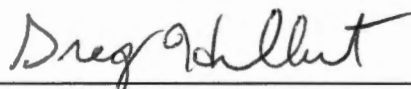


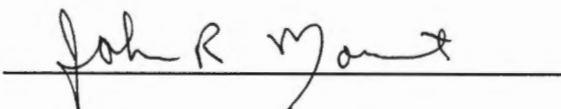
To the Graduate Council:

I am submitting herewith a thesis written by Hongyu Wu entitled "Effects of Ultrasound on Milk Homogenization and Fermentation with Yogurt Starter." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.



Greg Hulbert, Major Professor

We have read this thesis
and recommended its acceptance:



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**EFFECTS OF ULTRASOUND ON MILK HOMOGENIZATION
AND FERMENTATION WITH
YOGURT STARTER**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

HONGYU WU

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Thesis
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DEDICATION

**This thesis is dedicated to
my parents
who always have been giving me invaluable love.**

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ABSTRACT

Yogurt production was selected to study the effects of ultrasound on milk homogenization and fermentation. A microscope was used to check the size distribution of fat globules to evaluate ultrasound homogenization. The pH change during fermentation and the water holding capacity, viscosity and syneresis for finished yogurt were evaluated. It was found that ultrasound has a very good homogenization effect at high amplitude levels compared with conventional homogenization. Longer exposure times improved the ultrasound homogenization effect. If enough exposure time and amplitude level are provided, extremely small fat globules can be produced. The fermentation results showed that the method of sonicating bacteria had a significant effect on the pH change. Sonication after inoculation could reduce the total fermentation time by 0.5 hours. Ultrasound also significantly affected the water holding capacity, viscosity and syneresis. In general, increasing the ultrasound amplitude level improved the water holding capacity, and viscosity and reduced syneresis. However, sonication after inoculation did not decrease the syneresis effect. This may have been due to the lower pH of those samples.

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CHAPTER I

INTRODUCTION

The increasing interest in fermented dairy products has been due to the nutritional value and tastiness of the products. Fermented milk products are an important part of the human diet. Many countries have developed their own fermented products, however, yogurt is the most well known all over the world. It not only offers high levels of calcium, phosphorus, magnesium, vitamin A and riboflavin, but also can contain high levels of live bacterial cultures which are good for health. Yogurt manufacturers have enjoyed a rapidly growing market within the last three decades. To maintain this growth, the yogurt industry must be more innovative with regard to processing techniques.

All yogurt manufacturing procedures are basically the same. The milk is clarified and separated into cream and skim milk, then standardized to achieve the desired fat content. The mixture is then homogenized using high pressures (10-20 MPa) at a temperature of 55-70°C (Tamime and Robinson, 1985). Homogenization is a very important and necessary pretreatment for most dairy products. It can prevent creaming and

wheying off during incubation and storage. Stability, consistency and body of yogurt are enhanced by homogenization.

The milk is pasteurized by heating for 30 min at 85°C or 10 min at 95°C. Next the milk is cooled to about 43°C, which is an optimum growth temperature for the yogurt starter culture that is added to the milk in a fermentation tank. A temperature of 43°C is maintained for 4-6 h. The acid development of yogurt is carefully monitored until the pH reaches 4.0 to 4.7, and the fermentation is stopped by rapid cooling (Connolly, 1978).

Ultrasound applications in milk fermentation science and technology are noteworthy. Janovski and Pohlmann (1948) emphasized the economic advantages of the use of a type of ultrasound device (liquid whistle). Singiser and Beal (1960) considered ultrasound as a method of homogenization. A specially designed ultrasound device (a whistle type) has been applied to cheese production (Gaffney, 1996). The results showed this process reduced waste, lowered energy consumption and produced softer cheeses. Toba (1990) reported that ultrasound improved the action of lactobacillus by nearly 50% and also induced a sweetening effect in the yogurt without increasing the caloric content. Ultrasound shows potential advantages in yogurt manufacture. The process efficiency and yogurt quality could be improved.

Ultrasound is already well known to many people due to its wide applications in various fields, such as medical imaging, pasteurization, SONAR, non-destructive measurement, material processing and surface cleaning. Ultrasound can be divided into two main applications: at low power and high frequencies, it can be used as an analytical tool (such as imaging and sonar and non-destructive evaluation), and at high power and low frequencies (power ultrasound) it can assist processing. The ultrasound range used also can be divided into diagnostic and power ultrasound. Power ultrasound has been successfully used in the food industry for many years. For example, all ultrasound research mentioned above involved the use of power ultrasound.

Based on previous studies ultrasound is a promising tool for milk homogenization. Improvements over conventional homogenization could lead to yogurt of superior quality.

The objectives of this research were as follows:

1. To evaluate the effect of ultrasound on milk homogenization.
2. To determine and evaluate the effect of ultrasound on the pH changes in milk fermentation with yogurt starter.
3. To determine and evaluate the effect of ultrasound on the water holding capacity of the fermented yogurt product.
4. To determine and evaluate the effect of ultrasound on the viscosity of

the fermented yogurt product.

5. To determine and evaluate the effect of ultrasound on the syneresis of the fermented yogurt product.

CHAPTER II

LITERATURE REVIEW

I. MILK COMPONENTS AND THEIR FUNCTIONS

Introduction

Milk is a popular beverage due to its nutritional value. It is a very important food source for humans. Milk is typically from domesticated cows but can be obtained from humans, buffalo, goats, yak and sheep. All dairy foods are made from various milks, and the dairy components are the same as those of milk but in varying amounts. There are many factors that can affect milk composition such as breed, herd management including feed considerations, seasonal variation, and geographic variations. Milk contains proteins, lactose, butter fat, minerals, fat-soluble and water-soluble vitamins, enzymes and other organic compounds.

Milk is a very complex food with a high nutritional value. Protein and fat are the major functional components in milk products. An approximate composition of milk is: 87.3% water (range of 85.5% - 88.7%), 3.9 % milkfat (range of 2.4% - 5.5%), and 8.8% solids-non-fat (range of 7.9 - 10.0%). The approximate specific composition of the solids-non-fat is

3.25% (3/4 casein) protein, 4.6% lactose and 0.65% minerals other components include acids, enzymes, gases, vitamins, and microorganisms (Anon., 1980).

Water

Water is a major constituent in milk. The water content of milk can range from a low content in marine mammals to a high content in human milk. Cow milk is about 87% water, which is the average amount of water in most milk.

Carbohydrates

Milk products are the only food source of the carbohydrate lactose, and lactose is the most significant carbohydrate in milk. Other carbohydrates such as glucose and galactose are found in milk but at low concentrations. Lactose, a sugar, provides half of the total solids in milk and contributes 30% of the food energy in whole milk. Lactose is the major carbohydrate in the milk of most species, and the lactose content is the least variable component of milk. Lactose intolerance occurs in animals or animals that do not have lactase activity in their intestines. Yogurt and cheese are low in lactose, as the lactose is converted to lactic acid by bacterial cultures necessary to make these products (Mephram, 1983).

Milk Fat

Milk fat content can vary between whole milk and skim milk. Whole milk must contain not less than 3.25% milk fat and 8.25% milk solids-not-fat. Skim milk, also called nonfat milk, must have as much fat removed as technologically possible, bringing it to less than 0.5%. It must contain at least 8.25% solids-not-fat.

Milk is an oil-in-water type emulsion of fat globules and a suspension of casein micelles (casein, calcium, phosphorous). The aqueous phase contains solubilized lactose, whey proteins, and some minerals. Milk fat is 95 to 96% triglycerides (esters of fatty acids with glycerol), 0.8 to 1.0% phospholipids, 0.2 to 0.4% sterol - chiefly cholesterol, and very small amounts of free fatty acids, waxes, and squalene (an intermediary of cholesterol) and varying amounts of all the known fat-soluble vitamins. Milk's phospholipids are bound to the surface of the fat globules along with proteins. These are substances containing phosphorus, nitrogen and fatty acids.

The fat component of milk is a complex mixture of lipids. Allowing raw milk to stand for a period of time results in the cream rising to the top, and can change the stability of the fat emulsion in milk. This separation is due to a lower buoyant density of fat than water. The fat rises to the top resulting in the cream layer. There are so many fat globules that they also

carry some of the milk protein to the top, and the cream contains a small amount of protein in addition to the milk fat component. The process of homogenization of fluid milk is one in which the milk fat globules are broken into sizes small enough that they will not rise in the milk to form cream under normal milk storage conditions. This is important for processing, storage and consumption of milk (Walstra and Jenness, 1984).

Milk Protein

The total protein component of milk is composed of numerous specific proteins. Milk's principal proteins are casein (about 80%) and the whey proteins lactalbumin and lactoglobulin. These proteins are complete in that they contain all the essential amino acids required by humans. Milk protein has a high coefficient of digestibility (87 to 90%). In addition, when combinations of foods are eaten at the same meal, milk's high quality protein complements the incomplete proteins. This high quality protein in milk is one of the key reasons why milk is such an important human food.

The primary group of milk proteins is the caseins. There are 3 or 4 caseins in the milk of most species. The different caseins are distinct molecules, but are similar in structure. Caseins have an appropriate amino acid composition that is important for growth and development of the nursing baby.

Casein is composed of several similar proteins that form a multi-molecular, granular structure called a casein micelle. In addition to casein molecules, the casein micelle contains water, salts (mainly calcium and phosphorous) and enzymes. The micellar structure of casein in milk is an important factor influencing the mode of digestion of milk in the stomach and intestine. It is also the basis for our ability to easily separate some proteins and other components from milk (Kalab and Comer, 1982).

Casein is one of the most abundant organic components of milk.

Individual molecules of casein alone are not very soluble in the aqueous environment of milk. However, the casein micelle granules are maintained as a colloidal suspension in milk. If the micellar structure is disturbed, the micelles may come apart and the casein may come out of solution, forming the gelatinous material of the curd. This is part of the basis for the formation of all non-fluid milk products.

Because the casein micelle is in suspension, it can be separated from the skim milk by centrifugation at a very high speed. If casein is removed, then by definition every other protein left in the milk preparation is a whey protein. Whey is composed of the water, lactose and soluble non-casein proteins (Fox, 1992).

Minerals

Minerals in milk include calcium, phosphorus, potassium, magnesium and zinc. The major minerals in milk are calcium and phosphorous. Milk is considered one of the best sources of calcium in the diet. Calcium and phosphorous strongly associate with the casein micelle structure. Consequently, whey has relatively little calcium and phosphorous compared with whole milk. Milk also contains most other minerals found in the human body.

Vitamins

Milk contains all the major vitamins. The fat-soluble vitamins A, D, E, and K are found primarily in the milk fat. Vitamins A and D are the major fat-soluble vitamins present in milk. Vitamins are also found in the nonfat component of milk. The B vitamins, thiamin, riboflavin, niacin and B12 are water-soluble vitamins present in milk. Milk is particularly an excellent source of riboflavin (B2), which regulates the body's production of energy from dietary fat, carbohydrate and protein.

