. However, when total plate counts at pH 6.5 and 7.0 were compared, no significant differences were observed. Changes in the viscosity, pH, glucose concentration and growth in albumen fermented at 32^0C are shown in Figs. 15 and 16. No significant differences were observed between fermentations adjusted to pH 6.5 and 7.0 for any of the four parameters tested (Appendix 15). Interestingly, glucose depletion at 32^0C, regardless of albumen pH, was less pronounced compared with fermentation at 37^0C. This was particularly evident at 24 h.

B. FERMENTATION OF ALBUMEN SUPPLEMENTED WITH YEAST EXTRACT AND GLUCOSE

The effect of added yeast extract and carbohydrate (glucose) was also investigated in relation to fermentation of albumen. Studies were conducted at the optimum growth temperatures of the starter organisms and at a pH of approximately 6.0-6.4. Initial viscosity values, which ranged from 2.00 to 3.00 centipoise/sec decreased by approximately 0.50 to 1.00 units for all fermentations within 24 h (Table 1). The initial pH, which ranged from approximately 6.0 to 6.5, decreased to between 5.00 and 5.50 within 24 h (Table 2). Total plate counts increased by only 1 to 1.5 $\log_{10}$ cycles over the 24-h fermentation period (Table 3). No significant differences were found when results of the fermentations were compared. The viscosity, which was of the most interest, did not show any increases over the course of the fermentations.

Figure 13. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *K. pneumoniae* at 37^{0}C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

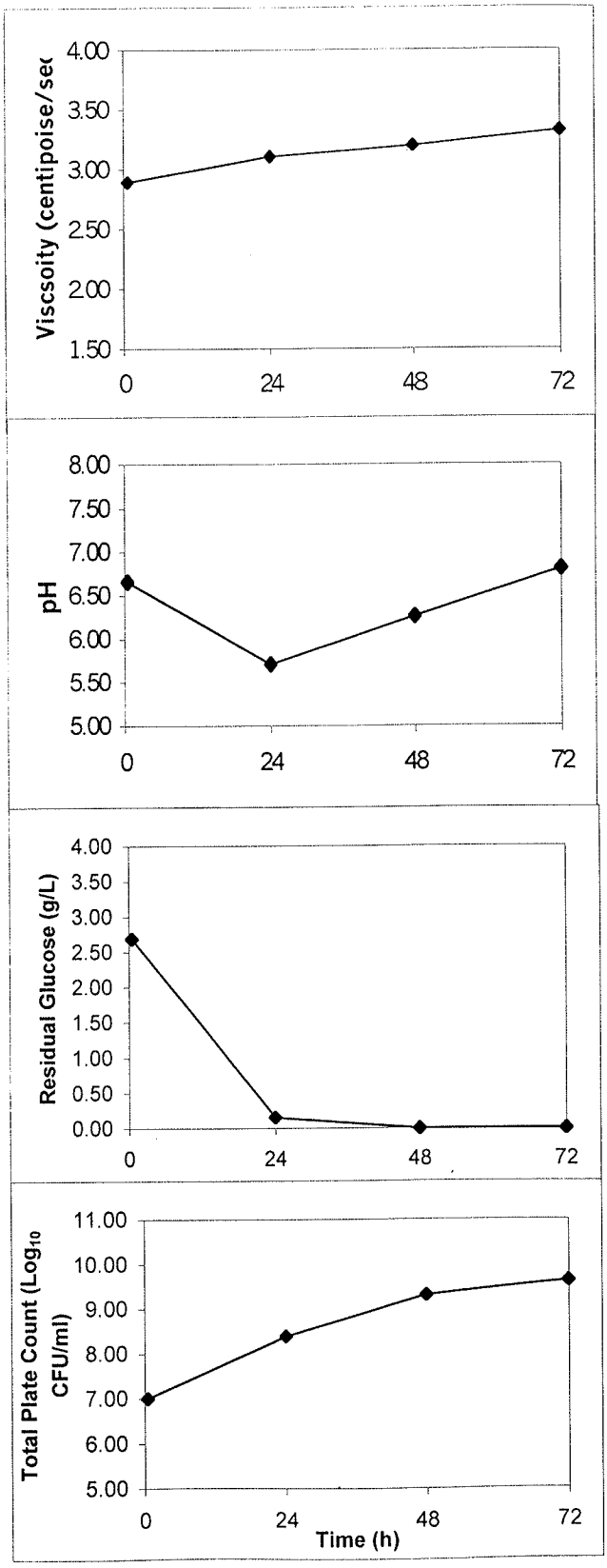


Figure 14. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *K. pneumoniae* at 37^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.

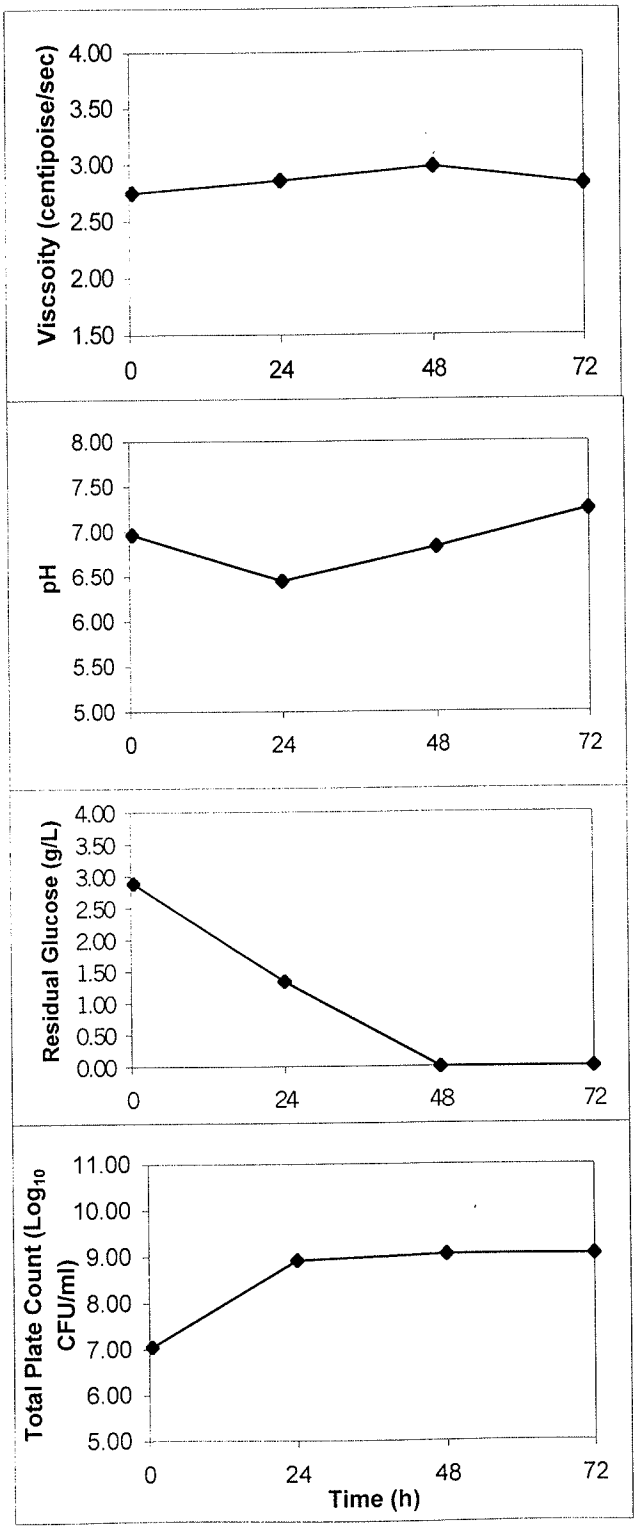


Figure 15. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by *K. pneumoniae* at 32^{0}C. Albumen was adjusted to approximately pH 6.5 prior to fermentation.

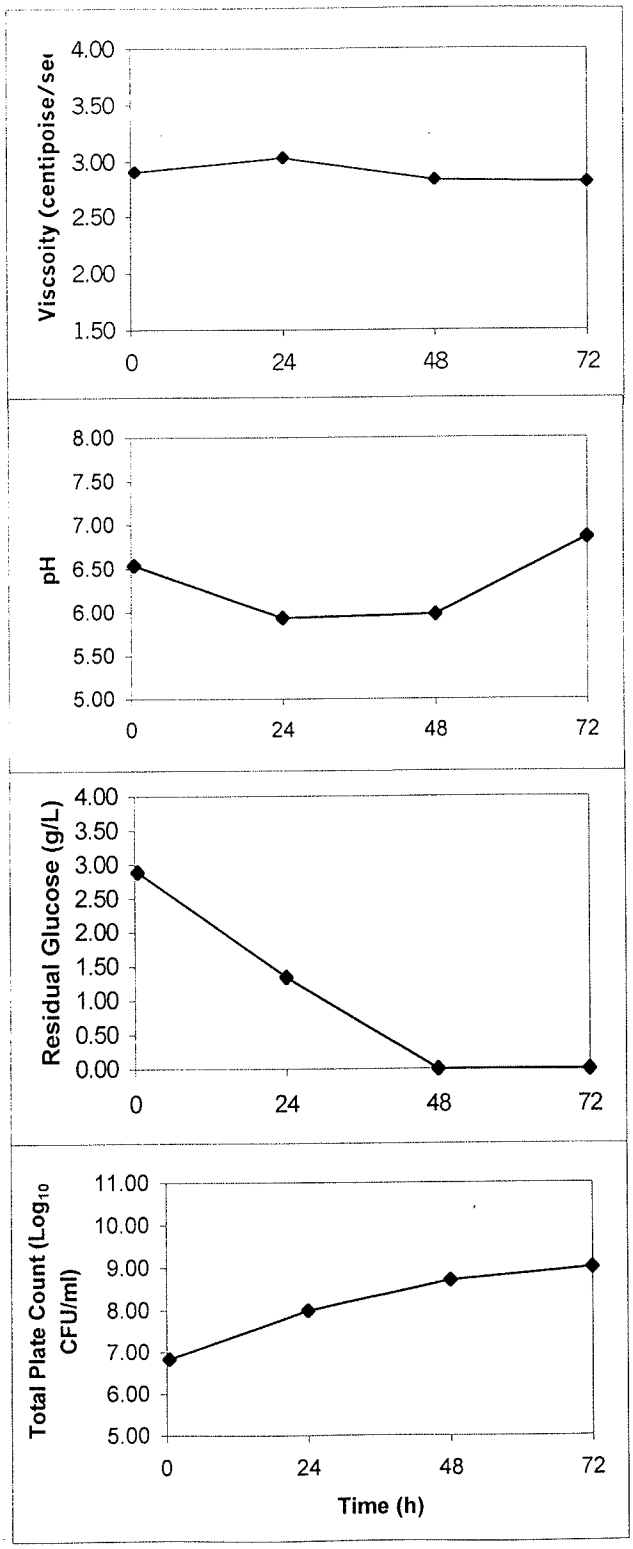
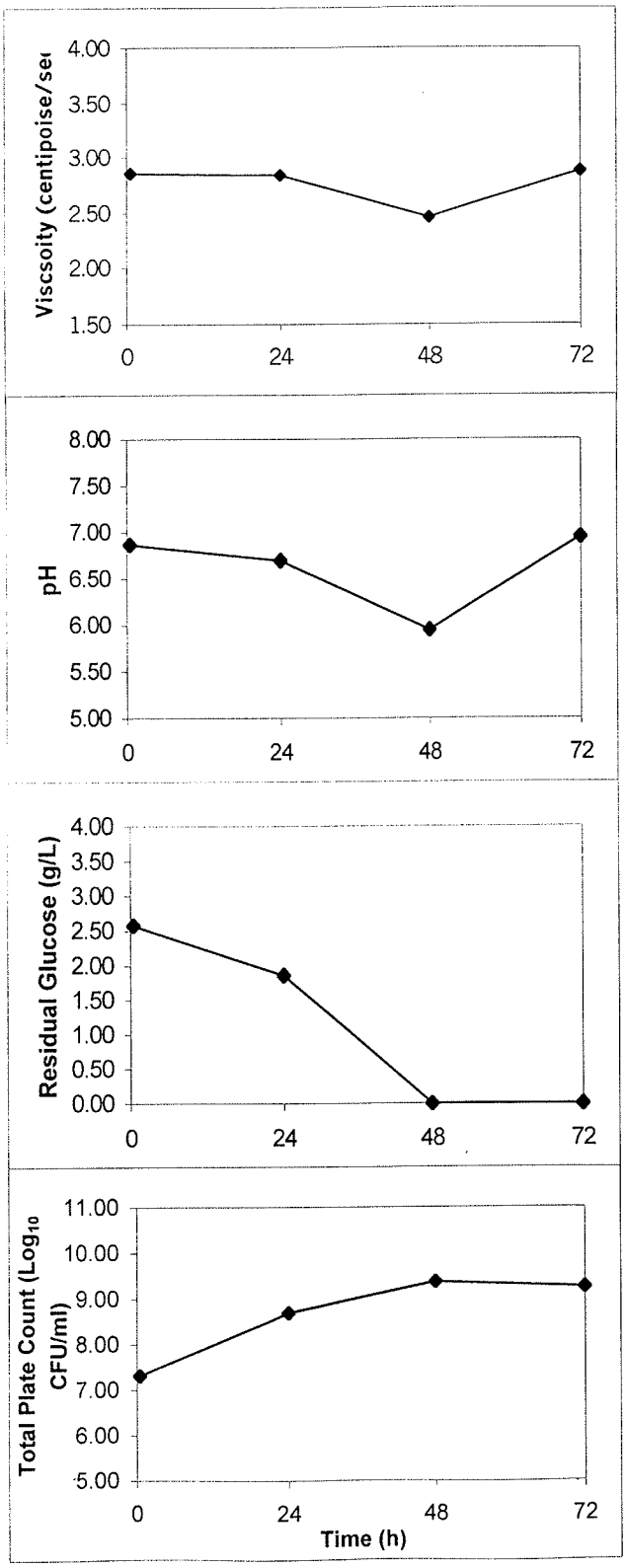


Figure 16. Time course changes in viscosity, pH, glucose concentration and microbial growth in albumen fermented by K. pneumoniae at 32^0C. Albumen was adjusted to approximately pH 7.0 prior to fermentation.



C. FERMENTATION OF ALBUMEN SUPPLEMENTED WITH YEAST EXTRACT AND SUCROSE

Yeast extract (0.1%) and sucrose (3, 4 and 5%) were added to albumen prior to fermentation with *L. mesenteroides* subsp. *mesenteroides* strain #1 to attempt to stimulate dextran production.

Changes in viscosity, pH and microbial growth are shown in Fig. 17. Although initial viscosity increased with increasing sucrose concentrations, further increases in viscosity were not observed over the course of the 48-h fermentation (Fig. 17, Appendix 16). The higher initial viscosity was likely due to the addition of sucrose.

Total plate counts increased by only 1.5 to 1.0 $\log_{10}$ cycles (Appendix 19). Expected outcomes of this set of trials, production of bacterial exopolysaccharide by *L. mesenteroides* in the presence of sucrose were not observed, as evidenced by microscopic examination using the Hiss method for capsule staining. A more concentrated addition of sucrose to albumen, as well as fermentation at pH 7.5, may induce the production of dextran by this microorganism, however, time restraints did not allow for this. These optimal conditions for dextran production may result in a more viscous medium with greater functional properties.