Other Components in Milk

Fresh milk also contains some enzymes, gases, acids, lactic acid bacteria and other microorganisms as contaminants, etc. (Jensen, 1995).

II. HOMOGENIZATION

Objectives of Homogenization

Milk has countless fat globules that vary from about 0.1 to 15 micrometers in diameter. These fat globules will separate easily from the milk upon standing (especially in the milk fermentation container during the incubation period). The fat globules float to the top of the solution, as what is commonly called cream. The remaining liquid is skim milk that has virtually no fat globules. In fact, milk is an oil-in-water emulsion, with the fat globules dispersed in a continuous skim milk phase (Mulder and Walstra, 1974). Milk fat globules have a tendency to gather into clumps and rise to form a creamline on the surface due to their lighter density than skim milk (Anon., 1980). This is a useful property that makes it easy to separate fat from milk. In the manufacture of certain dairy products such as yogurt, this property is not desirable. Homogenization can effectively prevent the problem.

Effects of Homogenization

Homogenization is the process of breaking up and dispersing milk fat throughout the milk, resulting in a smooth, uniform texture. Conventional homogenization is a mechanical treatment of the fat globules in milk brought about by passing milk under high pressure through a tiny orifice,

which results in a decrease in the average diameter (usually less than 2 micrometers in diameter) and an increase in number and surface area of the fat globules.

The major result of homogenization, from a practical view, is a much-reduced tendency for creaming of fat globules. Three factors contribute to this enhanced stability of homogenized milk: a decrease in the mean diameter of the fat globules, a decrease in the size distribution of the fat globules (causing the speed of rise to be similar for the majority of globules such that they don't tend to cluster during creaming), and an increase in density of the globules (bringing them closer to the continuous phase) due to the adsorption of a protein membrane (Banks and Evans, 1983).

Theories of Homogenization

A combination of two theories, turbulence and cavitation, explains the reduction in size of the fat globules during the homogenization process. Turbulence dissipating in the liquid going through the homogenizer valve generates intense turbulent eddies of the same size as the average globule diameter. Globules are thus torn apart by these eddie currents reducing their average size. Cavitation appears to be due to considerable pressure drop with the change in velocity of the fluid. Liquid cavitates because its vapor pressure is attained. Cavitation generates further eddies that also produce disruption of the fat globules. Increased pressure

increases velocity. The high velocity gives liquid a high kinetic energy that creates eddies. Dissipation of this energy leads to a high energy density. The resulting diameter of fat globules is a function of energy density (Waltra, 1993).

Homogenizer

The typical homogenizer consists of a high-pressure pump (usually a three-cylinder piston) and homogenizing valve. The pump is turned by an electric motor through connecting rods and a crankshaft. In a typical homogenizer, large fat globules in milk entering at the bottom are sheared as they are pumped under pressure. The globules emerge at the top about one-tenth of their original size (Anon., 1980).

Conventional homogenizer valves operate at 10-20 MPa depending on the product. As it first enters the valve, the liquid velocity is about 4 to 6 m/s. It then moves into the gap between the valve and the valve seat, and its velocity is increased to 120 m/s in about 0.2 μ s. The liquid then moves across the face of the valve seat (the land) and exits in about 50 μ s. The homogenization phenomena is completed before the fluid leaves the area between the valve and the seat, and therefore emulsification is initiated and completed in less than 50 μ s. The whole process occurs between 2 pieces of steel in a steel valve assembly (Anon., 1981). The product may then pass through a second-stage valve similar to the first stage. While

most of the fat globule reduction takes place in the first stage, there is a tendency for clumping or clustering of the reduced fat globules. The second stage valve permits the separation of those clusters into individual fat globules.

Homogenization is an important processing step in the treatment of food and dairy products. It provides improved product stability, shelf life, digestion, and taste. Homogenizing effects can also significantly reduce the amount of additives required. This is especially the case with many dairy products such as yogurt.

III. YOGURT PRODUCTION

Introduction

Fermented dairy products include yogurt, sour cream, buttermilk and cottage cheese, etc. They all are prepared by adding bacterial cultures to pasteurized milk to convert the lactose into lactic acid or the citric acid into diacetyl. The reduction in pH results in coagulation of the casein.

Milk components play a very big role in these dairy products. Casein micelles, whey proteins and milk fat globules are the most important constituents of cultured milk products (Tamime and Robinson, 1985). These products may be divided into products based on proteins (yogurt

and cheeses) and products based on fat (various forms of cream including whipped cream, ice cream and butter).

Yogurt is a most popular fermented milk product due to its health and nutritional benefits. Its popularity has grown, and it is now consumed in almost all regions of the world. Although the consistency, flavor and aroma may vary from one region to another, the basic ingredients and manufacturing are essentially the same (Anon, 1980).

Yogurt is made from whole milk, partially skimmed milk or skim milk (with non-fat milk powder added sometimes to raise the content of milk solids). Flavorings, fruit or fruit juice may be added into yogurt. Yogurt is made by pasteurizing homogenized milk, cooling to suitable temperatures and inoculating it with acid producing bacterial cultures.

Yogurt Starter

In yogurt manufacture, two essential bacteria are *Streptococcus salivarius subsp. thermophilus* (ST) and *Lactobacillus delbrueckii subsp. bulgaricus* (LB). In the milk they convert lactose to lactic acid (Ayres, et al., 1980). The lactic acid works the proteins into such a network structure that the whole solution curdles. This process causes milk to gel and also gives yogurt its clean, tart flavor (Tramer, 1973).

The starter culture for most yogurt production is a blend of ST and LB. A ratio of 1:1 is normally used with an optimum fermentation temperature

of 43°C. This temperature is a compromise between the optimums for the two microorganisms (39°C for ST, 45°C for LB) (Mocquot and Hurel, 1970). Although they can grow independently, the rate of acid production is much higher in combination than when either of the two organisms is used individually. ST grows faster and produces both acid and carbon dioxide, which stimulates LB growth. On the other hand, the proteolytic activity of LB produces stimulatory peptides and amino acids for use by ST. These microorganisms are ultimately responsible for the formation of typical yogurt flavor and texture (Sharp, 1979). The yogurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yogurt mixture to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0 (Tamine and Deeth, 1980).

Traditional lactic acid bacteria are not the only bacteria added to yogurt. Increasingly, there also are some other bacteria stirred in. These bacteria are normal inhabitants of the human intestinal tract, and eating more of them may be good for the gut (Mcdonough, 1987). Usually, they are strains of *Bifidobacterium* (bifido for short) and *Lactobacillus acidophilus*. When present in yogurt, they're usually listed on the label of the product. Unlike the other starter cultures, which are killed by stomach acids, these bacteria move through the stomach and settle in the small and

large intestines (Hilton, et al. 1992). Their ability to flourish in that environment is what makes these cultures special. In the intestines, bacteria form a beneficial ecosystem which is a key to health (Scarpignato, 1995). *Acidophilus* and *bifido* may help suppress infection by crowding spaces where bad bacteria would otherwise attach themselves and by releasing acids that kill the invading germs.

Acidophilus and bifidobacteria maintain a healthy balance of intestinal flora by producing organic compounds—such as lactic acid, hydrogen peroxide, and acetic acid—that increase the acidity of the intestine and inhibit the reproduction of many harmful bacteria. (Kawase, 1982). These bacteria also produce substances called bacteriocins, which act as natural antibiotics to kill undesirable microorganisms (Barefoot and Klaenhammer, 1983).

Yogurt Manufacturing Method

There are two types of yogurt in terms of processing procedures: stirred style yogurt and set style yogurt (Anon, 1980). In making stirred style yogurt, the milk is clarified and separated into cream and skim milk, then standardized to achieve the desired fat content. The mixture is then homogenized using high pressures of 10-20 MPa at a temperature of 55-70°C (Tamime and Robinson, 1985). Besides thoroughly mixing the stabilizers and other ingredients, homogenization also prevents creaming

and wheying off during incubation and storage. Stability, consistency and body of the yogurt are enhanced by homogenization. After homogenization, the viscosity of yogurt is improved, and there is a change in water holding capacity of the milk proteins which tends to reduce syneresis (Grigorov, 1966). The increased amount of fat globule membrane material may also improve the water holding capacity of the coagulum (Samuelsson and Christiansen, 1978).

The mixture is then heated in a continuous plate heat exchanger for 30 min at 85°C or 10 min at 95° C. These heat treatments, which are much more severe than fluid milk pasteurization, are necessary to achieve a relatively sterile and conducive environment for the starter culture (Gimour and Rowe, 1981). Another important purpose of the heat treatment is to denature and coagulate whey proteins to enhance the viscosity and texture (Lyster, 1979).

Once the homogenized mix has cooled to an optimum growth temperature, the yogurt starter culture is added to a fermentation tank. A temperature of 43° C is maintained for 4-6 h under non-agitated conditions. The acid development of yogurt is carefully monitored until the pH is 4.0 to 4.7, depending on the product (Connolly, 1978). At this time, the fermentation tank is cooled with water to stop the fermentation. Stirring is begun to maintain a smooth, cream-like consistency. The coagulated

product is cooled to around 5-20°C, depending on the product. The various ingredients are then blended together in a mix tank equipped with a powder funnel and an agitation system (Tamime, and Robinson, 1985). Fruit and flavor may be incorporated at this time, and then the product is packaged, cooled and stored at refrigeration temperatures (5°C) to slow down the physical, chemical and microbiological degradation. Yogurt has a storage life of 2 to 4 weeks when properly refrigerated (Anon, 1980).

In making a set custard type (a semi-solid consistency) yogurt, the warm inoculated milk is put directly into consumer containers, incubated under controlled conditions, then cooled to less than 5°C (Tamime and Robinson, 1985).

IV. POWER ULTRASOUND

Introduction

Ultrasound, which is a branch of acoustics, deals with vibratory waves at frequencies above those of the hearing range of the average person. Ultrasonic waves are stress waves, and for this reason they can exist only within mass media. They differ from light and electromagnetic radiation waves that travel freely through a vacuum. In fact, ultrasound is termed

elastic waves since it is the elastic property of the medium which is responsible for the sustained vibrations required for ultrasonic wave propagation.

The sound range used can be divided basically into two ranges: diagnostic and power sound. In general terms ultrasound has two main applications: at low power and high frequencies, it can be used as an analytical tool (such as imaging and sonar and non-destructive evaluation), and at high power and low frequencies it can assist processing. The former does not change materials and will not be covered herein. This research is concerned only with changing materials with power ultrasound.

Power Ultrasound and Cavitation

Power ultrasound processing is unlike underwater sonar, fetal imaging, thickness or level measuring, or non-destructive testing, which is done at very high frequencies, far above human hearing. But power ultrasonic processing works just above human hearing at 20 to 40KHz (20,000 to 40,000 cycles per second). In ultrasonic processing, sound actually changes materials (Povey, 1997).

Ultrasonic processing applies high intensity sound to liquids, producing intimate mixing and powerful chemical and physical reactions. The process ("cavitation"), in effect, is "cold boiling" and results from the creation and collapse of countless micro bubbles in the liquid, producing

shock waves. This work entails “blasting” liquids, usually water, with very intense sound at high frequency (compared with human hearing). The cavitation process which plays a very important part in so many reactions is activated by ultrasonic energy (Kelly, 1990).

Ultrasonic cavitation is the formation of bubbles or cavities in liquids during the low-pressure portion of a wave cycle. The bubbles may be filled with gas coming out of solution in the liquid under reduced pressure or with the formation and collapse of cavitation bubbles. That makes the molecules bounce back, creating a “shock wave” which runs outward from the collapsed bubble (Coupland, et al. 1993). The shock waves can wear away metal. At the same time, free chemical radicals are produced, and even very tough metals are eroded. Where shock waves meet each other, they can cause more bubbles to occur and collapse, creating even more cavitation.

Applications of Power Ultrasound

Ultrasound processing is used for cleaning, to accelerate reactions, treat wastes and ores, disrupt biological cells, and homogenize and emulsify various products. The use of ultrasound during an extraction process allows a greater forced penetration of solvent into cellular materials (Contreas et al., 1992), improves mass transfer to and from

interfaces and disrupts biological cell walls on the surface and within the plant material to facilitate the release of the target material (Archer et al. 1996).

CHAPTER III

MATERIALS AND METHODS

I. HOMOGENIZATION EXPERIMENT

Experimental Design

The purpose of homogenization is to subdivide the fat globules and clumps in milk to such a small size that they will no longer rise to the top of the milk as a distinct layer in the time before the milk is normally consumed. To test the efficiency of ultrasound homogenization, a microscope was used to check the fat globule size distribution. A conventional homogenizing method was used as a standard reference to compare the effect of ultrasound homogenization. Ultrasound amplitude level and exposure time were studied as independent variables against homogenization effectiveness.

Materials

Fresh cow milk (a mixture of Jersey and Holstein cow milk) was obtained from the dairy farm at the University of Tennessee, Knoxville. Before the homogenization treatment, milk was pasteurized to kill bacterial contaminants while not substantially altering the milk characteristics.

Heating just above 90°C for 15 seconds results in killing all vegetative microorganisms and inactivating most enzymes.

Homogenization by Conventional Homogenizer

A Schroder L100 homogenizer (Wilh. G. Schroder Nachfolger GmbH., Germany) was used to homogenize the whole milk under high pressure (12,410 kPa at 60°C).

Ultrasound Homogenization

An ultrasound generator (500w nominal power at 20KHz and 100 amplitude-cycles) with a 13 mm diameter probe (Model CP502, Cole-Parmer Instrument Company, U.S.) was used to homogenize 150ml milk for each trial. The ultrasound probe was immersed into the milk sample about 2.5 cm. The amplitude levels selected were 20, 50 and 100 respectively. Exposure times were 1, 6 and 10 minutes. All amplitude level and exposure time combinations were used. Therefore, there was a total of 9 treatments, which were denoted as 20/1(1 min at amplitude level 20), 20/6 (6 min at amplitude level 20), etc..

Sampling

Samples of all homogenized milk were taken without entrapping air. All samples were directly cooled to room temperature (about 20°C) and analyzed. Before analyzing, the sample was gently shaken. This

procedure was developed to prevent changes in fat globule size from taking place.

Microscope Method

A standard microscope with an oil immersion lens (magnification 1000 x) (Model Standard 14, Zeiss com., Oberkochen, Germany) was used. An eyepiece disc was ruled in such a manner that the fat globules could be measured in 0.5 micron increments. Slides and cover slips were cleaned to remove dust and other particles.

A very small drop of homogenized milk was placed onto the slide, and the cover slip was placed on top (pressed very slightly so that fat globules could still rise). A camera (Model 80, Zeiss com., Oberkochen, Germany) was attached to the microscope and used to take photographs of the samples. Fat globule sizes and distribution were estimated from the photographs.

II. FERMENTATION EXPERIMENTS

Experimental Design

To evaluate the ultrasound effects on yogurt fermentation, the total experiment had seven treatments (from preliminary experiments, the

optimum experimental conditions were pre-determined), which were divided into the following three categories:

Control (1 treatment)---conventional yogurt manufacturing method;

Ultrasound-homogenized milk (3 treatments)—ultrasound was applied before addition of inoculated yogurt starter including 20 amplitude level for 6 min (20/6), 50 amplitude level for 6 min (50/6), 100 amplitude level for 6 min (100/6);

Ultrasound-homogenized inoculated milk (3 treatments)—ultrasound was applied to inoculated milk before fermentation including 40 amplitude level for 8 min (M40/8), 60 amplitude level for 8 min (M60/8) and 100 amplitude level for 8 min (M100/8).

The assigned experimental numbers for those seven treatments was: (1) 20/6; (2) 40/6; (3) 100/6; (4) control; (5) M40/8; (6) M60/8; (7) M100/8. The pH change during fermentation, water holding capacity, viscosity and syneresis, of yogurt were measured and compared among treatments. The experiment was run in triplicate for statistical analysis.

Materials

Fresh raw milk (mixture of Jersey and Holstein cow milk) was acquired from the dairy farm at the University of Tennessee, Knoxville. Plain yogurt was purchased from the local grocery store and used as the yogurt starter culture. It contained live yogurt cultures (*Streptococcus thermophilus*,

Lactobacillus bulgaricus, *Bifidobacterium* and *Lactobacillus acidophilus*).

Before the yogurt starter culture was added it was well stirred.

An ultrasound generator (500w nominal power at 20KHz and 100 amplitude-cycles,) with a 13 mm diameter probe (Model CP502, Cole-Parmer Instrument Company, U.S.) was used to sonicate 150ml milk for each trial. The ultrasound probe was immersed into the milk sample about 2.5 cm. A Schroder L100 homogenizer (Wilh. G. Schroder Nachfolger GmbH., Germany) was used to homogenize the whole milk under high pressure (12,410 kPa at 60°C). Seven Salton yogurt jars were prepared to hold yogurt samples.

To prevent the presence of unwanted bacteria, all yogurt-making equipment and containers were thoroughly washed, and containers were boiled in water and air-dried. The ultrasound probe (diameter 13mm) was put into boiling water for 2 minutes and air-dried.

Milk sample preparation

Control

Fresh raw milk was milk was pasteurized to kill bacterial contaminants while not substantially altering the milk characteristics. Heating just above 90°C for 15 seconds results in killing all vegetative microorganisms and inactivating most enzymes. A Schroder L100 homogenizer (Wilh. G.

Schroder Nachfolger Gmbh., Germany) was used to homogenize the pasteurized milk under high pressure (12,410 kPa at 60°C).

After homogenization, milk was heated to about 95°C, stirred gently and held for another 10 minutes. The milk was then cooled rapidly to 20°C with cold water so that it would have the same starting conditions as the ultrasound treated samples. 150g milk was placed into a clean Salton yogurt jar, and then 30g yogurt starter were blended in. The jar was covered and placed in a water bath at 43°C for the 4.5-hour incubation.

Ultrasound-homogenized group

Fresh raw milk was heated to about 95°C, stirred gently and held for another 10 minutes. The milk was then cooled rapidly to 15°C with cold water. 150g milk was removed individually into each of 3 clean Salton yogurt jars. The ultrasound generator (20K, 500w, Cole Parmer) was used to homogenize 3 jars with 150g pasteurized milk individually at 20, 50 and 100 amplitude levels respectively (total 100 amplitude cycles) for 6 minutes in a water bath at 15°C (to offset a slight temperature increase due to ultrasounic cavation). Milk samples were then blended with 30g yogurt starter, and jars were covered and placed in the water bath at 43°C for the 4.5-hour incubation.

Ultrasound-homogenized inoculated milk

Fresh raw milk was heated to just before boiling, stirred gently and held for another 10 minutes. The milk was then cooled rapidly to 15°C with cold water. Milk samples (150g) were removed individually into each of 3 clean Salton yogurt jars. The milk samples were then blended with 30g yogurt starter. Ultrasound treatments were applied to the inoculated milk in a water bath at 15°C (to offset temperature increase due to ultrasonic cavitation) before fermentation. Treatments included 40 amplitude level for 8 min (M40/8), 60 amplitude level for 8min (M60/8) and 100 amplitude level for 8 min (M100/8). Samples were covered and placed in a water bath at 43°C for the 4.5-hour incubation.

After incubation, untested samples were refrigerated immediately at 5°C. Rapid cooling slowed down the production of acid.

Methods

pH testing

A pH meter (model 35624-30, Oakton, Singapore) was used to measure the pH values during fermentation (0.01 pH resolution, +/- 0.02 pH accuracy).

Calibration of pH meter:

At room temperature, the electrode was immersed into the standard buffer (pH=7) about 2.5 cm, stirred gently, and the displayed value was

read after 2 minutes. The “hold” button was pushed to confirm and finish the calibration. The same procedure was repeated with the pH 4 buffer, and then the pH 10 buffer.

Testing milk pH value:

Before starting the fermentation, the pH was measured for all 7 jars of milk. Then the values were measured every half-hour until the end the fermentation (total incubation time was 4.5 hours) for a total of 10 measurements.

After taking a reading, the electrode was rinsed immediately afterwards with deionized water to remove any residue.

Water Holding Capacity

Water holding capacity was measured by the centrifuge method (Parnell-Clunies et al, 1986). Within 12 hours of production, a 5-g sample was centrifuged at the highest power level—power level 7 (total 7 duty cycles) with the IEC centrifuge (115V, 50/60 Hz and 1.2 Amp) (Damon/IEC Division, Needham Hts., Mass.) for 30 minutes at 10°C. The supernatant was removed, and the pellet weight was recorded. Water holding capacity was calculated as (pellet weight/initial sample weight)*100.

Viscosity testing

Viscosity was measured in terms of flow ability as developed by Storgards (1964). A funnel (upper inside diameter = 12.5 cm, lower inside

diameter = 8mm), was marked 8cm below the upper orifice. Within 48 hours of production, the yogurt sample was stirred for one minute and then 40ml were placed in the funnel. The time required to reach the mark at the discharging hole was recorded.

Syneresis testing

The method of Mohamed and Morris (1987) was used to measure the syneresis of fermented milk. Within 24 hours after fermentation, a Whatman number 1 filter paper (Whatman Ltd., Maidstone, England) was used to cover a 17-cm diameter Buchner funnel (Coors Ceramicon Designs, Golden, CO.). Yogurt (20g) was spread in a thin layer over the surface. The sample was then vacuum filtered for 10 minutes. The liquid from the yogurt was collected and recorded. The syneresis was calculated as $(\text{liquid weight}/\text{initial sample weight}) \times 100$.