Table 1: Viscosity of albumen fermented with *S. cerevisiae* (control), *L. mesenteroides* (strain #1 and 11), *L. lactis and K. pneumoniae* for 24 h at 37^0, 30^0, 28^0 and 32^0C, respectively. Yeast extract and glucose were added to albumen prior to fermentation[1]

Yeast Extract (%)		0		0.1		0.2	
Glucose Added (%)		1	2	1	2	1	2
Microorganism	Time (h)	Viscosity (centipoise/sec) ± SD					
S. cerevisiae	0	2.87±0.20	2.82±0.18	2.76±0.25	2.76±0.02	2.86±0.29	2.54±0.67
	24	2.64±0.49	2.58±0.20	2.74±0.39	2.57±0.39	2.22±0.17	1.94±0.14
L. mesenteroides strain#1	0	2.26±0.99	2.23±1.00	2.23±0.92	2.42±0.64	2.48±0.48	2.59±0.67
	24	2.06±0.77	2.23±1.00	0.79±0.29	1.79±0.23	2.09±0.57	2.15±0.73
L. mesenteroides strain #11	0	3.00±0.99	2.68±0.59	2.31±0.30	2.14±0.05	2.96±1.16	2.67±0.99
	24	2.89±1.61	2.55±1.13	1.68±0.01	1.75±0.09	1.93±0.33	1.86±0.37
L. lactis	0	2.54±0.25	2.73±0.55	2.58±0.34	2.59±0.25	2.49±0.31	2.44±0.29
	24	1.96±0.51	1.96±0.31	1.73±0.10	1.81±0.04	1.74± 0.30	1.68±0.32
K. pneumoniae	0	2.78±0.44	2.79±0.42	2.83±0.50	2.77±0.59	2.64±0.27	2.57±0.26
	24	2.10±0.41	2.08±0.38	1.96±0.17	1.93±0.14	1.87±0.14	1.80±0.02

[1]Results are a mean of two trials (n = 2 ± SD)

No comparisons among fermentations at either time period were significantly different (student's t-test; α= 0.05)

Table 2: The pH of albumen fermented with *S. cerevisiae* (control), *L. mesenteroides* (strain #1 and 11), *L. lactis and K. pneumoniae* for 24 h at 37^0, 30^0, 28^0 and 32^0C, respectively. Yeast extract and glucose were added to albumen prior to fermentation[1]

Yeast Extract (%)		**0**		**0.1**		**0.2**	
Glucose Added (%)		**1**	**2**	**1**	**2**	**1**	**2**
Microorganism	**Time (h)**	**pH ± SD**					
S. cerevisiae	0	6.44±0.2	6.57±0.4	6.40±0.1	6.28±0.3	6.59±0.3	6.64±0.2
	24	6.39±1.2	6.19±0.9	6.53±1.3	6.16±1.1	7.71±2.8	5.41±0.1
L. mesenteroides strain#1	0	6.32±0.2	6.52±0.6	6.40±0.7	6.47±0.6	6.52±0.6	6.51±0.5
	24	6.10±0.7	6.20±1.0	5.28±0.4	5.19±0.4	5.16±0.5	5.24±0.4
L. mesenteroides strain #11	0	6.58±0.5	6.62±0.6	6.56±0.6	6.31±0.2	6.48±0.6	6.51±0.7
	24	6.54±1.6	6.55±1.6	5.23±0.3	5.09±0.5	5.11±0.4	5.21±0.3
L. lactis	0	6.49±0.2	6.56±0.1	6.15±0.7	6.08±0.7	6.13±0.7	6.17±0.6
	24	5.51±0.0	5.43±0.1	5.21±0.3	5.19±0.5	5.24±0.5	5.15±0.5
K. pneumoniae	0	6.21±0.5	6.28±0.5	6.02±0.8	5.98±0.8	6.00±0.9	5.95±0.9
	24	5.32±0.3	5.28±0.3	5.09±0.5	5.12±0.6	5.16±0.6	5.10±0.6

[1]Results are a mean of two trials (n = 2 ± SD)
No comparisons among fermentations at either time period were significantly different (student's t-test; α= 0.05)

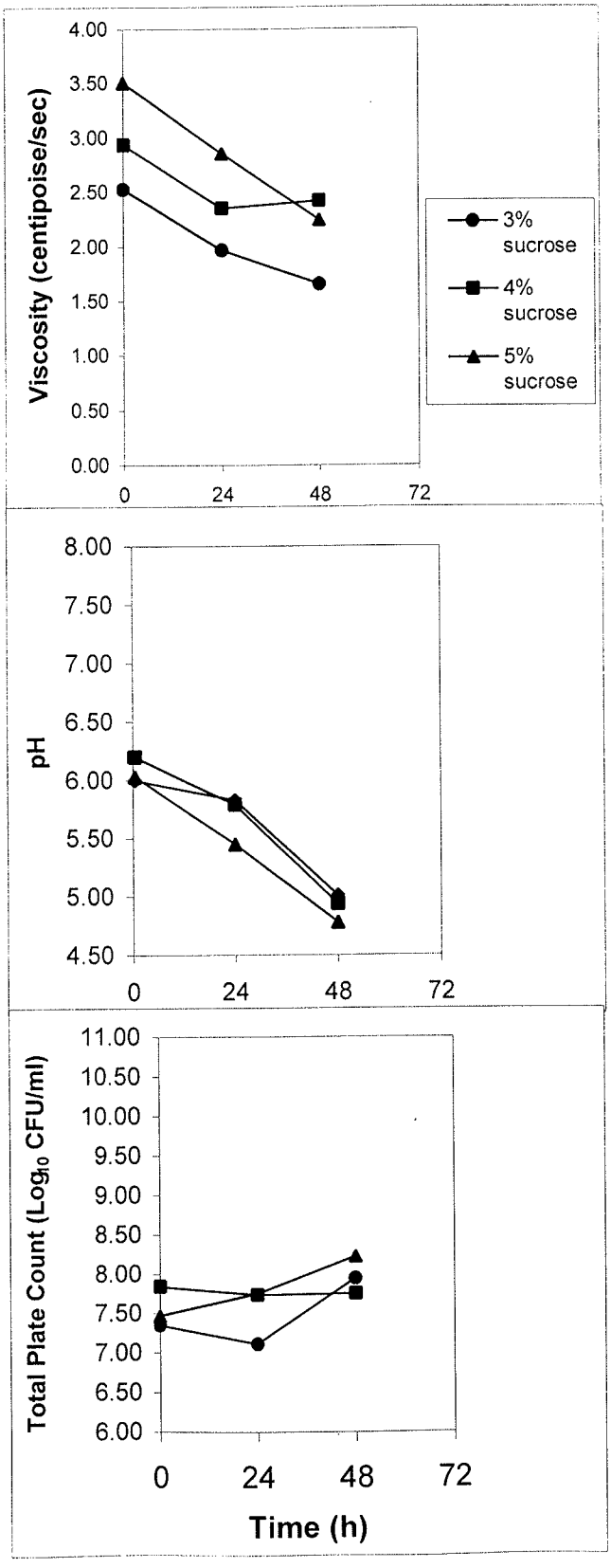
Table 3: Total plate count (Log_{10} CFU/ml) in albumen fermented with *S. cerevisiae* (control), *L. mesenteroides* (strain #1 and 11), *L. lactis and K. pneumoniae* for 24 h at 37^0, 30^0, 28^0 and 32^0C, respectively. Yeast extract and glucose were added to albumen prior to fermentation[1]

Yeast Extract (%)		**0**		**0.1**		**0.2**	
Glucose Added (%)		**1**	**2**	**1**	**2**	**1**	**2**
Microorganism	**Time (h)**	**Total Plate Count (Log_{10} CFU/ml) ± SD**					
S. cerevisiae	0	6.71±0.77	6.46±1.09	6.74±0.73	6.61±0.61	6.51±0.90	6.67±0.79
	24	6.42±2.13	7.31±0.82	6.99±0.88	7.09±0.86	6.75±1.16	6.86±0.99
L. mesenteroides strain#1	0	6.39±0.92	6.29±0.93	6.46±0.84	6.43±0.69	6.41±0.84	6.58±0.63
	24	7.39±0.58	6.57±1.21	7.67±0.27	7.10±0.83	7.12±0.93	7.15"0.03
L. mesenteroides strain #11	0	6.80±0.68	7.11±0.34	6.41±1.12	6.95±0.23	6.35±1.09	7.29±0.09
	24	7.64±0.38	7.61±0.22	6.91±0.97	7.35±0.56	7.47±0.34	7.69±0.11
L. lactis	0	6.82±0.30	6.71±0.35	6.83±0.24	6.76±0.30	6.75±0.31	6.76±0.40
	24	7.83±0.62	7.97±0.52	7.99±0.14	8.10±0.39	7.89±0.07	8.07±0.34
K. pneumoniae	0	7.33±0.39	7.35±0.54	7.42±0.46	7.38±0.41	7.31±0.33	7.26±0.20
	24	8.12±0.27	8.07±0.40	7.88±0.26	8.14±0.35	7.86±0.12	8.12±0.30

[1]Results are a mean of two trials (n = 2 ± SD)

No comparisons among fermentations at either time period were significantly different (student's t-test; α= 0.05)

Figure 17. Time course changes in viscosity, pH and microbial growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C. Yeast extract (0.1%) and sucrose (3, 4 and 5%) were added to albumen prior to fermentation.



D. FUNCTIONAL PROPERTIES OF FERMENTED ALBUMEN

Following scale-up fermentation with the five test microorganisms, albumen was spray-dried and pasteurized in hot rooms prior to functional testing. The purpose of the functionality tests was to determine whether the different fermentations carried out improved the functionality of albumen. To compare functionality before and after heat treatment, most tests were performed on both unpasteurized and pasteurized albumen.

1. Whip Height

Whip height measurements are a good indication of foaming capacity of proteins (Yang and Baldwin, 1995). Whip height results for unpasteurized albumen are shown in Fig. 18. *L. mesenteroides* (strains #1 and #11), and *L. lactis* had significantly higher whip heights than the control (Appendix 20). Fig. 19 shows whip heights of pasteurized albumen fermented by the five microorganisms. All four test bacteria had significantly higher whip heights than the control; however, no significant differences were observed among albumens fermented by the 4 test bacteria (Appendix 21). Comparison of whip heights in this study showed that pasteurized samples had significantly higher values than non-pasteurized (Appendix 22). Kato *et al.* (1990b) observed similar results when foaming capacity of dried egg white, globulin, albumin and purified albumin fractions were measured by conductivity measurements, following dry-heating for 10 days. Although methods of heat treatment differed, both methods allowed partial denaturation of protein molecules.

2. Angel Cake Height

Angel cakes are another method for evaluating the foaming properties of egg albumen. Angel cake height can be used to determine the foaming capacity of egg albumen, in addition to providing information about tenderness, texture and elasticity of the cake crumb. One of the most important functional properties of egg whites is their ability to coagulate during baking, while retaining their solubility. Starch gelatinization and protein denaturation increase batter viscosity, preventing coalescence and promoting strong crumb structure (Raeker and Johnson, 1995). In this study, angel cakes were tested to evaluate the foaming capacity of pasteurized albumen fermented by the five test organisms. Due to the large volume of sample required for cake baking, cake heights were tested only on pasteurized albumen, as it has been proven that cake height is improved with heat treatment (Martinez *et al.*, 1995). Results for angel cake heights are shown in Fig. 20. Albumen fermented using *L. lactis* had a cake height significantly higher than that of *L. mesenteroides* strain #11, but there were no significant differences among cakes prepared with albumen fermented by the other microorganisms (Appendix 23). Martinez *et al.* (1995) studied ultrapasteurization, and found that the average angel cake height prior to heat treatment was 6.04 cm. As shown in Fig. 20, cake heights achieved with pasteurized albumen were greater than 6.04 cm, confirming that heat treatment improves cake height.

The viscosity and specific gravity of raw angel cake batters were also determined. Specific gravity is an indirect measurement of cake volume, and batter viscosity determines how much air can be incorporated during whipping (Yang and Baldwin, 1995). *K. pneumoniae* yielded a significantly higher specific gravity than both the control and *L. mesenteroides* strain #1; however, no significant differences

Figure 18. Whip heights of spray-dried unpasteurized albumen fermented by *S. cerevisiae, L. mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae*