Statistical Analysis

The experiment was replicated three times. Data were recorded and analyzed using SAS (SAS institute Inc., 1990). The pH measurements were analyzed as a randomized block design with seven different treatments (20/6, 50/6, 100/6, control, M40/8, M60/8 and M100/8). The water holding capacity, viscosity and syneresis were analyzed as a completely randomized design with seven different treatments (20/6, 50/6,

100/6, control, M40/8, M60/8 and M100/8). Treatment differences were determined by the Fisher's protected mean comparison ($p < 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

I. THE EFFECT OF HOMOGENIZATION

The effect of ultrasound homogenization was studied. Homogenization efficiency was examined by observing the size of fat globules under the microscope (Emery, 1953). In this study, microscopic observations were sufficient to achieve the experimental objectives: To determine whether ultrasound can produce uniform solutions and dispersions with size-reduced fat globules in comparison to conventional homogenization, and to select the proper amplitude levels for the fermentation experiments.

The ultrasound wave does not directly induce vibration of the globules, but produces intense cavitation and consequently liquid bubble disruption. The power levels for ultrasonic homogenization were not determined in these experiments because of the operating mode of the sonicator. The AMPLITUDE control allowed the ultrasonic vibrations at the probe tip to be set to any desired level, and the power was automatically adjusted to maintain the amplitude at the pre-selected level. Thus the amplitude level also reflects the energy needed by the treated samples. An adequate homogenization effect will occur beyond a specified

amplitude level and exposure time combination. Longer exposure times can produce smaller fat globule sizes similar to repeated passes with a conventional homogenizer. Hence longer exposure time was expected to induce a better effect.

Microscopic observations were mainly to see if the fat globules were broken up finely enough (Emery, 1953). The microscope also allows one to check for fat globule clusters or other abnormalities (Walstra, 1975).

Figure 1 shows that the milk homogenized by the conventional homogenizer had many more fat globules than the milk without homogenization (Fig. 2). From these figures, the average diameter of fat globules was estimated. In Fig1, the total fat globules had an average diameter equal to $2\mu\text{m}$ or less, which was far less than the average size of fat globules (approximately $5\mu\text{m}$) in Fig.2.

As shown in Fig. 3, Fig. 4 and Fig. 5, the use of ultrasound at amplitude level 20 for 10 min (20/10) (Fig. 5) resulted in a smaller average diameter of fat globules than the samples homogenized at the same amplitude level for 6 min and 1 min (20/6 in Fig. 3 and 20/1 in Fig. 4). In Fig 5, there are only two or three fat globules greater than $5\mu\text{m}$ in size, but in Fig. 2, there are numerous fat globules larger than $5\mu\text{m}$. However, overall Fig. 3 and Fig. 4 showed almost the same fat globule distribution as

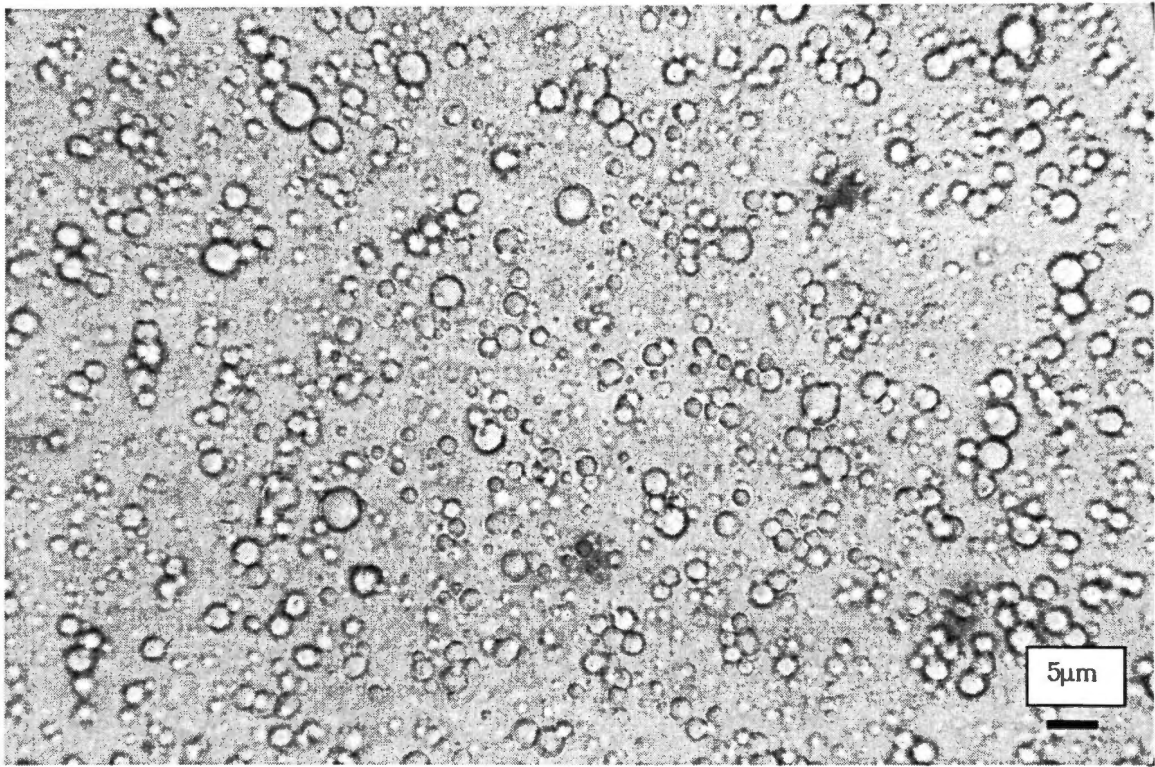


Fig. 1 Micrograph of cow milk homogenized with a conventional homogenizer under 12,410 kPa at 60°C

Magnification 1000 x under light microscope

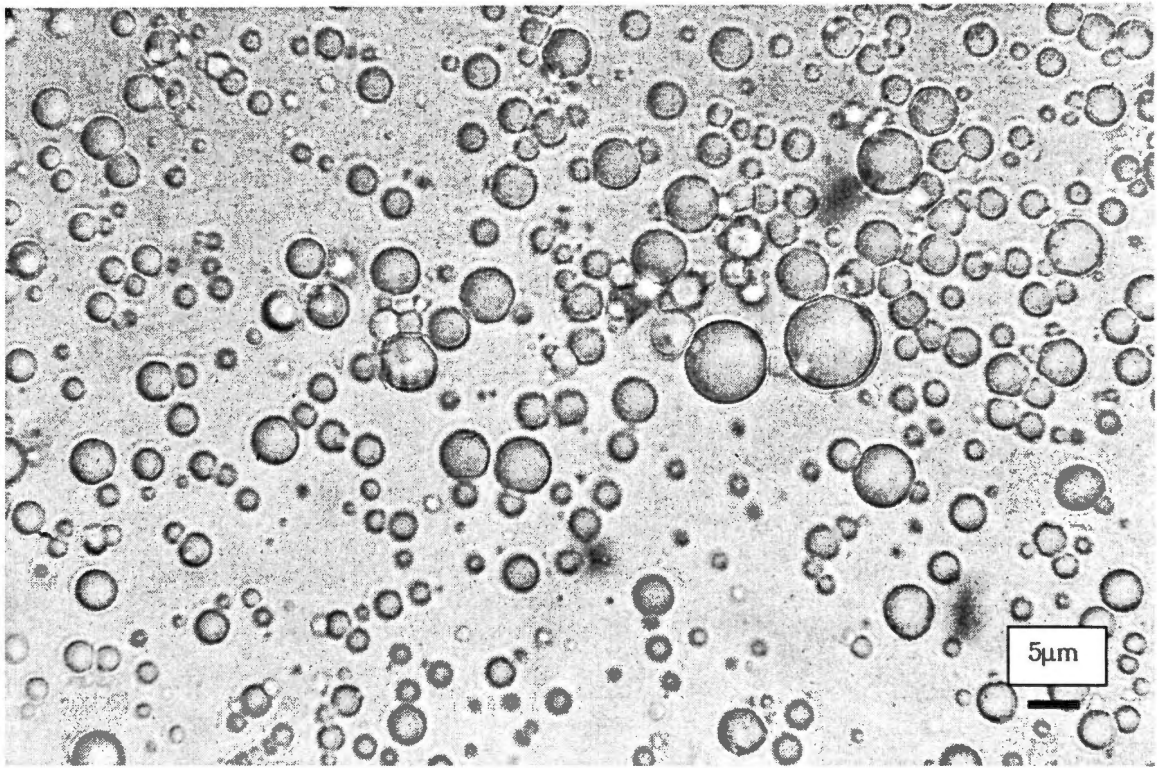


Fig.2 Micrograph of cow milk without homogenization

Magnification 1000 x under light microscope

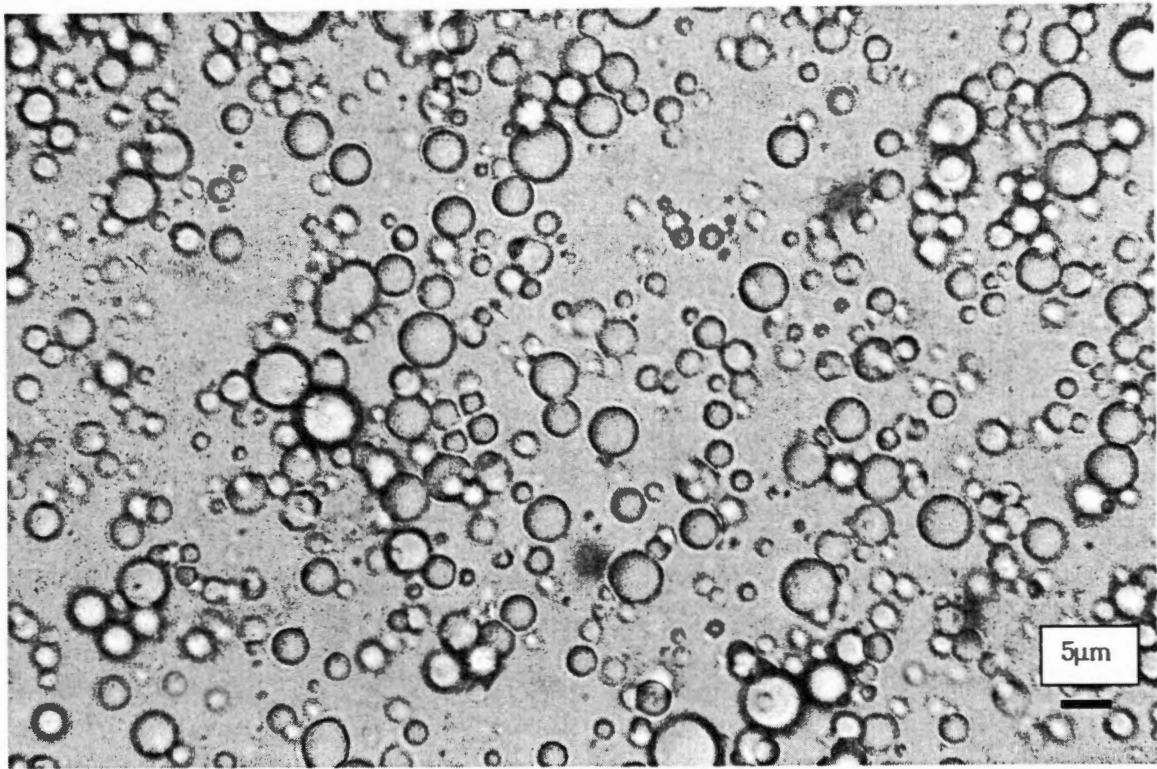


Fig. 3 Micrograph of cow milk homogenized with ultrasound at amplitude level 20 for 1 min