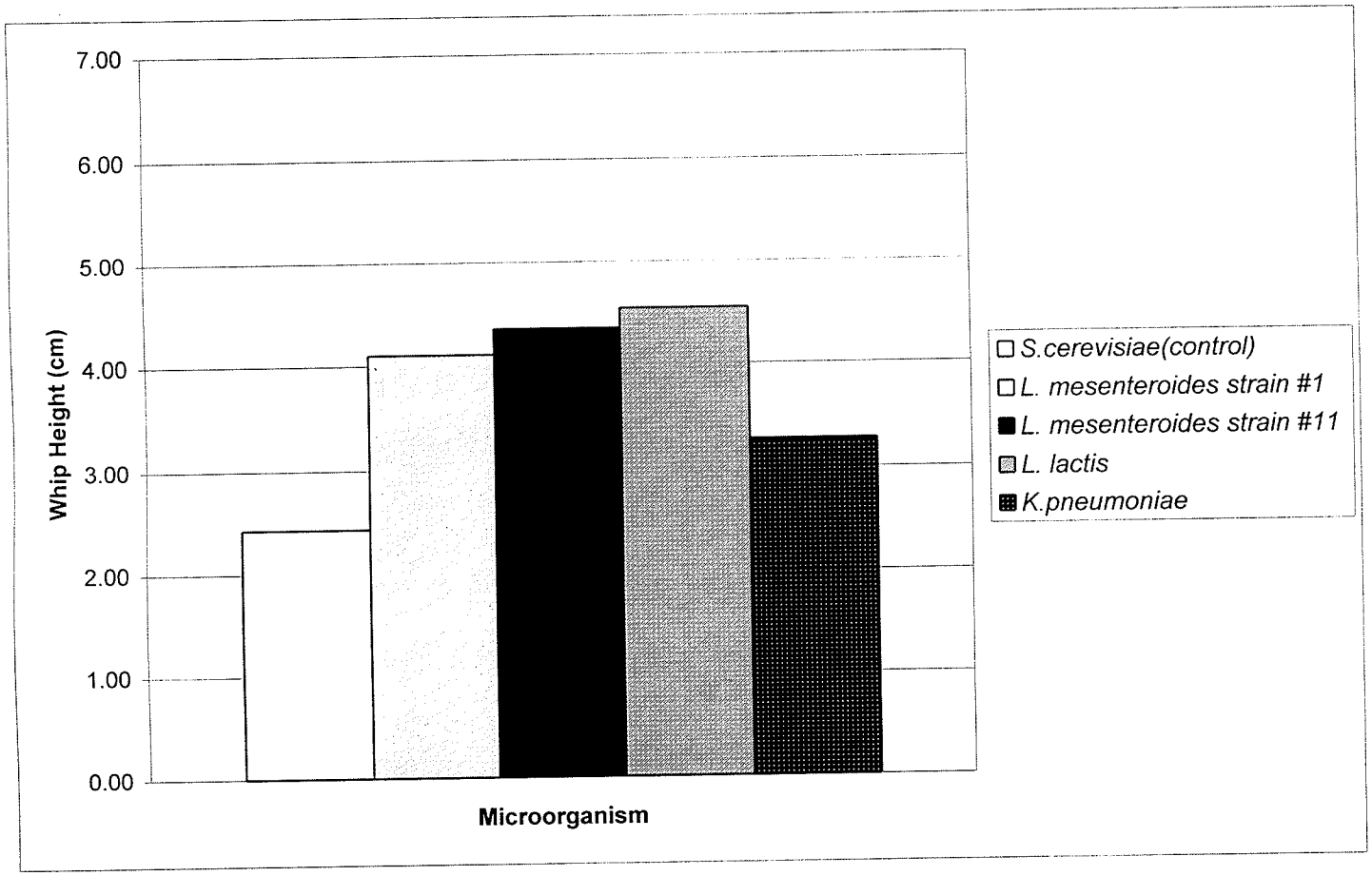
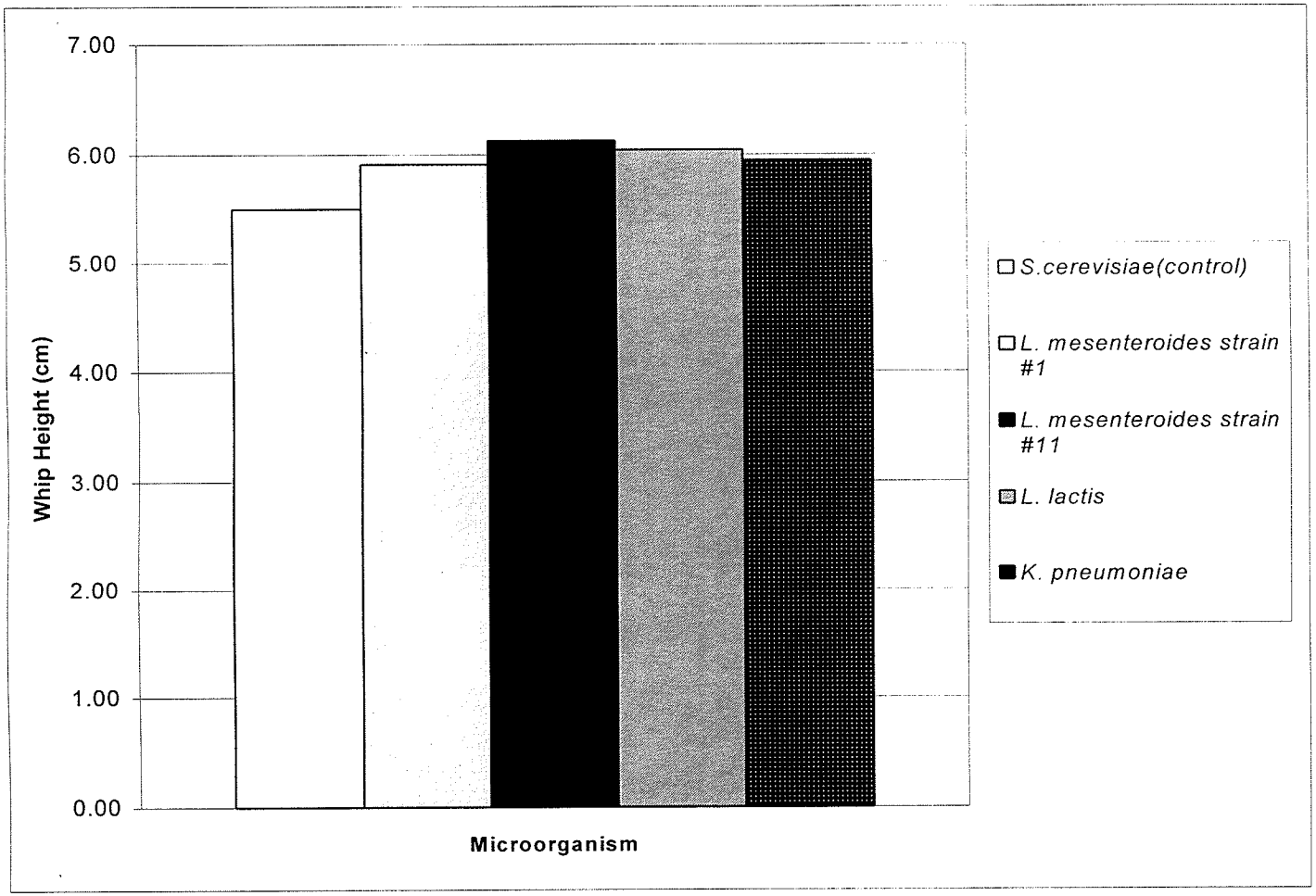


Figure 19. Whip heights of spray-dried pasteurized albumen fermented by *S. cerevisiae, L. mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae*



were observed among the other organisms (Table 4). No significant differences in viscosity were observed among angel cake batter prepared with the five test microorganisms (Table 5).

Table 4: Specific gravity of raw angel cake batter prepared with spray-dried, fermented pasteurized egg albumen[1]

Fermenting Microorganism	**Specific Gravity(g)** [2]
S. cerevisiae (control)	134.09±0.03[a]
L. mesenteroides subsp. *mesenteroides* strain #1	133.94±0.02[a]
L. mesenteroides subsp. *mesenteroides* strain #11	135.73±0.02[ab]
L. lactis	136.22±0.02[ab]
K. pneumoniae	138.60±0.01[b]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

Figure 20. Angel cake height of spray-dried pasteurized albumen fermented with *S. cerevisiae* (control), *L. mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae*

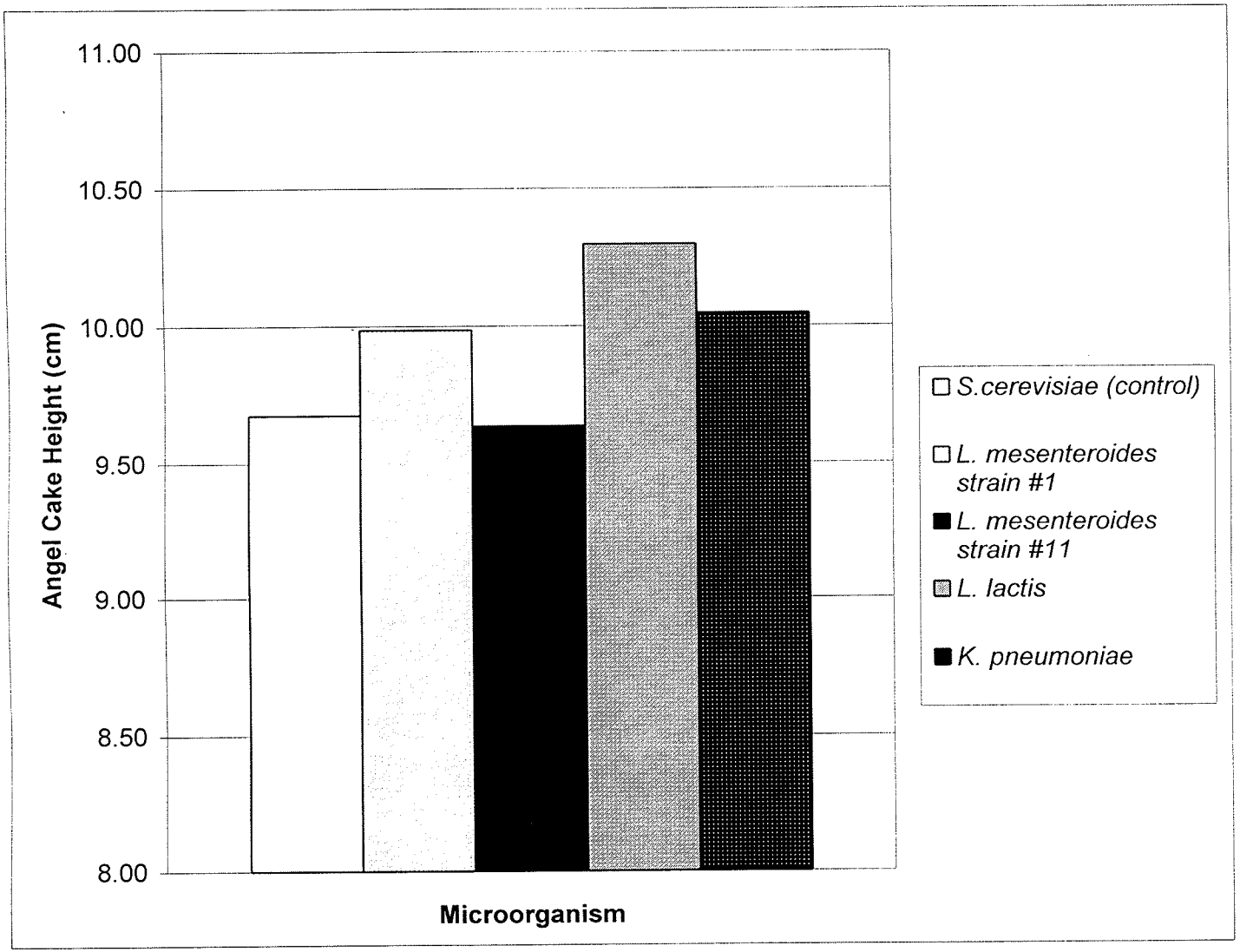


Table 5: Viscosity of raw angel cake batter prepared with spray-dried, fermented pasteurized albumen

Fermenting Microorganism	**Viscosity (Dynes)** [2]
S. cerevisiae (control)	23800±3023[a]
L. mesenteroides subsp. *mesenteroides* strain #1	22283±3767[a]
L. mesenteroides subsp. *mesenteroides* strain #11	22492±4053[a]
L. lactis	23400±2727[a]
K. pneumoniae	22433±3349[a]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ± SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

3. Gel Strength

Gel strength is one of the most important functional properties of albumen because high gel strength is a parameter often demanded by customers involved in food processing. In this study, the strength of albumen gels was measured using two cooking temperatures. Gel strength results for unpasteurized and pasteurized albumen cooked at 80^0C for 40 minutes are shown in Figs. 21 and 22, respectively. For unpasteurized albumen, *K. pneumoniae* had significantly higher gel strength than both the control and *L. mesenteroides* strain #1 (Appendix 26). Gel strengths of pasteurized albumen were all significantly higher than those made with unpasteurized albumen, as expected (Appendix 27).

Comparison of gels formed with pasteurized albumen, cooked at 80^0C for 40 min revealed that the gel strength achieved with *L. mesenteroides* strain #1 was

Figure 21. Gel strength of spray-dried unpasteurized albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* (strains #1and #11), *L. lactis* and *K. pneumoniae*. Gels were cooked at 80^0C for 40 min

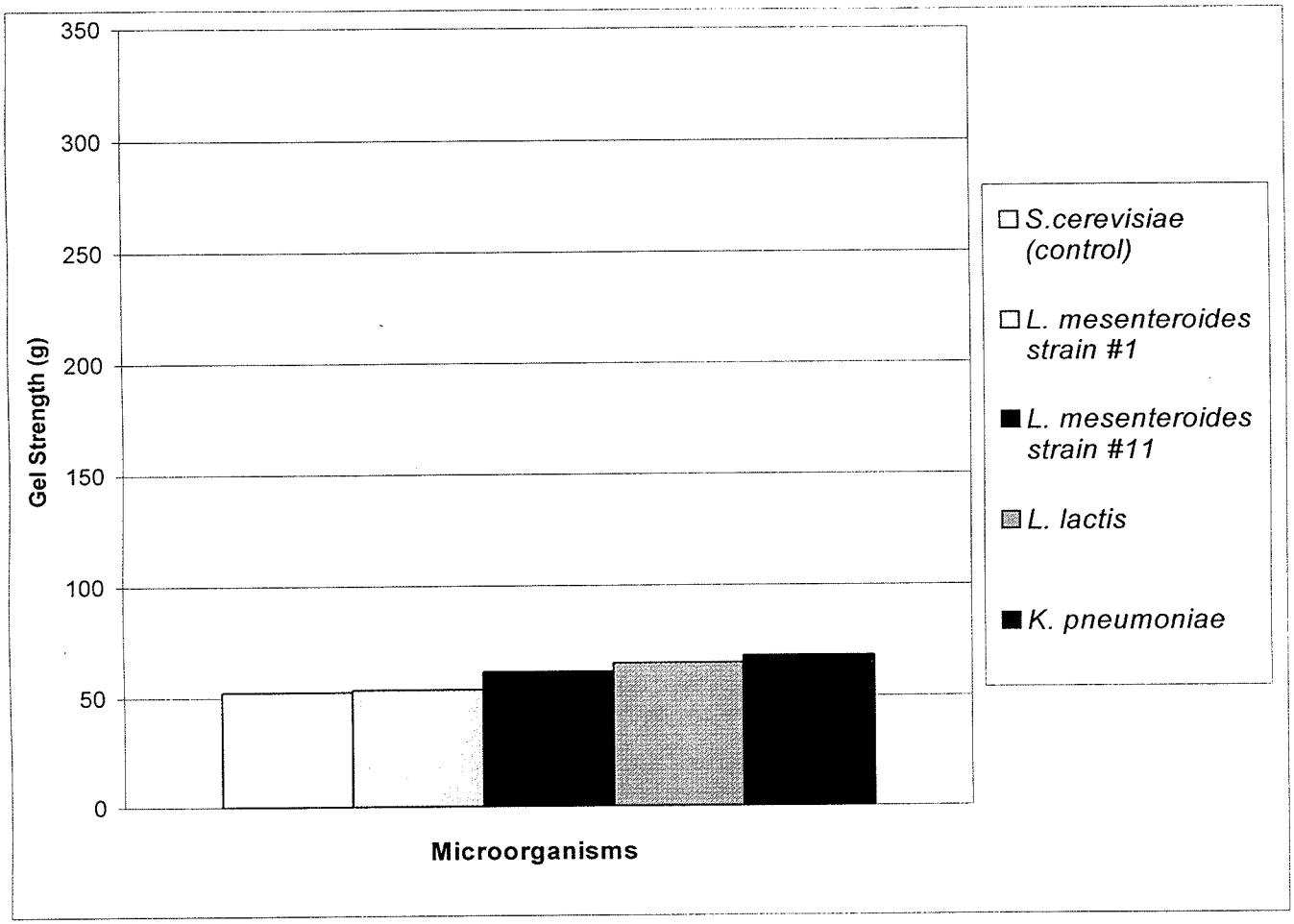
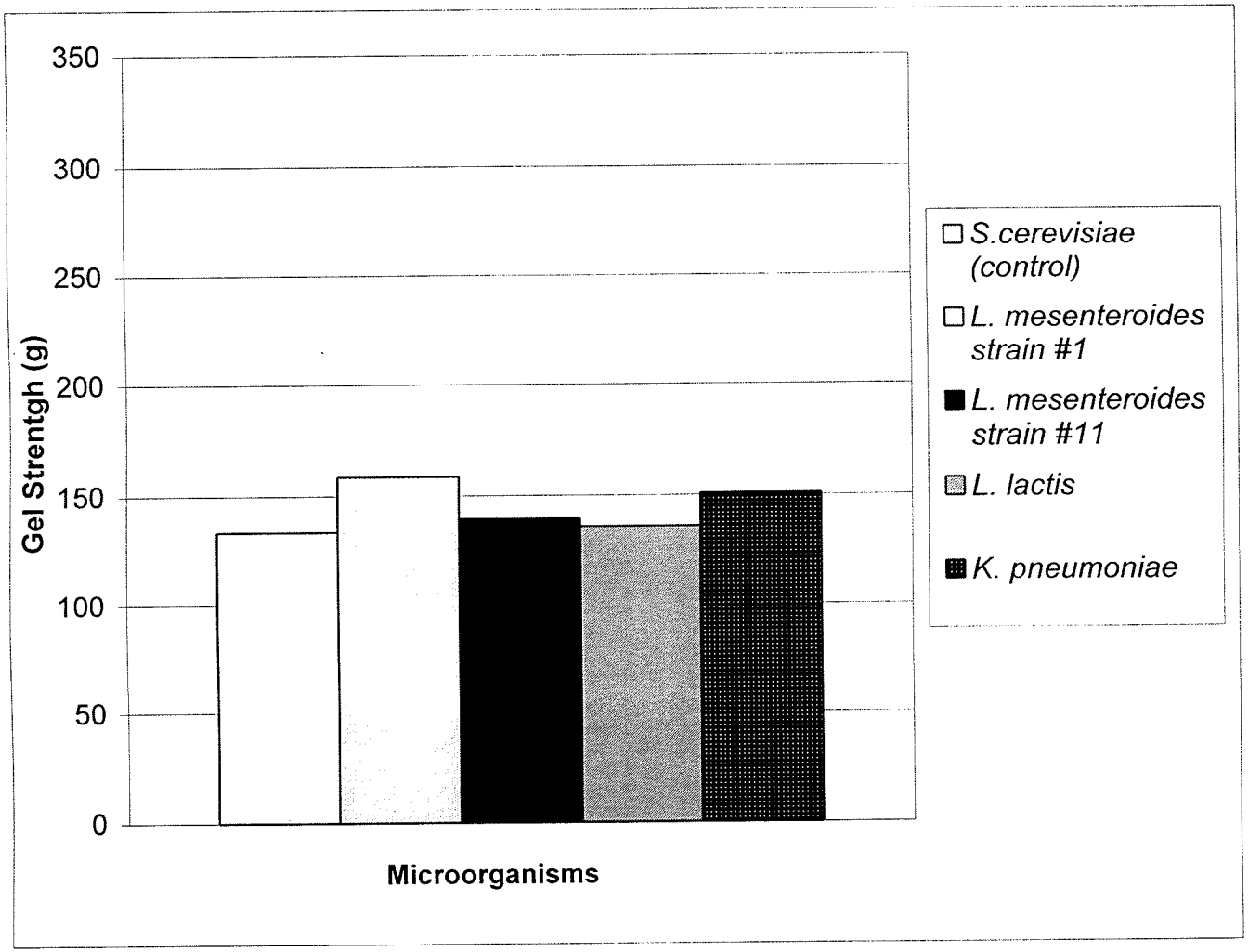