Magnification 1000 x under light microscope

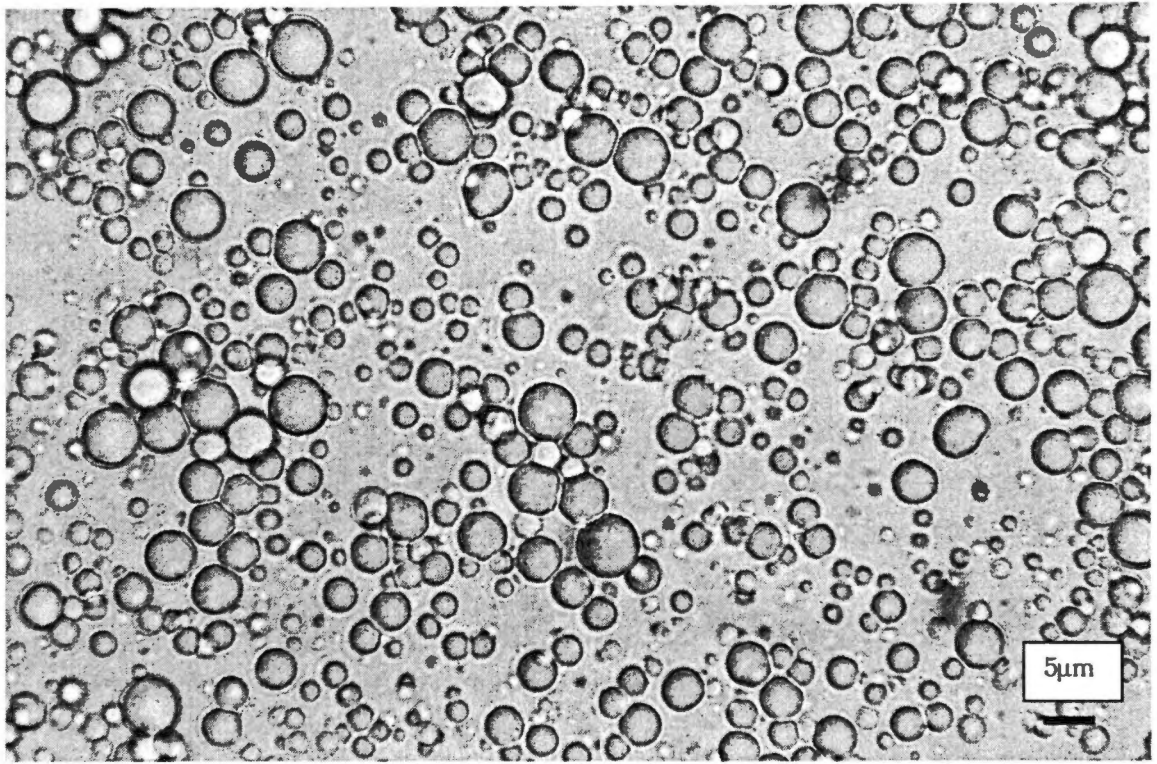


Fig. 4 Micrograph of cow milk homogenized with ultrasound at amplitude level 20 for 6 min

Magnification 1000 x under light microscope

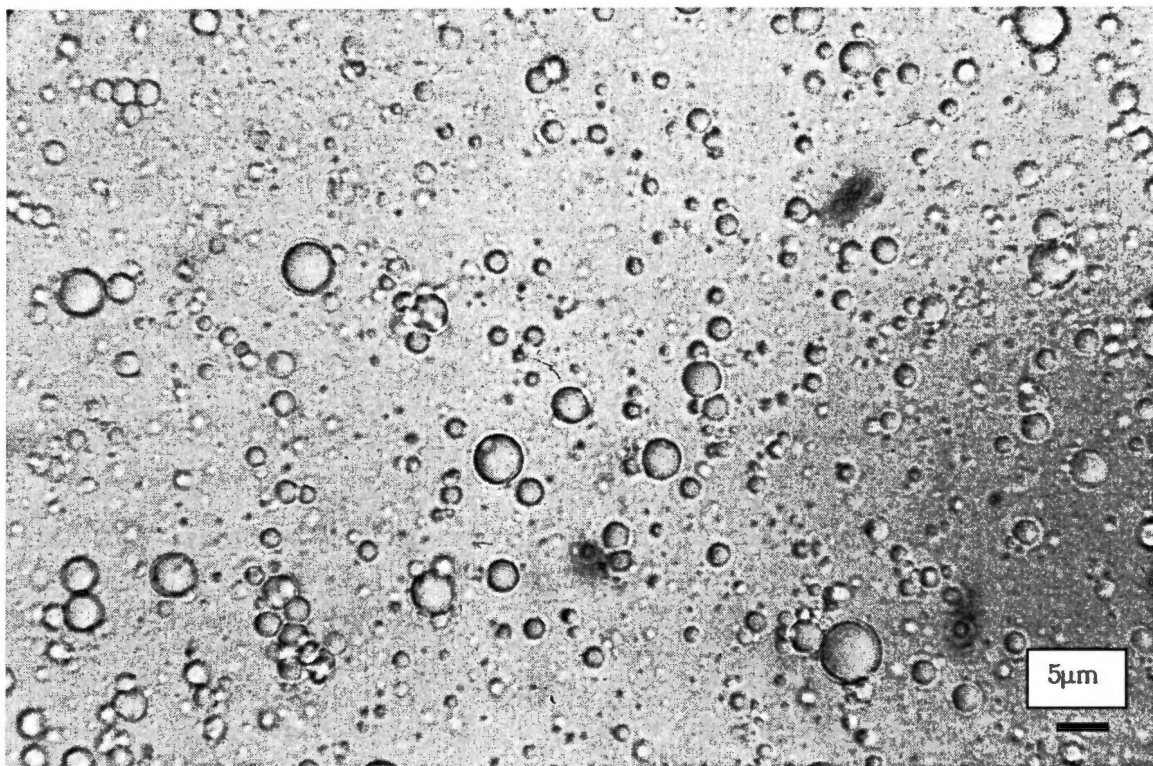


Fig. 5 Micrograph of cow milk homogenized with ultrasound at amplitude level 20 for 10 min

Magnification 1000 x under light microscope

that of non-homogenized milk (Fig. 2). In other words, the only beneficial effect was to break some of the larger fat globules. The (20/1) and (20/6) treatments were clearly not as effective as conventional homogenization (Fig. 1). However, the (20/10) treatment resulted in a fat globule size distribution similar to that for conventional homogenization.

Similarly, at amplitude levels 50 and 100, the figures (from Fig.6 to Fig.11) showed that the longer exposure time induced smaller sized fat globules and more uniform size distributions.

At amplitude level 50, the three different exposure time treatments showed considerable differences in the effect of homogenization. For the 1-min exposure time (Fig. 6), the fat globules had almost no effect on the fat globule size distribution compared with non-homogenized milk (Fig. 2). But for the 6-min exposure time, only a few fat globules were larger than $2\mu\text{m}$, and most of them were smaller than $2\mu\text{m}$. For the 10-min exposure time, the fat globules were dispersed very finely and disrupted very thoroughly. This was much better than the control group (Fig.1).

At amplitude level 100 even a 1-min exposure time (Fig. 9) worked adequately. The fat particle sizes were similar to the control (Fig. 1). For 6-min and 10 min exposure times (Fig.10 and Fig. 11), milk fat globules

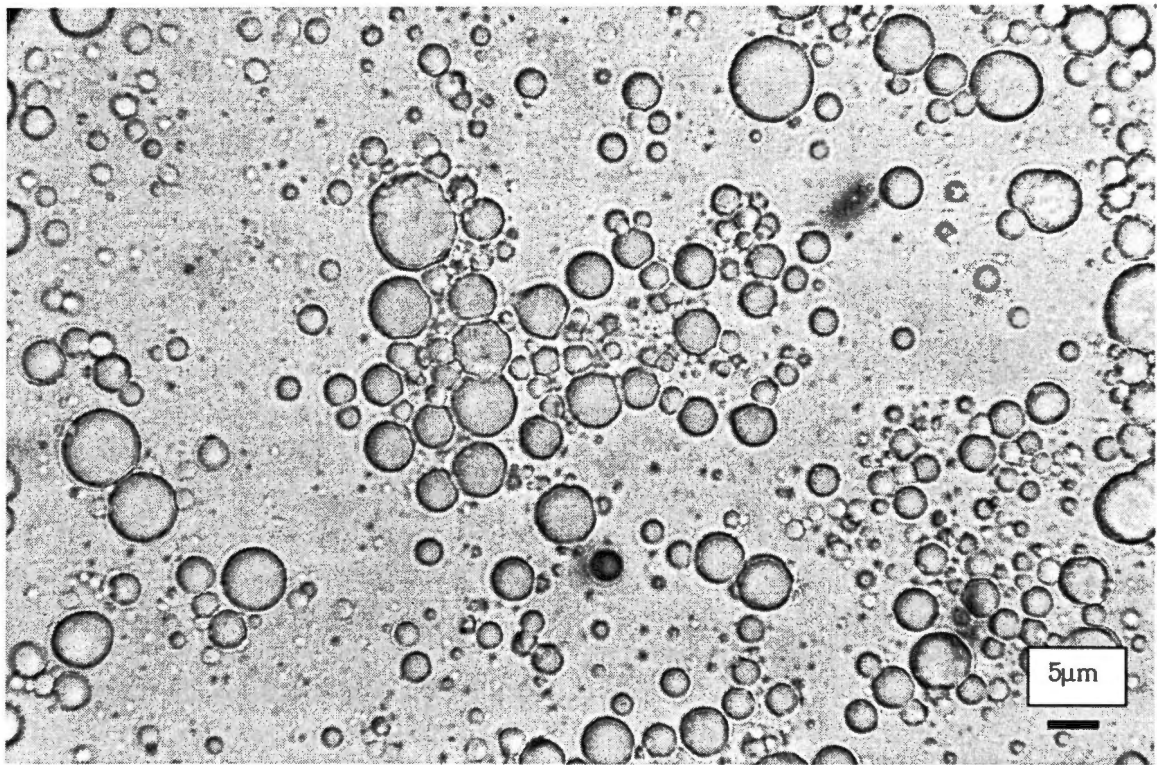


Fig. 6 Micrograph of cow milk homogenized with ultrasound at amplitude level 50 for 1 min

Magnification 1000 x under light microscope

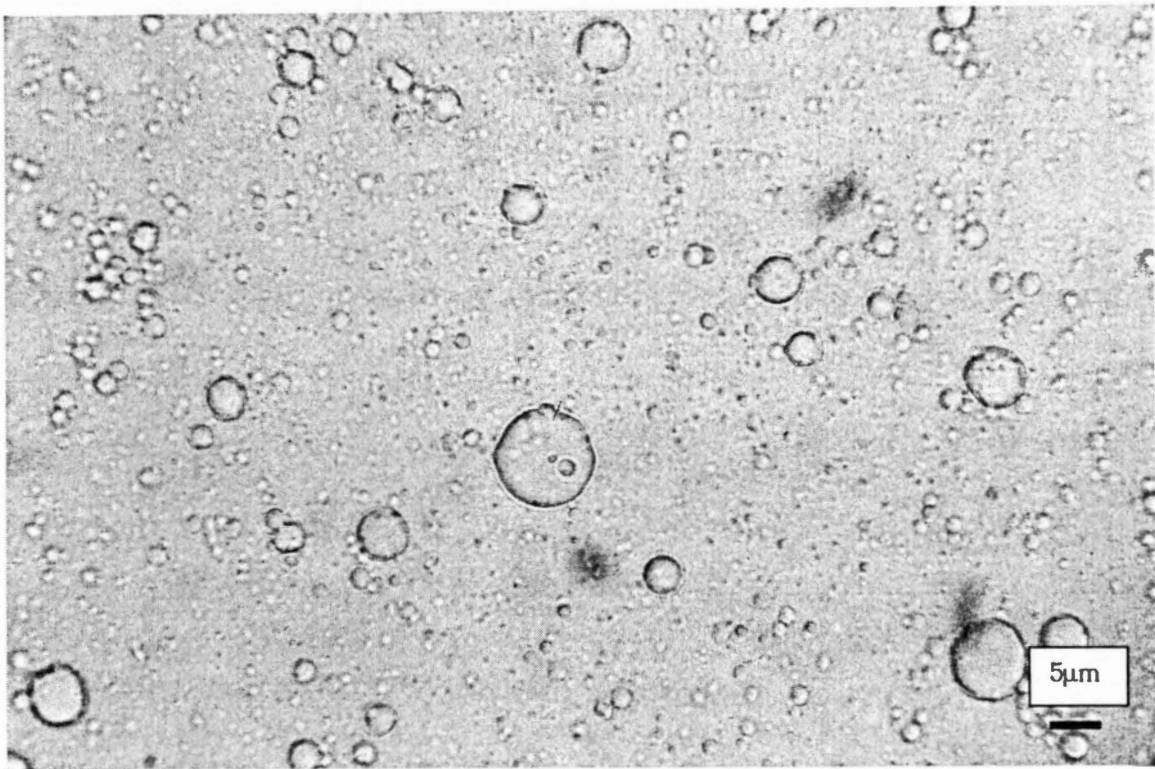


Fig. 7 Micrograph of cow milk homogenized with ultrasound at amplitude level 50 for 6 min

Magnification 1000 x under light microscope

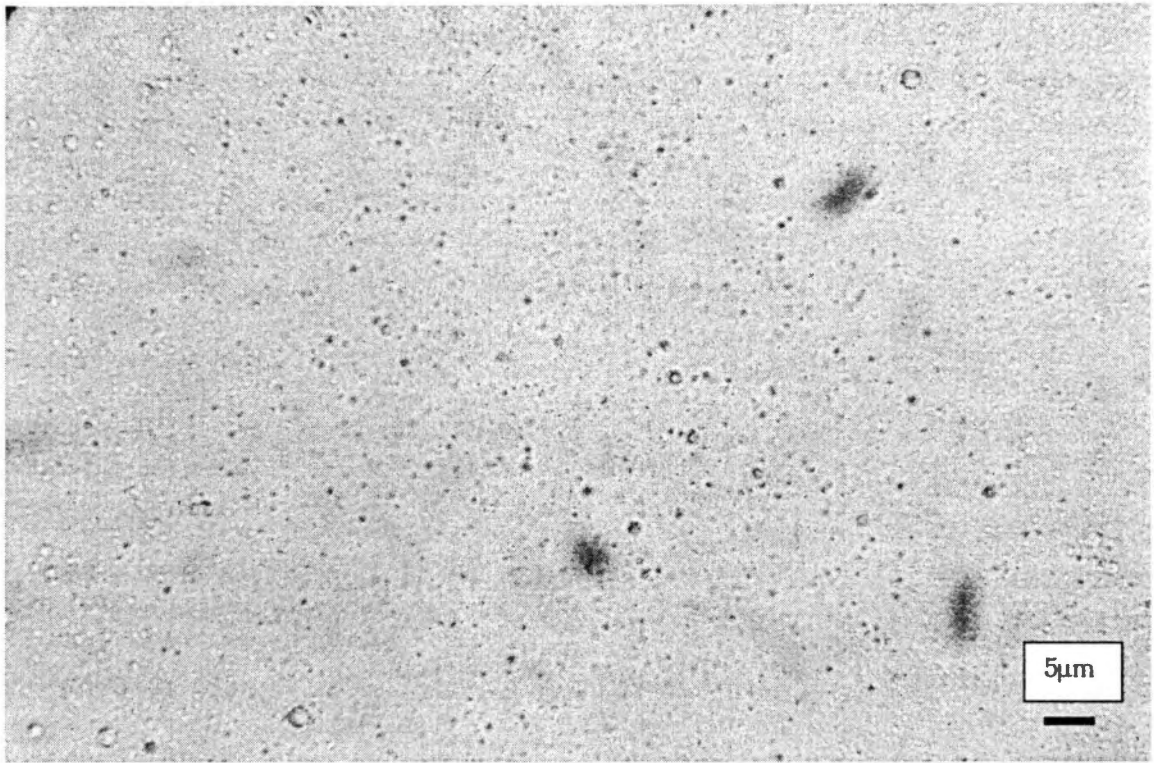


Fig. 8 Micrograph of cow milk homogenized with ultrasound at amplitude level 50 for 10 min

Magnification 1000 x under light microscope

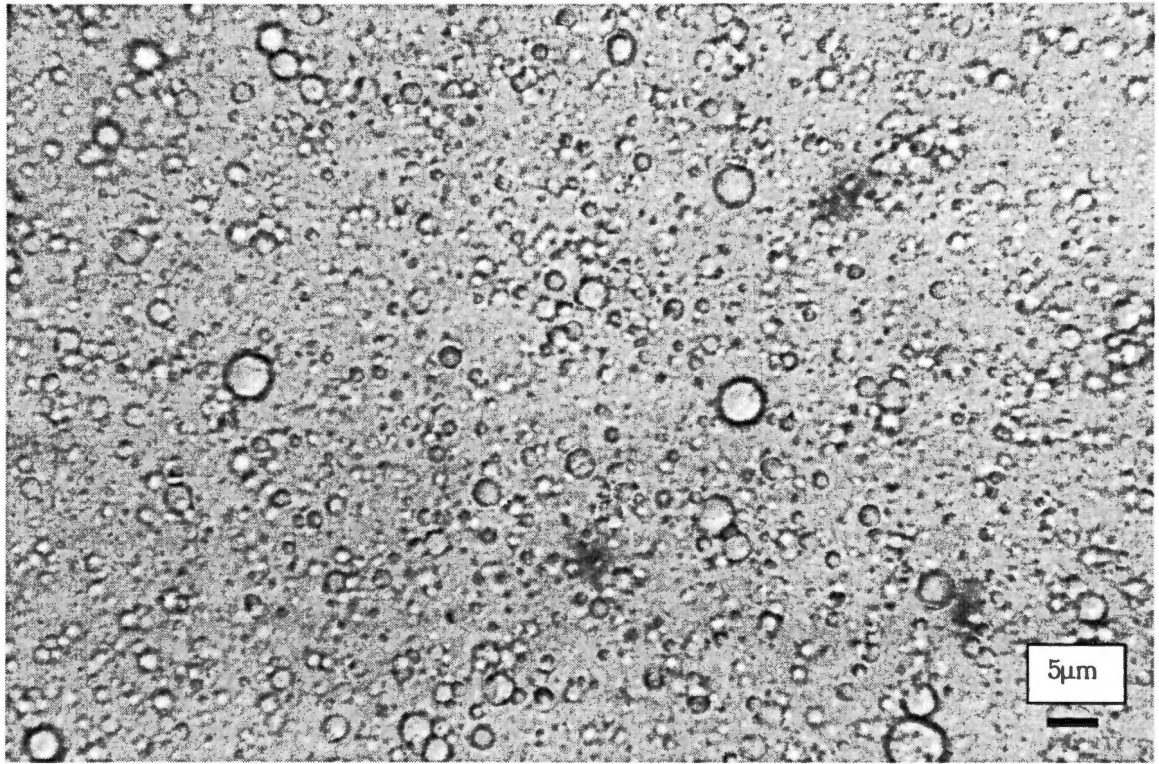


Fig. 9 Micrograph of cow milk homogenized with ultrasound at amplitude level 100 for 1 min

Magnification 1000 x under light microscope

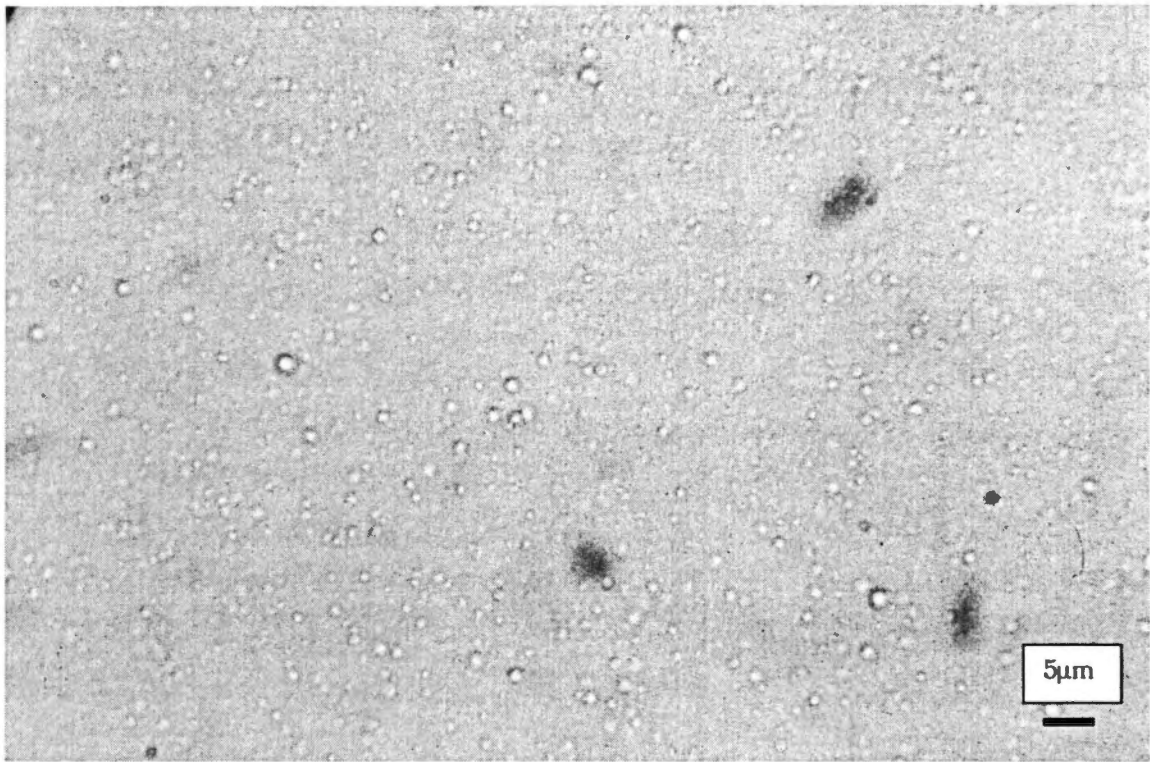


Fig. 10 Micrograph of cow milk homogenized with ultrasound at amplitude level 100 for 6 min