Figure 22. Gel strength of spray-dried pasteurized albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* (strains #1and #11), *L. lactis* and *K. pneumoniae*. Gels were cooked at 80^0C for 40 min



significantly higher than the control (Appendix 25). However, no significant differences were observed among gel strength values of albumen fermented by the four test bacteria at 80^0C for 40 minutes (Appendix 25). The gel strength of pasteurized albumen gels cooked at 75^0C for 60 min. is shown in Fig. 23. At this cooking temperature, which is also used for commercial quality control assessment, *K. pneumoniae* had a lower gel strength than both the control and *L. mesenteroides* strain #11 (Appendix 26). Since gel strength is such an important commercial parameter, *K. pneumoniae* should not be included as a fermenting organism in current processing protocols.

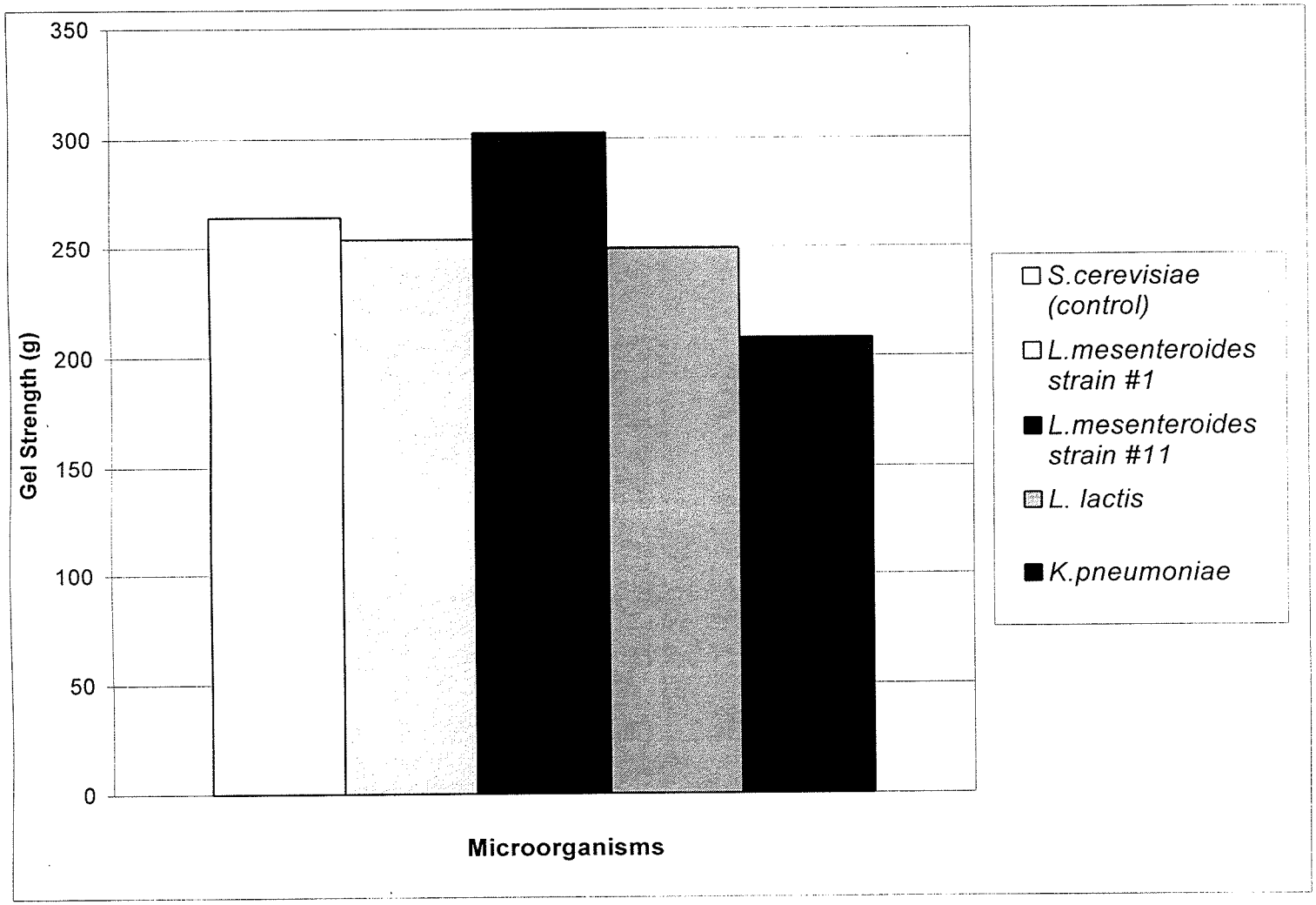
E. PHYSICOCHEMICAL CHARACTERISTICS OF FERMENTED ALBUMEN

The physicochemical characteristics of proteins include properties such as surface hydrophobicity and the changes that occur during heat denaturation. In this study, the purpose of assessing these physicochemical properties was to help explain the changes observed in functionality of albumen following fermentation with various microorganisms.

1. Thermal Denaturation Characteristics

Differential scanning calorimetry (DSC) is used to reflect the denaturation characteristics of proteins when they are heated. The denaturation temperature (T_d) reflects the temperature at which denaturation occurs, while enthalpy (ΔH) measures the amount of energy required to denature proteins. The heat flow required for denaturation is reflected in the size of the endothermic peak (Kato *et al.*, 1990b; Donovan, 1975). DSC reveals valuable information about the rate of protein denaturation and the identity of major protein fractions, such as conalbumin and

Figure 23. Gel strength of spray-dried pasteurized albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* (strains #1and #11), *L. lactis* and *K. pneumoniae*. Gels were cooked at 75^0C for 60 min



ovalbumin present in egg albumen. Disadvantages of more traditional DSC methods include their inability to detect components that exist in small concentrations; micro DSC is more appropriate to analyze these small protein components. In addition, some protein fractions, such as ovomucin, are not denatured by heat and therefore, will not be present on a thermogram (Donovan *et al.*, 1975). The typical shape of a DSC thermogram of lysozyme-extracted egg white has two characteristic endothermic peaks, conalbumin (lower temperature) followed by ovalbumin. The distinctly larger size of the ovalbumin peak reflects its dominance in egg white, comprising 60% of the total protein content (Donovan, 1975; Raeker and Johnson, 1995).

DSC was carried out on both unpasteurized and pasteurized spray-dried fermented albumen. DSC performed on unpasteurized albumen reflects the thermal characteristics of proteins in their native conformation. Figure 24 shows a typical DSC thermogram of native albumen. Table 6 shows the T_d and ΔH of the conalbumin and ovalbumin fractions of native egg albumen. The conalbumin T_d of the control was significantly higher than the other albumen samples, indicating that it had the most tightly coiled protein conformation. By comparison, the T_d of the ovalbumin fraction was higher than conalbumin (Table 6), showing the higher heat requirement for denaturation of ovalbumin, as it is more heat-stable than conalbumin. These results are in accordance with literature values, where the T_d of conalbumin is usually 60-65^{0}C and the T_d of ovalbumin is usually in the range of 80-85^{0}C (Donovan *et al.*, 1975). The T_d of ovalbumin for *L. lactis* was significantly higher than *L. mesenteroides* strain #1. The enthalpy of denaturation (ΔH) of conalbumin for unpasteurized samples ranged from 0.83 to 1.01 J/g, while the ΔH of ovalbumin was much higher than conalbumin, and ranged between 6.14 - 7.04 J/g (Table 7). This can

Figure 24: Typical DSC thermogram of albumen in its native conformation

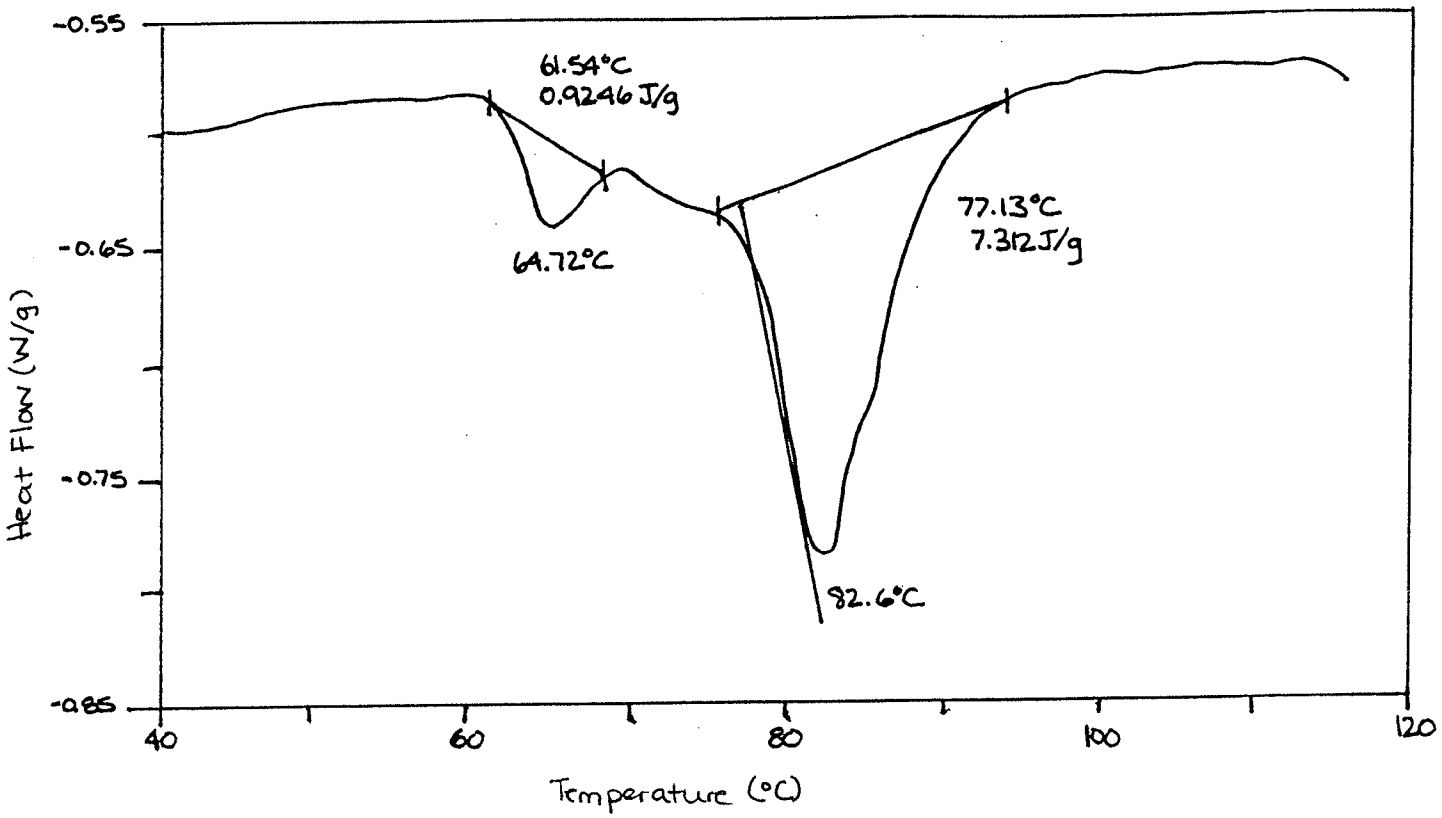


Table 6: Thermal denaturation parameters of fermented, spray-dried unpasteurized albumen

Fermenting Microorganism	T_d[3] Conalbumin (^{0}C) ±SD[2]	ΔH[4] Conalbumin (J/g) ±SD	T_d Ovalbumin (^{0}C) ±SD	ΔH Ovalbumin (J/g) ±SD
S. cerevisiae (control)	65.65±0.45[a]	0.91±0.24[a]	82.87±0.24[ab]	6.14±0.93[a]
L. mesenteroides subsp. *mesenteroides* strain #1	64.47±0.54[b]	1.01±0.10[b]	82.36±0.69[a]	7.36±0.86[a]
L. mesenteroides subsp. *mesenteroides* strain #11	64.68±0.47[b]	0.83±0.12[ab]	82.65±0.69[ab]	7.04±0.82[a]
L. lactis	64.98±0.33[b]	0.90±0.20[ab]	83.10±0.25[b]	6.86±0.92[a]
K. pneumoniae	64.66±0.51[b]	0.87±0.61[ab]	82.86±0.46[ab]	6.76±0.70[a]