Magnification 1000 x under light microscope

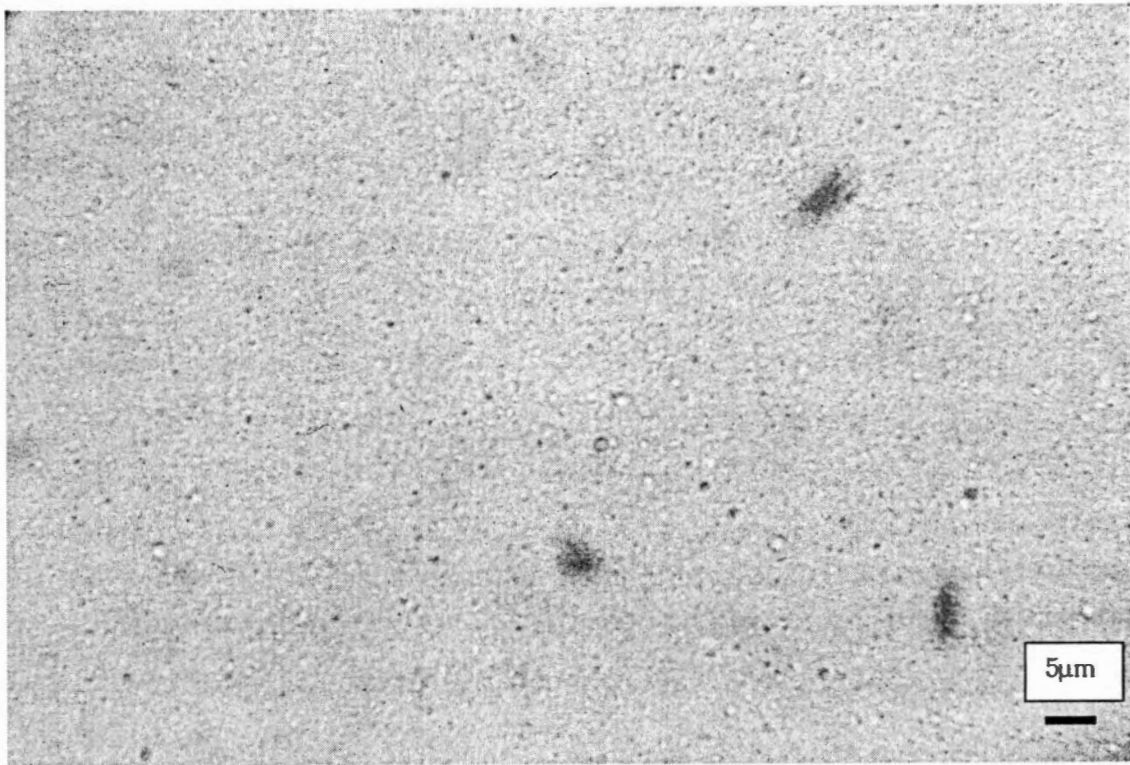


Fig. 11 Micrograph of cow milk homogenized with ultrasound at amplitude level 100 for 10 min

Magnification 1000 x under light microscope

were broken to very fine fragments. It is difficult to observe the fragments in the pictures.

In Fig. 4, Fig. 7 and Fig. 10 (treatments 20/6, 50/6 and 100/6), the samples had the same exposure time (6 min). The fat globule size distribution shows that larger amplitude levels induce smaller size fat globules at the same exposure time. At the 1-min exposure time (20/1 in Fig. 3, 50/1 in Fig. 6 and 100/1 in Fig. 9) 20/1 and 50/1 hardly altered the fat globule sizes, but 100/1 had a much better effect. In Fig. 5, Fig. 8 and Fig. 11, the 20/10, 50/10 and 100/10 treatments produced extremely small fat globules (much smaller than $2\mu\text{m}$) and uniform dispersion in Fig. 8 and Fig. 11.

From the above, as expected, it was found that ultrasound has a very good homogenization effect at high amplitude levels compared with conventional homogenization. Low amplitude levels did not have efficient homogenization effects. Longer exposure time can improve the ultrasound homogenization effect. If enough exposure time and amplitude level are provided, extremely small fat globules are produced. The results imply that the ultrasound-homogenizing effect increases with increasing amplitude level, but energy consumption should also be considered.

In this study, all samples treated at amplitude level 100 had a good homogenizing effect. Compared with the control sample, samples treated

at amplitude level 20 had a poor homogenizing effect. 50/1 had a poor effect, but 50/10 had a very good homogenizing effect. 50/6 had a fair effect in between that of 50/1 and 50/10. For the fermentation experiments, 20/6, 50/6 and 100/6 were selected to produce samples with poor homogenization, fair homogenization and good homogenization, respectively.

II. THE EFFECTS ON FERMENTATION

The pH Changes

The pH decline curves (Fig. 12) show difference between the sonicated mixture (M40/8, M60/8 and M100/8) and the control. The means of pH measurements are shown in Table 1.

From the pH decline curves (Fig. 12), all ultrasound-homogenized groups (20/6, 50/6 and 100/6) and the control group overlapped each other during the first 3.5 hours of fermentation. No significant difference was found at that time. During the last hour of fermentation, a statistical difference was found between 100/6 and the control group. During the last half-hour, the pH value of 50/6 also was statistically different from the control group. Overall, all ultrasound-homogenized groups (20/6, 50/6 and 100/6) showed a pH change tendency similar to that of the control group.

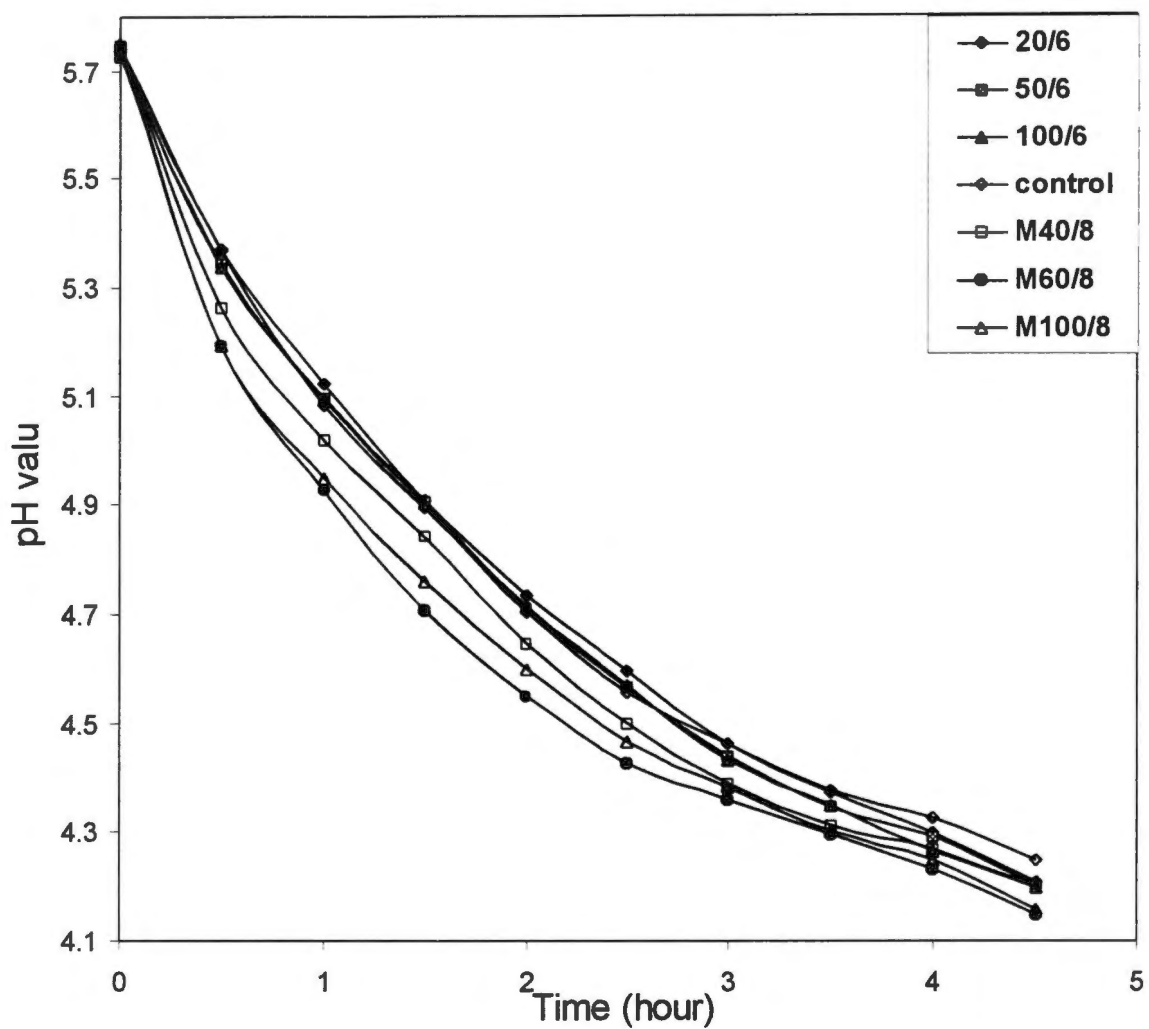


Fig.12 The pH decline curves for milk undergoing ultrasonic treatment before addition of starter culture (20/6, 50/6, 100/6), or after addition of starter culture (M40/8, M60/8, M100/8)

Table 1. Means* of pH measurements for milk undergoing ultrasonic treatment before addition of starter culture (20/6, 50/6, 100/6) or after addition of starter culture (M40/8, M60/8, M100/8)

Time	20/6	50/6	100/6	Control	M40/8	M60/8	M100/8
0.0 h	5.74 a	5.73 a	5.73 a	5.75 a	5.75 a	5.74 a	5.75 a
0.5 h	5.37 a	5.34 a	5.34 a	5.37 a	5.26 b	5.19 c	5.19 c
1.0 h	5.12 a	5.10 a	5.09 a	5.08 a	5.02 b	4.92 c	4.95 c
1.5 h	4.91 a	4.90 a	4.90 a	4.89 a	4.84 b	4.71 d	4.76 c
2.0 h	4.73 a	4.71 a	4.71 a	4.70 a	4.65 b	4.55 d	4.60 c
2.5 h	4.60 a	4.57 a	4.57 a	4.56 a	4.50 b	4.43 c	4.47 bc
3.0 h	4.46 a	4.44 a	4.43 a	4.46 a	4.39 b	4.36 b	4.38 b
3.5 h	4.37 a	4.34 ab	4.35 ab	4.37 a	4.31 bc	4.29 c	4.30 c
4.0 h	4.30 ab	4.29 ab	4.26 bc	4.32 a	4.27 bc	4.23 c	4.25 c
4.5 h	4.21 ab	4.20 b	4.20 bc	4.25 a	4.20 b	4.15 d	4.16 cd

*Means are significantly different from the means with the different letter in the same row at $p < 0.05$.