[1]Results are a mean of three duplicate trials (n = 6±SD)
[2]SD; standard deviation
[3]T_d; denaturation temperature
[4]ΔH; enthalpy of denaturation
Means with the same letter within a column are not significantly different (student's t-test; α = 0.05)

be mainly attributed to the higher concentration of ovalbumin than conalbumin in egg white. Upon heat treatment, the ΔH and T_d values would be expected to decrease, indicating unfolding of the tertiary protein structure (Kato *et al.,* 1990b). The shift to a lower denaturation temperature following pasteurization indicates that the ovalbumin was partially denatured because less heat was required for denaturation. However, the endothermic peak of ovalbumin experienced extreme band widening compared with unpasteurized samples, causing the conalbumin peak to be masked (Fig. 25). This band widening was caused by partial denaturation of ovalbumin, while other protein fractions present were not denatured. The endothermic peaks of these protein fractions overlapped resulting in a wider ovalbumin peak, rather than a sharp peak at a lower Td value. When proteins are denatured, they unfold, therefore, it is expected that DSC analysis would reveal lower ΔH values because less energy is required to denature partially unfolded proteins (Kato *et al.*, 1990b). Calculation of

Figure 25: Typical DSC thermogram of pasteurized albumen

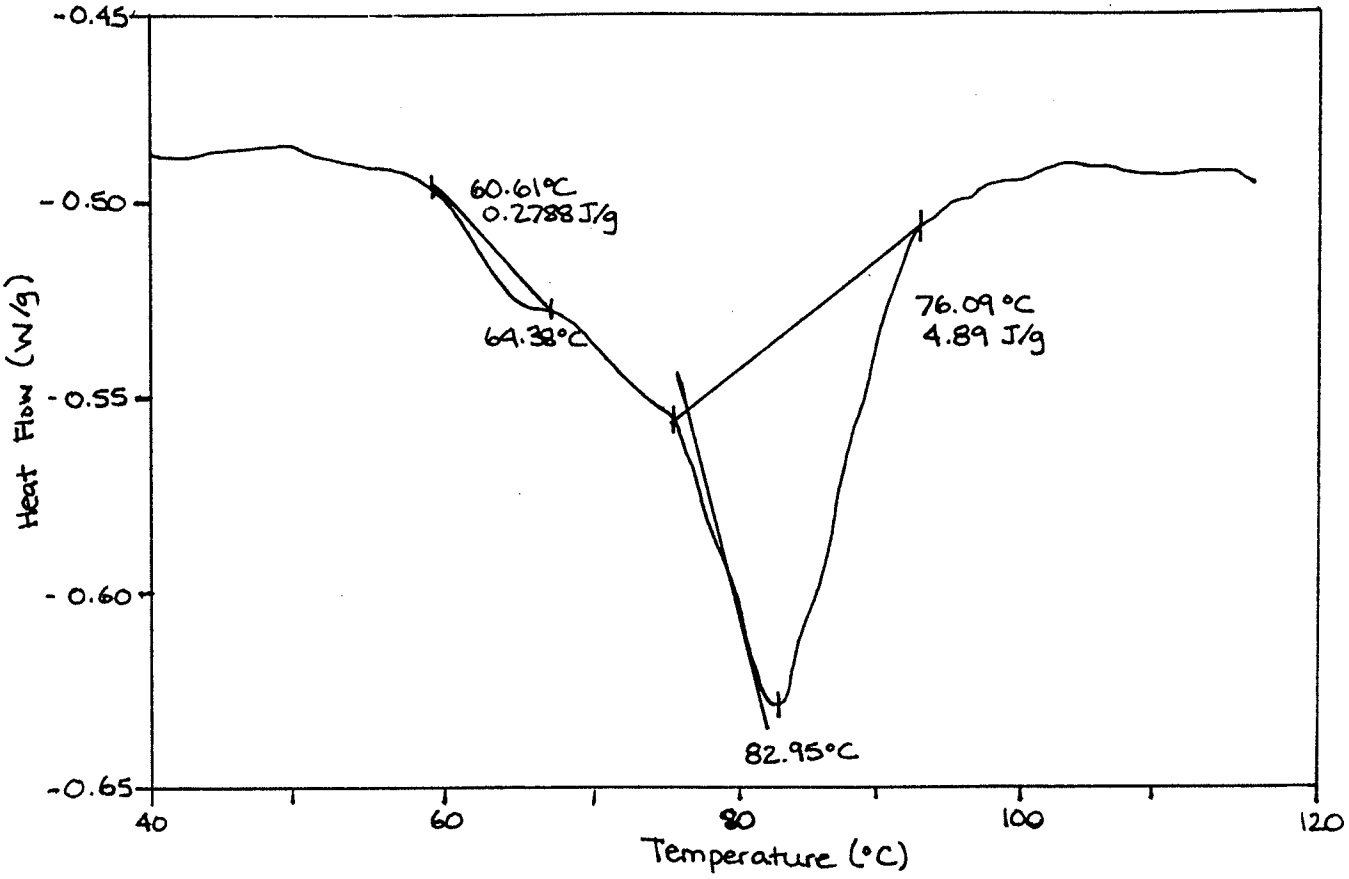


Table 7: Thermal denaturation parameters of spray-dried, fermented pasteurized albumen

Fermenting Microorganism	**T_d[2] Ovalbumin (^{0}C)**	**ΔH[3] Ovalbumin (J/g)**
S. cerevisiae (control)	82.35±0.35[a]	ND[4]
L. mesenteroides subsp. *mesenteroides* strain #1	82.10±0.75[a]	ND
L. mesenteroides subsp. *mesenteroides* strain #11	81.76±0.66[a]	ND
L. lactis	82.56±0.34[a]	ND
K. pneumoniae	82.49±0.32[a]	ND

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]T_d - denaturation temperature
[3]ΔH - enthalpy of denaturation
[4]ND - not determined
Results followed by the same letter within a column are not significantly different (student's t-test; $\alpha = 0.05$)

ΔH values for ovalbumin from pasteurized albumen could not be determined (Appendix 28).

Comparison of ovalbumin peaks for unpasteurized and pasteurized albumen samples revealed that in the case of the control, *L. mesenteroides* strain #11 and *L. lactis*, the T_d values were significantly lower in pasteurized samples. Similarly, significant differences were observed in the ΔH of ovalbumin for *S. cerevisiae* (control), as well as *L. mesenteroides* strain #11, *L. lactis* and *K. pneumoniae* with pasteurized albumen (Appendix 28).

2. Surface Hydrophobicity

Surface hydrophobicity (So) is used to determine the surface hydrophobic amino acid content in a sample, using techniques such as fluorescence spectroscopy. Originally, total hydrophobicity was measured; however, the So properties of proteins

have greater significance for protein functionality in biological systems (Kato and Nakai, 1980). The So of egg white was determined using the extrinsic fluorescent probe method according to Kato and Nakai (1980) with the probe anilinonapthalene-8-sulphonate (ANS). This probe was chosen primarily because it was easier to work with due to its stability. Determination of So by fluorometric methods is often used because it is an easier and faster method than either hydrophobic partition or hydrophobic interaction chromatography techniques (Kato and Nakai, 1980).

Results of spray-dried unpasteurized albumen (Fig. 26) show that *L. lactis* had a significantly higher So than the control (Appendix 29). No significant differences were observed among pasteurized albumen samples fermented by the five microorganisms (Figure 27, Appendix 30). When unpasteurized and pasteurized albumen samples were compared, pasteurized samples had significantly greater So values for all microorganisms (Appendix 31).

Figure 26. Surface hydrophobicity of spray-dried unpasteurized albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae*

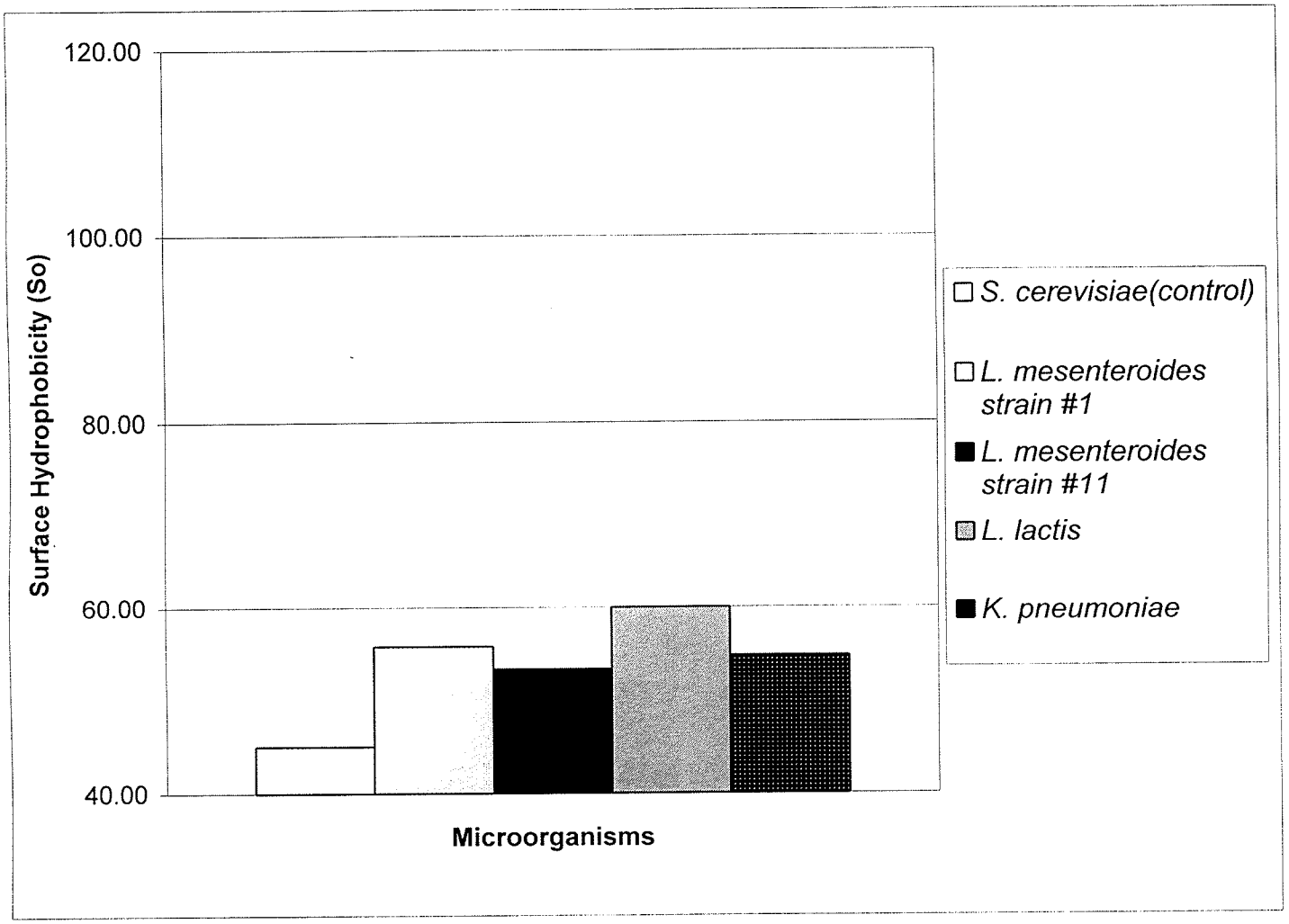
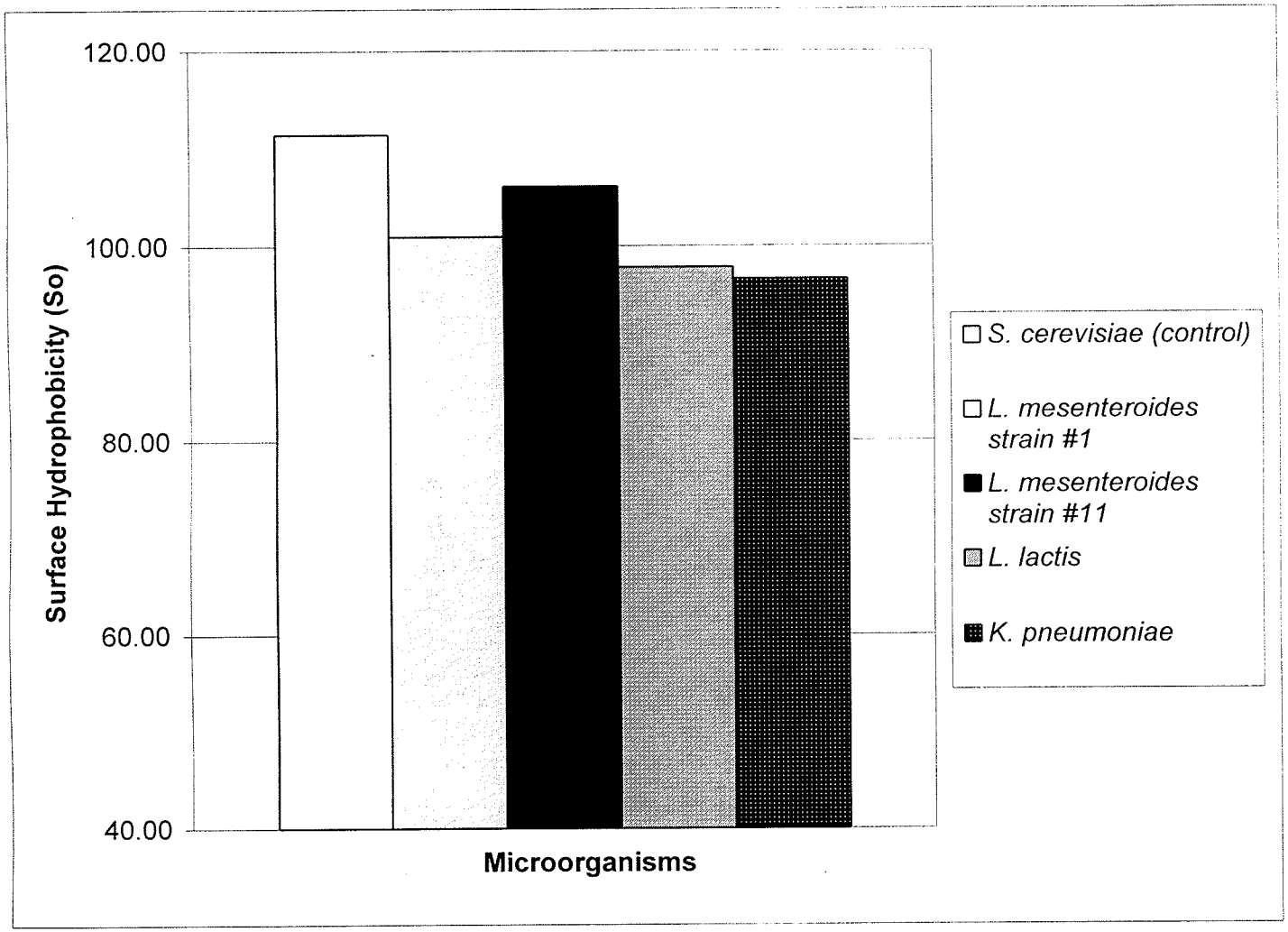


Figure 27. Surface hydrophobicity of spray-dried pasteurized albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae*



DISCUSSION

PRELIMINARY EXPERIMENTS

Production of bacterial exopolysaccharide (EPS) is of interest in foods such as egg albumen because of its potential use as a texturizer and viscosifier, as well as its water-binding capacity (Degeest and de Vuyst, 1999). In addition, the increased viscosity caused by production of EPS results in enhanced foaming and gelling capacities. Some bacterial exopolysaccharides such as dextran and xanthan are an attractive alternative to additives in the food industry because they have GRAS (generally regarded as safe) status (de Vuyst and Degeest, 1999). Degeest and de Vuyst (1999) reported that EPS may be produced in two ways; *in vivo* (in a fermentor as was used in this study) or *in situ* (in a food matrix such as during yoghurt processing). Studies have shown that certain nutritional requirements are necessary for production of bacterial EPS, including carbohydrate source, mineral salts, amino acids, vitamins and nucleic acid bases (Grobben *et al.*, 1998). For instance, Looijesteijn *et al.* (1999) reported that *Lactococcus lactis* subsp. *cremoris* is likely to produce nine times more EPS with glucose than with fructose as the carbon source. Physical culture conditions such as pH (6.0 to 7.0) and temperature (25^0-30^0C) are also important to EPS production (Degeest and Vuyst, 1999). However, production of EPS was not observed in this study, likely due to the fact that albumen lacks nutrients such as essential amino acids, vitamins (riboflavin, pantothenate, thiamine and vitamin B12), and minerals (potassium, calcium, acetate, iron and zinc) necessary for EPS production (Grobben *et al.*, 1998). Various studies have been performed to investigate the production of EPS in selective media (Marshall *et al.*, 1995; Ludbrook *et al.*, 1997; Grobben *et al.*, 1998). However, to date, no studies have been conducted where EPS was either added to albumen as a thickening agent, or produced by EPS-producing bacteria growing in egg albumen.

Results of this study showed that no net increase in viscosity was observed when albumen was fermented by any of the four test bacteria. The pH of albumen, which was initially adjusted to 6.5 and 7.0 prior to fermentation, decreased during the first 24-48 hours, indicating acid production by the fermenting bacteria. Glucose depletion was used to monitor the fermentation process. Its utilization paralleled bacterial growth. No EPS was produced during fermentation of egg albumen, verified by the lack of capsular material upon microscopic examination. Marshall *et al.* (1995) found that polysaccharide production by *Lactococcus lactis* subsp. *cremoris*, indicated by higher viscosity, was favoured at 25^0C when the pH of a selective growth medium was not controlled. Similar increases in viscosity were not observed in this study. Results in this study with *Leuconostoc mesenteroides* did not show similar results to Ludbrook *et al.* (1997), who observed a thickened modified EPS selective medium due to production of EPS during fermentation by lactic acid bacteria such as *L. mesenteroides, L. dextranicum* and *Streptococcus thermophilus.* The difference in nutrient levels (amino acids, vitamins and minerals) between the EPS-selective medium compared with egg albumen may explain the lack of EPS production in albumen. In addition, Marshall *et al.* (1995) reported that EPS production takes place at the end of the exponential and in the stationary phases of bacterial growth. Results of this study indicated that during the 72-hour fermentation, the test bacteria remained in the exponential phase of growth. Therefore, conditions were not optimal for EPS production.

When Marshall *et al.* (1995) grew *Lactococcus lactis* subsp. *cremoris* as a batch culture in an EPS-selective medium, they observed maximum bacterial populations of $5.6x10^8$ CFU/ml, slightly higher than those observed in this study. EPS cells were isolated using centrifugation and the supernatant was dialysed; the total carbohydrate present was measured spectrophotometrically at 490 nm with phenol-sulphuric acid against a series of glucose

standards (Marshall *et al.*, 1995). Landon *et al.* (1993) observed production of dextran (60g/L) by *L. mesenteroides* after a 21-h fermentation in EPS-selective media. However, dextran production was not observed in this study when varying concentrations of sucrose were added to egg albumen, indicating that the EPS selective medium used by Landon *et al.* (1995) is more conducive to EPS production than albumen. The EPS-selective media used by Marshall *et al.* (1995) contained all 20 essential amino acids, in addition to many vitamins and minerals. They reported that all 20 essential amino acids were essential for growth and production of EPS, in particular asparagine, glutamine and threonine. Egg albumen lacks asparagine and glutamine and EPS production by lactic acid bacteria in albumen may therefore, not be favoured unless supplemented by these amino acids. Ammonium chloride was not essential for growth, however, EPS production was enhanced when it was included in the growth medium (Grobben *et al.*, 1998). Additionally, sodium acetate, magnesium sulphate and manganese chloride were essential for optimal growth as they contribute to biosynthetic pathways. (Grobben *et al.*, 1998). The chemical medium utilized by Grobben *et al.* (1998) also included vitamins such as aminobenzoic acid, biotin, folic, lipoic and nicotinic acids, calcium pantothenate, pyridoxamine, pyridoxine, riboflavin, thiamine and vitamin B_{12}. Egg albumen lacks amino-benzoic acid, and lipoic and nicotinic acids, which may also contribute to its inability to support production of adequate amounts of EPS to cause significant increases in viscosity. For example, a 10-fold increase (50-60 g/L) in EPS production by lactic acid bacteria is essential to achieve the desired functionality as a food additive (de Vuyst and Degeest, 1999).

Additionally, the microorganisms chosen for this study may not have been ideal for production of EPS. Bacteria such as *L. lactis* may differ among strains and species. Studies have shown that *L. lactis* subsp. *cremoris* is able to produce significant amounts of EPS (Marshall *et*

al., 1995), whereas in this study, *L. lactis* subsp. *lactis* was not found to produce EPS. Similar phenomena may occur with bacteria such *Leuconostoc mesenteroides* and *Klebsiella* spps.

FUNCTIONAL AND PHYSICOCHEMICAL PROPERTIES OF ALBUMEN

Foaming

It is generally accepted that favourable foaming properties are achieved under conditions where a balance of hydrophobic and hydrophilic groups exists. Since proteins are surface-active agents, the exposure of hydrophobic residues has great impact on the overall functionality of proteins, especially foaming and emulsification (Kato *et al.*, 1990b). Heat denaturation of proteins causes an increase in surface hydrophobicity (So) due to the increasing exposure of hydrophobic amino acid residues, which are mainly buried in the interior of proteins in their native protein configuration (Kato and Nakai, 1980). Since Townsend and Nakai (1983) observed good correlation between So and emulsification, it is reasonable to assume that similar observations would be seen with foaming properties. In this study, when native albumen was evaluated, both species of *L. mesenteroides* and *L. lactis* had higher whip heights than the yeast control. Although no significant differences were observed among the five microorganisms, whip heights were found to improve after heat treatment of albumen compared with non-heat treated albumen. The improved whip heights observed here are similar to the findings of Raeker and Johnson (1995) who showed that heat denaturation of proteins such as ovalbumin resulted in improved foaming properties without the loss of solubility. The increased molecular flexibility and So, along with protein-protein interactions facilitate enhanced functionality (Raeker and Johnson, 1995). The relationship between foaming properties and So is attributed to a reduction of surface tension through adsorption of protein films at the air-liquid interface, and the

amphiphilic nature of protein molecules (Townsend and Nakai, 1983). Horiuchi *et al.* (1978) observed a correlation between foam stability and So using fluorescence spectroscopy. Townsend and Nakai (1983) did not observe a significant relationship between foaming capacity and surface hydrophobicity, because extensive unfolding of proteins at the air-liquid interface caused complete denaturation of the proteins. A significant relationship was, however, observed between foam capacity and total hydrophobicity (Townsend and Nakai, 1983). Kato *et al.* (1980) confirmed the findings of Horiuchi *et al.* (1978), when they observed an increase in So upon heat denaturation of ovalbumin, thereby improving protein functionality.

Kato *et al.* (1990b) showed that compared with other protein fractions, dried egg white had the greatest foaming capacity. Researchers have found high negative correlation between enthalpy of denaturation (ΔH) and foaming properties, indicating that denatured proteins have enhanced foaming abilities (Kato *et al.*, 1990b; Raeker and Johnson, 1995). Hayakawa *et al.* (1992) found that native ovalbumin from egg albumen had a T_d of 77^0C and a ΔH value of 11.3 mJ/mg. Comparison of native and denatured samples revealed that the ΔH decreased by 61% in denatured samples (Hayakawa *et al.*, 1992). This decreased ΔH may encourage the formation of films at the surface, by increasing the diffusion rate of denatured protein molecules, thereby resulting in increased foaming capacity (Kato *et al.*, 1990b). Our study confirms this concept, as improved foaming properties were observed following pasteurization. The improved whipping properties observed with pasteurized samples can be attributed to denaturation of albumen proteins during heat treatment; DSC analysis confirmed that partial denaturation had occurred. It is important to examine changes that occur during heating of egg albumen because structural changes can help explain the increased functionality observed following heat treatment of fermented albumen.

In this study, the height of angel cakes made with albumen fermented by *L. lactis* were significantly higher than those made with albumen fermented by *L. mesenteroides* strain #11. Previous study has shown that cake height is significantly higher following partial heat denaturation (Martinez *et al.*, 1995). The hydrophobic amino acid residues are exposed at the air-liquid interface, promoting incorporation of air into the albumen, resulting in a higher cake height following baking (Martinez *et al.*, 1995). The denaturation temperature of ovalbumin, a major constituent of egg albumen, parallels the starch gelatinization of angel cake batter. Under storage at high temperatures ovalbumin is converted to a more heat stable form, S-ovalbumin. Because this form of ovalbumin is not as easily denatured by heat, structural collapse and oven spring occur, resulting in decreased cake volume. However, denaturation that occurs during cake baking prevents this transformation to S-ovalbumin, indicating that the denaturation that occurs during baking is important to the functionality of albumen (Raeker and Johnson, 1995).

Gel Strength

The thermal denaturation properties of proteins affect the gelation characteristics of egg albumen. Protein denaturation is essential for gelation and is studied by observing the structural changes that occur during heat treatment such as pasteurization and dry-heating of egg albumen (Gossett *et al.*, 1984). In non heat-treated albumen, *K. pneumoniae* had higher gel strength than *S. cerevisiae* (control). However, following heat treatment, *L. mesenteroides* strain #1 had higher gel strength than the control, and no differences were observed among the other test bacteria. Overall, a reduction in T_d paralleled an increase in gel strength following heat treatment by pasteurization, indicating a relationship between thermal denaturation and gelation. When Kato *et al.* (1990c) studied the effects of heating on dried egg albumen, they also observed

decreased T_d values, along with broadening of endothermic peaks during DSC analysis. They attributed the broader peaks and decreasing Td values to unfolding of protein molecules. The denaturation of proteins promotes intermolecular interactions that are important in the gelation process (Kato *et al.*, 1990c). Good correlation was also observed between the ΔH and gel strength of albumen. A decrease in ΔH paralleled improved gel strength, suggesting that unfolding of protein molecules promotes the intermolecular interactions that play an important role in the gelation process (Kato et al., 1990c).

The improved gel strength of heat-treated albumen observed in this study confirms the work of Kato *et al.* (1994). They found that heat treatment improved the gel strength of dried egg white gels, confirming their earlier findings that gel strength of dried egg white increased with heating time. When dried egg albumen was heated in the dry state at 80^0C for 10 d, the gel strength of albumen was reported to be four times that of native albumen (Kato *et al.*, 1989). Structural and conformational changes in albumen explain the increased functionality. Kato *et al.* (1989) studied the effects of heating proteins in the dry state and found a 7-fold increase in So upon heating albumen at 80^0C for 10 days. This confirms our findings where pasteurization resulted in increased So. The increased So of heated albumen suggests unfolding of secondary protein structure, hydrophobic residues are exposed at the surface following partial denaturation of protein molecules. When a large complement of hydrophobic residues are exposed at the surface, protein solubility is compromised, causing hydrophobic interactions which result in aggregation of protein molecules, indicating that increased So is likely to parallel higher gel strength (Nakai, 1983).

CONCLUSIONS

Although preliminary results did not show signs of exopolysaccharide production during fermentation of albumen, functional and physicochemical testing did reveal that improved functionality of egg albumen was achieved following pasteurization. Of the four test bacteria, *L. mesenteroides* subsp. *mesenteroides* strain #1 and *L. lactis* showed the most promising results. Fermentation of egg albumen with these two microorganisms showed consistently improved functionality over the control. Future research should concentrate on production of EPS in albumen in order to elicit improvements in functionality by natural processes. The albumen medium may be supplemented with nutrients and amino acids necessary for growth. EPS may also be produced by microorganisms such as lactic acid bacteria in selective media and inoculated into the albumen fermentation as microbial growth approaches the stationary phase, allowing for optimal EPS production. The ensuing increases in viscosity would likely lead to improvements in functionality.

CONCLUSIONS AND RECOMMENDATIONS

A. GENERAL CONCLUSIONS

1. Preliminary trials

In general, preliminary trials revealed that fermentation of albumen with the four chosen bacteria did not result in improved viscosity compared with the control, indicating that production of bacterial extracellular polysaccharide likely did not occur. Addition of yeast extract and varying concentrations of sugar also did not lead to increases in viscosity.

The capillary method for determination of viscosity of egg albumen provided a great source of variation, likely due to build-up of albumen in the small capillary. Therefore, it is recommended that an alternate method be employed for future research. Such methods may include using a Brookfield viscometer. However, large volumes are required for this method, one of the reasons it was not used for this research. A comparable method should be developed for use with small volumes.

All microorganisms tested were effective in depleting carbohydrate from albumen, which is the primary purpose of fermentation in a commercial setting. Overall, their growth was better than the control. No correlation among the four parameters tested (viscosity, pH, residual glucose or microbial growth) proved meaningful.

2. Functional Properties and Physicochemical Characteristics

Determination of the functional (whip height, angle cake height and gel strength) and physicochemical (thermal denaturation and surface hydrophobicity) properties of egg albumen elucidated several relationships. In general, heat-treatment by pasteurization resulted in enhanced functionality. In addition to killing most pathogenic microorganisms present in the albumen following fermentation, the enhanced functionality achieved during pasteurization is essential in the processing of powdered egg albumen. Whip heights of

pasteurized albumen fermented with the four test bacteria were all significantly higher than the control and cake height obtained with albumen fermented by *L. lactis* was significantly higher than that obtained with *L. mesenteroides* strain #11. Since whip height and angel cake height are both determinants of foaming properties, it is reasonable to conclude that an essential heat treatment such as pasteurization enhances the foaming capacity of fermented egg albumen. Gel strength was also significantly higher in pasteurized albumen. The gel strength of *Leuconostoc mesenteroides* strain #1 was significantly higher than the control in gel cooked at 80^0C for 40 minutes.

Analysis of the ΔH values of pasteurized albumen was inconclusive due to masking of the conalbumin fraction by widening of the ovalbumin peak. However, the shift to lower T_d values in heat-treated ovalbumin suggests that partial protein denaturation occurred, therefore, less heat was required to denature the proteins. The So increased significantly upon pasteurization of albumen. This suggests that denaturation of proteins allows exposure of hydrophobic amino acid residues at the air-liquid interface, which facilitates the foaming process. The increased So paralleled increases in whip and angel cake heights obtained with fermented, pasteurized albumen, and indicated that denaturation of proteins does indeed contribute to the foaming properties of albumen.

Results revealed that both *L. lactis* and *L. mesenteroides* subsp. *mesenteroides* strain #1 showed the greatest improvement in functionality when all functional and physicochemical properties were taken into consideration. However, more study is needed before a recommendation for change in current processing practices can be made.

B. RELATIONSHIPS AMONG FUNCTIONALITY AND PHYSICOCHEMICAL CHARACTERISTICS OF ALBUMEN

Many relationships may be drawn which interconnect the functional and physicochemical properties of egg albumen. A positive correlation was found between ΔH of ovalbumin and whip height, indicating that increases in whip height parallel the decrease in ΔH of ovalbumin. Significant increases in whip height also corresponded with increased So, confirming the theory that exposure of hydrophobic residues at the surface plays a large role in the formation of foam. Gel strength and the T_d of ovalbumin showed a negative correlation, confirming the theory that partially heat denatured proteins, evidenced by a downward shift in the T_d of ovalbumin, form stronger gels. Angel cake height and So also showed a strong correlation. The increased So in pasteurized albumen indicates denaturation of albumen proteins and confirms the fact that denatured proteins enhance angel cake height through the exposure of hydrophobic amino acid residues at the air-liquid interface.

C. RECOMMENDATIONS FOR FUTURE RESEARCH

There are several areas of research warranting further investigation. Conducting similar fermentation trials using various other extracellular polysaccharide-producing bacteria such as *Leuconostoc dextranicum*, or *Lactococcus lactis* subsp. *cremoris*, for example, is necessary in order to achieve a fermentation process where dextran or other exopolysaccharide is produced. Although viscosity did not prove to relate directly with protein functionality, it is still a good indication of exopolysaccharide production. Because egg processors do not like to include chemical additives on food labels, the production of exopolysaccharide should be a natural process. Further research is needed to explore production of bacterial exopolysaccharide in a selective medium, which could be subsequently inoculated into partially fermented egg albumen. Addition of this

polysaccharide to egg white in the latter portion of the fermentation may induce improved functionality, without adding artificial chemicals to the fermenting medium. Amino acids, vitamins and minerals such as glutamine, asparagine, vitamin B_{12} and aminobenzoic acid are necessary for EPS production, however, these are lacking in albumen. Therefore, addition of these ingredients to the albumen during fermentation may provide a growth medium more conducive to production of EPS, which would in turn, facilitate improved functionality.

Preliminary results showed that fermentation of albumen with the microorganisms chosen did not yield increased viscosity. However, functional testing showed that the four test bacteria chosen were superior to the yeast control currently used in egg albumen processing. Future studies should be conducted with the pH of albumen adjusted to 6.0 prior to fermentation, as this is more favourable for growth of acid-producing microorganisms. In most cases, optimal growth was observed when fermentation was carried out at the optimum growth temperature for each microorganism. No significant differences were observed when the albumen was supplemented with various concentrations of yeast extract and carbohydrate. However, it is thought that more trials with different combinations may elucidate favourable relationships among viscosity of fermented albumen, and addition of yeast extract and carbohydrate. Different species of fermentative microorganisms within the same genera may also provide favourable results.

Although favourable results of functional tests were achieved, the overall gel strength values were lower than expected. It was thought that the pasteurization process was not completely successful, as gel strength values were lower than observed in previous work at Canadian Inovatech. Pasteurization could be undertaken on the remaining unpasteurized samples to determine whether gel strength values could be improved compared with those obtained in this study. If stimulation of exopolysaccharide production can be achieved in an egg white medium, functional testing should subsequently be conducted to compare with the

results of this study. Fermentation practices that are both economical and provide products with improved functionality are critical to maintaining Canada's increasing role in the export of egg products to foreign markets.

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SECTION VII

APPENDICES

Appendix 1. Viscosity of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 6.5 prior to fermentation[1]

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Viscosity (centipoise/sec) ±SD[2]						
0	2.81±0.24^{ab}	2.81±0.16 ^{a}	2.93±0.06 ^{a}	2.94±1.65 ^{a}	2.49±0.05^{a}	2.90±0.42^{b}	2.90±0.02^{b}
24	2.58±0.29^{ab}	2.36±0.18 ^{a}	2.39±0.39 ^{a}	2.29±0.225 ^{a}	2.48±0.11^{a}	3.11±0.13^{b}	3.03±0.03^{b}
48	3.04±0.152^{ab}	2.43±0.14 ^{a}	2.38±0.31 ^{a}	2.09±0.23 ^{a}	2.51±0.16^{a}	3.20±0.21^{b}	2.83±0.10^{b}
72	2.57±0.10^{ab}	2.40±0.05 ^{a}	2.58±0.21 ^{a}	2.25±0.33 ^{a}	2.58±0.01^{a}	3.33±0.66^{b}	2.82±0.15^{b}

[1]Results are a mean of three trials (n = 3 ±SD)
[2]Results followed by the same superscript within a row are not significantly different (student's t-test, α= 0.05)
No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 2. The pH of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 6.5 prior to fermentation[1]

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	pH ±SD[2]						
0	6.36±0.11^a	6.50±0.21^b	6.77±0.18^b	6.65±0.40^{ab}	6.52±0.06^{ab}	6.66±0.30^{ab}	6.54±0.04^{ab}
24	6.45±0.20^a	5.34±0.20^b	5.34±0.08^b	6.22±1.16^{ab}	5.83±0.11^{ab}	5.71±0.10^{ab}	5.94±0.00^{ab}
48	6.72±0.16^a	5.49±0.23^b	5.55±0.01^b	6.17±0.13^{ab}	5.91±0.21^{ab}	6.29±0.36^{ab}	5.98±0.06^{ab}
72	6.94±0.12^a	5.66±0.07^b	5.97±0.23^b	7.42±0.09^{ab}	6.69±0.15^{ab}	6.81±0.99^{ab}	6.86±0.21^{ab}

[1]Results are a mean of three trials (n = 3 ±SD)

[2]Results followed by the same superscript within a row are not significantly different (student's t-test, $\alpha = 0.05$)

No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 3. Glucose concentration of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 6.5 prior to fermentation[1]

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Glucose Concentration (g/L) ±SD[2]						
0	2.05±0.82^a	2.40±0.05^a	1.62±1.89^a	2.52±0.42^a	2.68±0.08^a	2.69±0.26^a	2.89±0.76^a
24	0.00^a	0.07±0.07^a	0.11±0.08^a	0.22±0.03^a	0.92±0.85^a	0.16±0.22^a	1.34±0.13^a
48	0.00^a	0.00	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a
72	0.00^a	0.00^a	0.00^a	0.15±0.21^a	0.00^a	0.00^a	0.00^a

[1]Results are a mean of three trials (n = 3 ± SD)
[2]Results followed by the same superscript within a row are not significantly different (student's t-test, $\alpha = 0.05$)
No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 4. Growth in albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 6.5 prior to fermentation[1]

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Total Plate Count (Log_{10} CFU/ml) ±SD[2]						
0	5.59±0.11[a]	6.64±0.24 [b]	6.94±0.27 [b]	7.31±0.21 [b]	7.04±0.03[b]	7.01±0.60 [b]	6.84±0.07[b]
24	6.05±0.39[a]	7.76±0.42 [b]	7.95±0.02 [b]	8.87±0.12 [b]	8.17±0.14[b]	8.39±0.45 [b]	7.99±0.05[b]
48	6.11±0.35[a]	8.43±0.82 [b]	7.95±0.11 [b]	9.14±0.07 [b]	9.19±0.02[b]	9.31±0.63 [b]	8.71±0.04[b]
72	5.97±0.36[a]	9.00±0.41 [b]	9.00±0.67 [b]	9.53±0.29 [b]	9.18±0.03[b]	9.64±1.20 [b]	9.01±0.31[b]

[1]Results are a mean of three trials (n = 3 ± SD)

[2]Results followed by the same superscript within a row are not significantly different (student's t-test, α= 0.05)

No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 5. Viscosity of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 7.0 prior to fermentation[1]

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Viscosity (centipoise/sec) ± SD[2]						
0	2.94±0.65^{a}	3.03±0.00^{ab}	2.96±0.01^{a}	2.82±0.65^{ac}	2.28±0.01^{ad}	2.75±0.96^{ab}	2.85±0.09^{ab}
24	2.63±0.44^{a}	2.95±0.12^{ab}	2.66±0.03^{a}	2.93±0.45^{ac}	2.41±0.06^{ad}	2.86±0.41^{ab}	2.84±0.01^{ab}
48	2.45±0.46^{a}	2.53±0.15^{ab}	2.61±0.32^{a}	2.46±0.14^{ac}	2.18±0.01^{ad}	2.98±0.28^{ab}	2.46±0.05^{ab}
72	2.18±0.25^{a}	2.63±0.18^{ab}	2.69±0.38^{a}	2.47±0.16^{ac}	1.77±0.08^{ad}	2.83±0.71^{ab}	2.88±0.06^{ab}

[1]Results are a mean of three trials (n = 3 ±SD)

[2]Results with the same superscript within a row are not significantly different (student's t-test, α= 0.05)

No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 6. The pH of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 7.0 prior to fermentation[1].

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	pH ± SD[2]						
0	6.92±0.09^a	7.29±0.08^b	7.02±0.20^b	7.10±0.11^a	7.16±0.00^a	6.97±0.21^{ab}	6.87±0.00^{ab}
24	7.44±0.10^a	6.47±0.13^b	6.02±0.36^b	7.25±0.86^a	7.59±0.04^a	6.45±0.59^{ab}	6.70±0.02^{ab}
48	7.73±0.12^a	5.94±0.13^b	6.02±0.03^b	6.92±0.16^a	7.15±0.11^a	6.83±0.34^{ab}	5.95±0.02^{ab}
72	7.68±0.06^a	6.21±0.01^b	6.19±0.12^b	7.54±0.39^a	6.66±0.40^a	7.25±0.49^{ab}	6.95±0.04^{ab}

[1]Results are a mean of three trials (n = 3 ±SD)

[2]Results with the same superscript within a row are not significantly different (student's t-test, α= 0.05)

No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 7. Glucose concentration of albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* for 72 h. Albumen was adjusted to approximately pH 7.0 prior to fermentation[1].

Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Glucose Concentration (g/L) ±SD[2]						
0	2.46±0.24^a	2.60±0.33^a	1.67±1.83^a	3.07±0.35^a	2.69±0.19^a	2.78±0.03^a	2.58±0.09^a
24	0.00^a	0.66±0.63^a	0.14±0.01^a	0.51±0.25^a	0.97±0.16^a	0.68±0.97^a	1.86±0.24^a
48	0.00^a	0.00^a	0.00^a	0.37±0.52^a	0.10±0.05^a	0.00^a	0.00^a
72	0.00^a	0.00^a	0.00^a	0.00^a	0.01±0.01^a	0.00^a	0.00^a

[1]Results are a mean of three trials (n = 3 ± SD)
[2]Results with the same superscript within a row are not significantly different (student's t-test, α= 0.05)
No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 8. Growth in albumen fermented by *S. cerevisiae* (control), *L. mesenteroides* subsp. *mesenteroides* (strains #1 and #11), *L. lactis* and *K. pneumoniae* in albumen for 72 h. Albumen was adjusted to approximately pH 7.0 prior to fermentation[1].

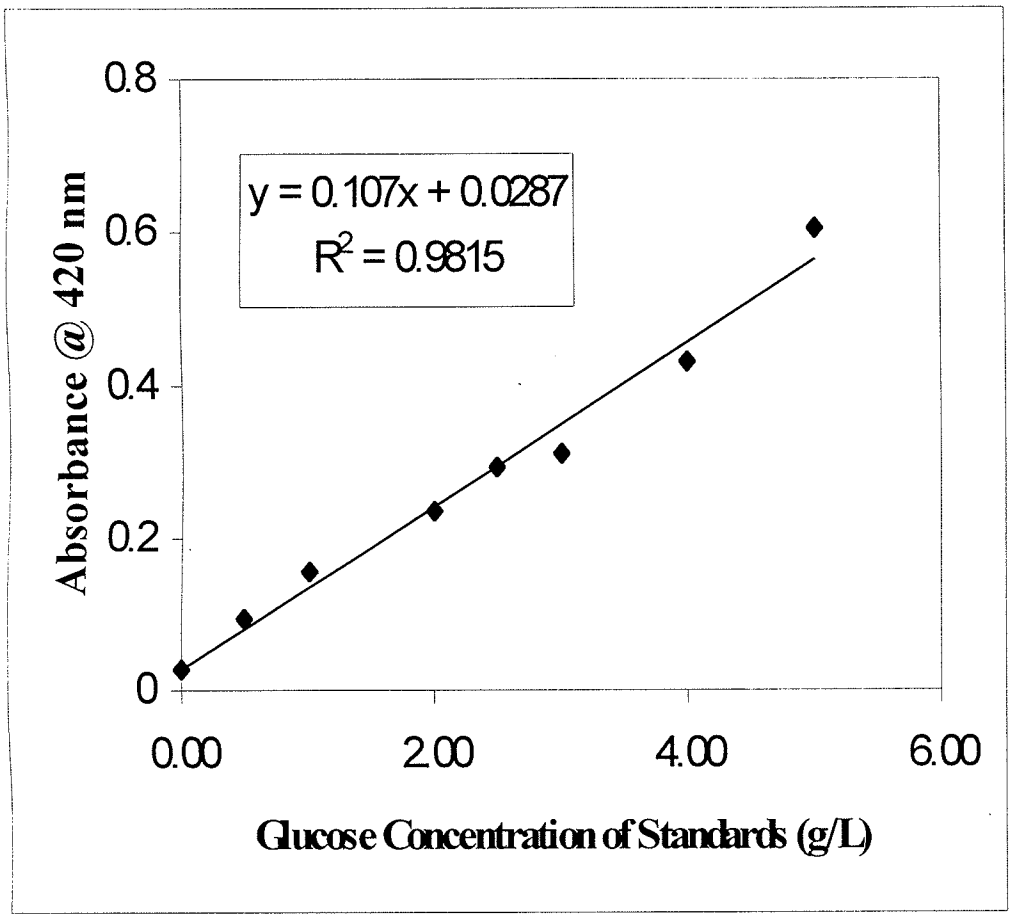
Microorganism	*S. cerevisiae*	*L. mesenteroides* strain #1	*L. mesenteroides* strain #11	*L. lactis*		*K. pneumoniae*	
Temperature (^{0}C)	37	30	30	28	32	37	32
Time (h)	Total Plate Count (log_{10} CFU/ml) ±SD[2]						
0	5.48±0.15^a	6.75±0.16^b	6.96±0.27^b	7.00±0.35^b	6.78±0.10^b	7.06±0.28^b	7.32±0.41^b
24	5.55±0.26^a	7.32±0.63^b	7.98±0.11^b	9.18±0.27^b	8.46±0.29^b	8.92±0.31^b	8.69±0.08^b
48	5.65±0.20^a	8.12±1.14^b	7.63±0.64^b	9.36±0.95^b	8.66±0.19^b	9.05±0.24^b	9.38±0.27^b
72	5.71±0.18^a	9.32±0.38^b	9.26±0.74^b	10.44±0.3^b	9.49±0.11^b	9.06±0.08^b	9.26±0.42^b

[1]Results are a mean of three trials (n = 3 ± SD)

[2]Results with the same superscript within a row are not significantly different (student's t-test, α= 0.05)

No significant differences were found when fermentation of albumen by *L. lactis* and *K. pneumoniae* at different temperatures were compared

Appendix 9: Standard curve for determination of glucose concentration in egg albumen



Appendix 10. Viscosity, pH, glucose concentration and total yeast & mould growth in albumen fermented by *S. cerevisiae* (control) at 37^{0}C for 72 h. Albumen was adjusted to approximately pH 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5	**pH 7.0**	**pH 6.5** ***	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5** ***	**pH 7.0**
0	2.81±0.24	2.94±0.65	6.36±0.11	6.92±0.09	2.05±0.82	2.46±0.24	5.59±0.11	5.48±0.15
24	2.58±0.29	2.63±0.44	6.45±0.20	7.44±0.10	0	0	6.05±0.39	5.55±0.26
48	3.04±0.15	2.45±0.46	6.72±0.16	7.73±0.12	0	0	6.11±0.35	5.65±0.20
72	2.57±0.10	2.18±0.25	6.94±0.12	7.68±0.06	0	0	5.97±0.36	5.71±0.18

[1]Results are a mean of three trials (n = 3 ± SD)

*** Comparison is significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 11. Viscosity, pH, glucose concentration and total growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^{0}C for 72 h. Albumen was adjusted to approximately 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5 ***	pH 7.0	pH 6.5 ***	pH 7.0	pH 6.5	pH 7.0	pH 6.5	pH 7.0
0	2.82±0.16	3.03±0.00	6.50±0.21	7.29±0.08	2.40±0.05	2.60±0.33	6.64±0.24	6.75±0.16
24	2.39±0.18	2.95±0.12	5.34±0.20	6.47±0.13	0.07±0.07	0.66±0.63	7.76±0.42	7.32±0.63
48	2.43±0.14	2.53±0.15	5.49±0.23	5.94±0.13	0	0	8.43±0.82	8.12±1.14
72	2.40±0.05	2.63±0.18	5.66±0.07	6.21±0.01	0	0	9.00±0.41	9.32±0.38

[1]Results are a mean of three trials (n = 3 ± SD)

*** Comparison is significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 12. Viscosity, pH, glucose concentration and total growth in albumen fermented by *L. mesenteroides* subsp. *mesenteroides* strain #11 at 30^0C for 72h. Albumen was adjusted to approximately pH 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**
0	2.93±0.06	2.96±0.01	6.65±0.18	7.02±0.20	1.62±1.89	1.67±1.83	6.94±0.27	6.96±0.27
24	2.34±0.39	1.66±0.03	5.40±0.08	6.02±0.36	0.11±0.08	0.14±0.01	7.95±0.02	7.98±0.11
48	2.38±0.31	2.61±0.32	5.56±0.01	6.02±0.03	0	0	7.95±0.11	7.63±0.64
72	2.58±0.21	2.69±0.38	5.81±0.23	6.19±0.12	0	0	9.00±0.67	9.26±0.74

[1]Results are a mean of three trials (n = 3 ± SD)
No comparisons were significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 13. Viscosity, pH, glucose concentration and total growth in albumen fermented by *L. lactis* at 28^{0}C for 72h. Albumen was adjusted to approximately 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**
0	2.94±1.65	2.82±0.65	6.65±0.40	7.10±0.11	2.52±0.42	3.07±0.35	7.31±0.21	7.00±0.35
24	2.29±0.22	2.93±0.45	6.22±1.16	7.25±0.86	0.22±0.03	0.51±0.25	8.87±0.12	9.18±0.27
48	2.09±0.23	2.46±0.14	6.17±0.13	6.92±0.16	0	0.37±0.52	9.14±0.07	9.36±0.95
72	2.25±0.33	2.47±0.16	7.42±0.09	7.54±0.39	0.15±0.21	0	9.53±0.29	10.44±0.38

[1]Results are a mean of three trials (n = 3 ± SD)
No comparisons were significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 14. Viscosity, pH, glucose concentration and total plate count in albumen fermented by *L. lactis* at 32^{0}C for 72h. Albumen was adjusted to approximately pH 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log CFU/ml)	
	pH 6.5 ***	pH 7.0	pH 6.5 ***	pH 7.0	pH 6.5	pH 7.0	pH 6.5	pH 7.0
0	2.49±0.05	2.28±0.01	6.52±0.06	7.16±0.00	2.68±0.08	2.69±0.19	7.04±0.03	6.78±0.10
24	2.48±0.11	2.41±0.06	5.83±0.11	7.59±0.04	0.92±0.85	0.97±0.16	8.17±0.14	8.46±0.29
48	2.51±0.16	2.18±0.01	5.91±0.21	7.15±0.11	0	0.10±0.05	9.19±0.02	8.66±0.19
72	2.58±0.01	1.77±0.08	6.69±0.15	6.66±0.40	0	0.01±0.01	9.18±0.03	9.49±0.11

[1]Results are a mean of three trials (n = 3 ±SD)

*** Comparison is significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 15. Viscosity, pH, glucose concentration and total growth in albumen fermented by *K. pneumoniae* at 37^{0}C for 72h. Albumen was adjusted to approximately pH 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5	pH 7.0	pH 6.5	pH 7.0	pH 6.5	pH 7.0	pH 6.5	pH 7.0
0	2.90±0.42	2.75±0.96	6.66±0.30	6.97±0.21	2.69±0.26	2.78±0.03	7.01±0.60	7.06±0.28
24	3.11±0.13	2.86±0.41	5.71±0.10	6.45±0.59	0.16±0.22	0.68±0.97	8.39±0.45	8.92±0.31
48	3.20±0.21	2.98±0.28	6.29±0.36	6.83±0.34	0	0	9.31±0.63	9.05±0.24
72	3.33±0.66	2.83±0.71	6.81±1.00	7.25±0.49	0	0	9.64±1.20	9.06±0.08

[1]Results are a mean of three trials (n = 3 ± SD)
No comparisons were significantly different at all time intervals (student's t-test, $\alpha = 0.05$)

Appendix 16. Viscosity, pH, glucose concentration and total growth in albumen fermented by *K. pneumoniae* at 32^0C for 72h. Albumen was adjusted to approximately pH 6.5 and 7.0 prior to fermentation[1]

Time (h)	Viscosity (centipoise/s)		pH		Glucose Concentration (g/L)		Total Plate Count (log_{10} CFU/ml)	
	pH 6.5	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**	**pH 6.5**	**pH 7.0**
0	2.90±0.02	2.85±0.09	6.54±0.04	6.87±0.00	2.89±0.76	2.58±0.09	6.84±0.07	7.32±0.41
24	3.03±0.03	2.84±0.01	5.94±0.00	6.70±0.02	1.34±0.13	1.86±0.24	7.99±0.05	8.69±0.08
48	2.83±0.10	2.46±0.05	5.98±0.06	5.95±0.02	0	0	8.71±0.04	9.38±0.27
72	2.82±0.15	2.87±0.06	6.86±0.21	6.95±0.04	0	0	9.01±0.31	9.26±0.42

[1]Results are a mean of three trials (n = 3 ±SD)
No comparisons were significantly different at all time intervals (student's t-test, α= 0.05)

Appendix 17: Viscosity of albumen fermented with *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C for 48 h. Yeast extract (0.1%) and sucrose were added to albumen prior to fermentation[1]

Time (h)	Viscosity (centipoise/sec) [2]		
	Sucrose (%)		
	3 ± SD*	4 ± SD	5 ± SD
0	2.53±0.56[a]	2.94±0.00[a]	3.51±0.78[b]
24	1.97±0.13[a]	2.35±0.56[a]	2.86±1.53[b]
48	1.66±0.08[a]	2.42±1.01[a]	2.25±0.71[b]

[1]Results are a mean of two trials (n = 2 ±SD)
[2]Results followed by the same superscript within a row are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 18: The pH of albumen fermented with *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^{0}C for 48 h. Yeast extract (0.1%) and sucrose were added to albumen prior to fermentation[1]

Time (h)	pH [2]		
	Sucrose (%)		
	3 ± SD*	4 ± SD	5 ± SD
0	6.00±1.26[a]	6.20±1.09[a]	6.03±1.08[a]
24	5.83±1.93[a]	5.79±1.91[a]	5.45±1.51[a]
48	5.01±1.01[a]	4.94±0.93[a]	4.78±0.78[a]

[1]Results are a mean of two trials (n = 2 ±SD)
[2]Results followed by the same superscript within a row are not significantly different (student's t-test; α= 0.05)

Appendix 19: Total growth in albumen fermented with *L. mesenteroides* subsp. *mesenteroides* strain #1 at 30^0C for 48 h. Yeast extract (0.1%) and sucrose were added to albumen prior to fermentation[1]

Time (h)	Total Plate Count (log_{10} CFU/ml) [2]		
	Sucrose (%)		
	3 ± SD*	4 ± SD	5 ± SD
0	7.35±0.21[a]	7.84±0.47[a]	7.46±0.46[a]
24	7.11±1.32[a]	7.73±0.65[a]	7.74±0.30[a]
48	7.94±0.65[a]	7.75±1.05[a]	8.23±0.52[a]

[1]Results are a mean of two trials (n = 2 ±SD)
[2]Results followed by the same superscript within a row are not significantly different (student's t-test; α= 0.05)

Appendix 20: Whip heights of spray-dried, fermented unpasteurized egg albumen[1]

Fermenting microorganism	**Whip height (cm)** [2]
Saccharomyces cerevisiae (control)	6.18±0.76[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	10.42±3.09[b]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	11.05±1.15[b]
Lactococcus lactis	11.54±1.44[b]
Klebsiella pneumoniae	8.32±2.95[ab]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 21: Whip heights of spray-dried, fermented pasteurized egg albumen[1]

Fermenting microorganism	**Whip height (cm)** [2]
Saccharomyces cerevisiae (control)	13.99±0.97[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	15.02±0.64[b]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	15.57±0.25[b]
Lactococcus lactis	15.35±0.55[b]
Klebsiella pneumoniae	15.11±0.40[b]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 22: Comparison of whip heights for unpasteurized and pasteurized spray-dried, fermented albumen

Fermenting microorganism	**Whip height (cm)**		
	Unpasteurized		**Pasteurized**
Saccharomyces cerevisiae (control)	6.18±0.76	***	13.99±0.97
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	10.42±3.09	***	15.02±0.64
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	11.05±1.15	***	15.57±0.25
Lactococcus lactis	11.54±1.44	***	15.35±0.55
Klebsiella pneumoniae	8.32±2.95	***	15.11±0.40

***Comparison is significantly different (t-test; $\alpha = 0.05$)

Appendix 23: Angel cake height prepared with spray-dried, fermented pasteurized egg albumen[1]

Fermenting microorganism	**Cake height (cm)** [2]
Saccharomyces cerevisiae (control)	9.68 ± 0.52^{abc}
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	9.99 ± 0.32^{abc}
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	9.64 ± 0.63^{ab}
Lactococcus lactis	10.30 ± 0.38^{ac}
Klebsiella pneumoniae	10.05 ± 0.69^{a}

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 24: Gel strength of spray-dried, fermented unpasteurized egg albumen. Gels were cooked at 80^0C for 40 minutes[1]

Fermenting microorganism	**Gel strength (g)** [2]
Saccharomyces cerevisiae (control)	52.33 ± 17.51^{a}
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	53.33 ± 22.71^{ab}
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	61.42 ± 22.53^{abc}
Lactococcus lactis	65.11 ± 14.73^{abc}
Klebsiella pneumoniae	68.34 ± 25.07^{c}

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 25: Gel strength of spray-dried, fermented pasteurized egg albumen. Gels were cooked at 80^0C for 40 minutes[1]

Fermenting microorganism	**Gel strength (g)** [2]
Saccharomyces cerevisiae (control)	133.67±42.42[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	158.67±61.39[b]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	139.67±19.38[ab]
Lactococcus lactis	135.89±16.99[ab]
Klebsiella pneumoniae	150.78±23.75[ab]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; α= 0.05)

Appendix 26: Gel strength of spray-dried, fermented pasteurized egg albumen. Gels were cooked at 75^{0}C for 60 minutes[1]

Fermenting microorganism	**Gel strength (g)** [2]
Saccharomyces cerevisiae (control)	264.11±60.76^a
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	253.67±15.56^{abc}
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	302.78±91.91^{ab}
Lactococcus lactis	249.22±84.92^{abc}
Klebsiella pneumoniae	208.33±23.24^c

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; α= 0.05)

Appendix 27: Comparison of gel strength for unpasteurized and pasteurized spray-dried albumen. Gels were cooked at 80^0C for 40 minutes

Fermenting microorganism	**Gel strength (g)**		
	Unpasteurized		**Pasteurized**
Saccharomyces cerevisiae (control)	52.33±17.51	***	133.67±42.42
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	53.33±22.71	***	158.67±61.39
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	61.42±22.53	***	139.67±19.38
Lactococcus lactis	65.11±14.73	***	135.89±16.99
Klebsiella pneumoniae	38.34±25.07	***	150.78±23.75

*** Comparison is significantly different (student's t-test; α= 0.05)

Appendix 28: Comparison of thermal denaturation parameters for the ovalbumin protein fraction of unpasteurized and pasteurized spray-dried, fermented albumen

Fermenting microorganism	T_d[2] Ovalbumin (^{0}C)			ΔH[3]Ovalbumin (J/g)	
	Unpasteurized		Pasteurized	Unpasteurized	Pasteurized
Saccharomyces cerevisiae (control)	82.87±0.24	***	82.35±0.35	6.14±0.93	ND[4]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	82.36±0.69		82.10±0.75	7.36±0.86	ND
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	82.65±0.69	***	81.76±0.66	7.04±0.82	ND
Lactococcus lactis	83.10±0.25	***	82.56±0.34	6.86±0.92	ND
Klebsiella pneumoniae	82.86±0.46		82.49±0.32	6.76±0.70	ND

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]T_d - denaturation temperature
[3]ΔH - enthalpy of denaturation
[4]ND - not determined
Results followed by the same superscript within a column are not significantly different (student's t-test; $\alpha = 0.05$)

Appendix 29: Surface hydrophobicity (S_O) of spray-dried, fermented unpasteurized albumen[1]

Fermenting microorganism	S_O[2]
Saccharomyces cerevisiae (control)	45.0±15.5[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	55.6±11.3[ab]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	53.3±10.2[ab]
Lactococcus lactis	60.0±5.2[b]
Klebsiella pneumoniae	54.7±6.5[ab]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ± SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; α= 0.05)

Appendix 30: Surface hydrophobicity (S_O) of spray-dried, fermented pasteurized albumen[1]

Fermenting microorganism	**S_O** [2]
Saccharomyces cerevisiae (control)	111.4±19.9[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	101.0±12.5[a]
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	106.1±18.7[a]
Lactococcus lactis	97.9±21.7[a]
Klebsiella pneumoniae	96.6±16.2[a]

[1]Results are a mean of three replicates, each performed in duplicate (n = 6 ±SD)
[2]Results followed by the same superscript are not significantly different (student's t-test; α= 0.05)

Appendix 31: Comparison of surface hydrophobicity (S_O) for unpasteurized and pasteurized albumen

Fermenting microorganism	S_O		
	Unpasteurized		**Pasteurized**
Saccharomyces cerevisiae (control)	45.0±15.5	***	111.4±19.9
Leuconostoc mesenteroides subsp. *mesenteroides* strain #1	55.6±11.3	***	101.0±12.5
Leuconostoc mesenteroides subsp. *mesenteroides* strain #11	53.3±10.2	***	106.1±18.7
Lactococcus lactis	60.0±5.2	***	97.9±21.7
Klebsiella pneumoniae	54.7±6.5	***	96.6±16.2

*** Comparison is significantly different (student's t-test; $\alpha = 0.05$)