Therefore these treatments would not result in an appreciative reduction in fermentation time.

For all groups of sonicated inoculated mixture (M40/8, M60/8 and M100/8), the pH curves (Fig. 12) show significant differences after only 0.5 hours of fermentation. They all had a faster acid development than the control. After 1.5 hours of fermentation, M60/8 had the biggest pH-drop. M100/8 also showed a relatively rapid drop in the pH. All treatments eventually reached the desired pH, but the M40/8, M60/8 and M100/8 treatments would allow a shorter fermentation time to be used. This could increase productivity and potentially reduce operating costs.

M40/8, M60/8 and M100/8 showed a faster acid development than the other groups. The results show that high power ultrasound did not inactivate the yogurt bacteria, and somehow stimulated the acid development. The results also showed that only the proper power level could cause the optimum pH development. A reasonable explanation for stimulating the acid development is a release of intracellular enzyme from the yogurt bacteria cells due to sonication. The catabolism of lactose by yogurt bacteria takes place inside the cell, and hence the initial step is to transport the lactose molecule across the cell wall membrane. The transport of lactose is mediated by the enzyme β -galactosidase which hydrolyses the lactose inside the cell (Tamime and Robinson, 1985). The

ultrasound cavitation caused the intracellular β -galactosidase to be released from the lactic bacteria cells. The released β -galactosidase showed a higher lactose hydrolysis activity than that in the cell, and hence more lactic acid is produced (Wang et al., 1995).

During the entire fermentation, all groups of sonicated mixture (M40/8, M60/8 and M100/8) were significantly different than the control in terms of acid development. This method could reduce the total fermentation time by 0.5 hours.

Water Holding Capacity

The results of the Water Holding Capacity experiments and statistical analysis (Fig. 13) showed that all six groups with ultrasound treatment were significantly different from the control. Higher amplitude levels (50/6, 100/6, M40/8, M60/8, and M100/8) resulted in a higher water holding capacity. Those groups had a higher water holding capacity than the control. However, the M40/8 treatment resulted in only a slight increase in water holding capacity.

Use of lower amplitude levels (20/6 and M40/8) had a less beneficial effect on the water holding capacity. In fact the 20/6 treatment resulted in a lower water holding capacity than the control. The result from the 20/6 treatment was expected because of the poor homogenization in the previous study.

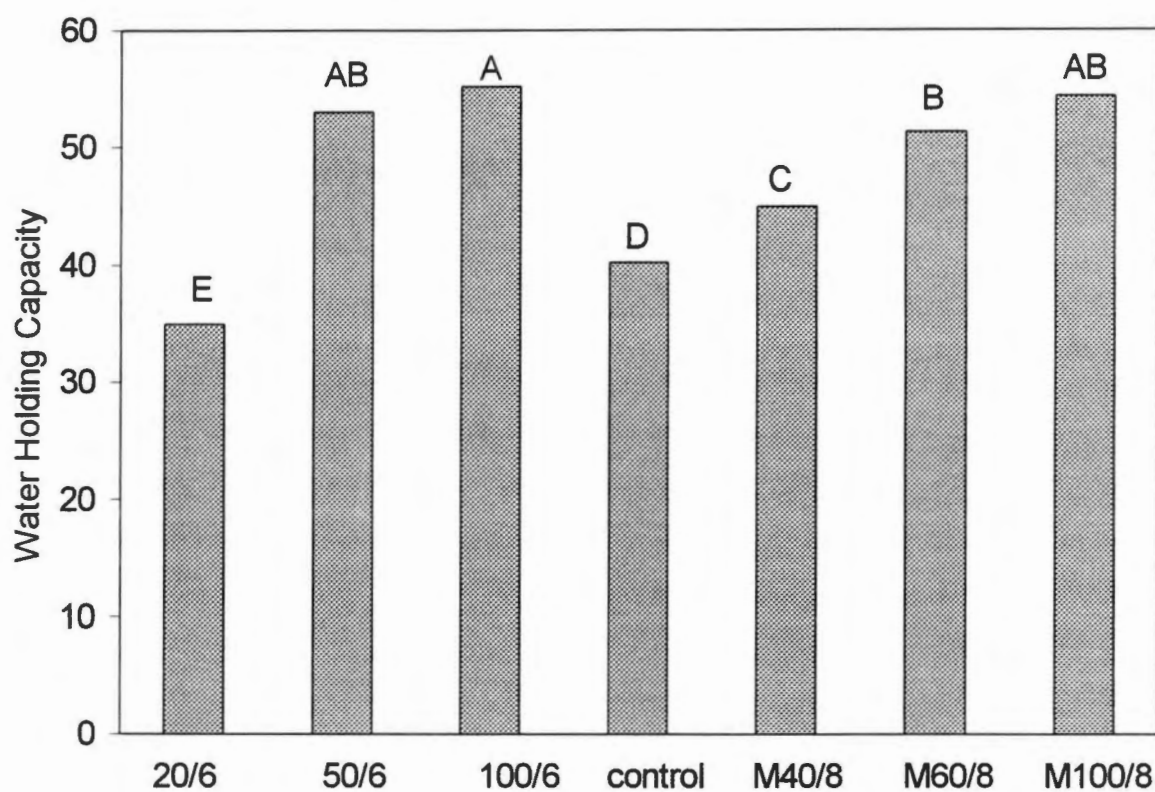


Fig. 13 Water holding capacity of yogurt which was pretreated with ultrasound before (20/6, 50/6, 100/6) or after (M40/8, M60/8, M100/8) addition of starter culture

The results showed that ultrasound can significantly change the water holding capacity when it is applied either before or after inoculation with starter culture. All treatments had the same protein content before incubation, so the fat globules in the milk must play an important role. Ultrasound cavitation produced sized-reduced fat globules, hence total fat membrane surface area increased. The increased amount of membrane included a lot of new-bonded casein, which would be hydrophilic. This should be the main reason why all the high-amplitude ultrasound treatments increased the water holding capacity. This explanation agrees with the research of Kinsella (1984), which concluded water holding capacity is attributed to the ability of the proteins to retain water within the yogurt structure.

Viscosity

The results of the viscosity experiments and the statistical analysis are shown in Fig. 14. All 4 middle and high amplitude level groups (50/6, 100/6, M60/8, and M100/8) showed significant difference from the control. They all had a higher viscosity than the control. The viscosity of yogurt increased as ultrasound level increased. Low amplitude level groups had no significant difference from the control group.

From the above, it was concluded that ultrasound can significantly change the viscosity of yogurt whether it is applied before or after

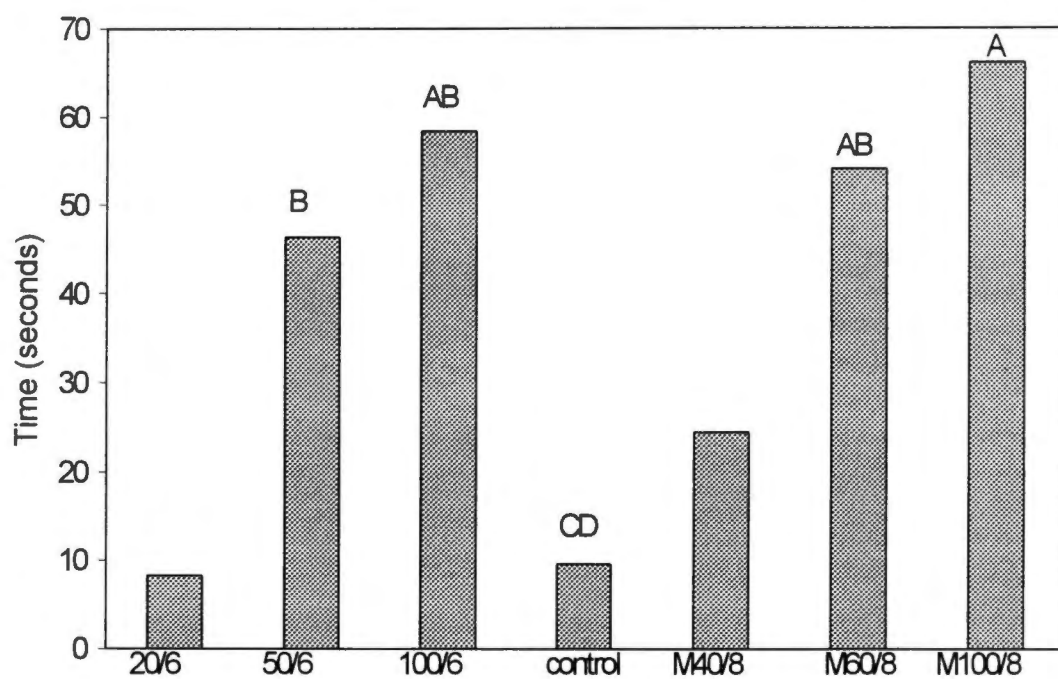


Fig. 14 Viscosity of yogurt which was pretreated with ultrasound before (20/6, 50/6, 100/6) or after (M40/8, M60/8, M100/8) addition of starter culture

inoculation as long as a high enough amplitude is used. The viscosity increases with the viscosity of continuous liquid (whey) and the volume fraction of gel fragments. The volume fraction of casein particles increases with the use of ultrasound, so the viscosity of yogurt increases.

Syneresis

Syneresis is the effect of liquid separating from the yogurt curd, which is an undesirable property. Syneresis of milk curd has been determined by various methods. In this experiment, the vacuum filter was used under a vacuum of 80.6 kPa. Due to the suction applied to the yogurt samples, some free whey trapped between the curd particles was also removed. Different vacuum pressure causes slightly different syneresis values, however, it is sufficient to compare the effect of syneresis because of the same pressure applied to all samples. This method (Mohamed and Morris, 1987) is still a valid indication of the tendency of syneresis although the recorded measurements could be a little different from syneresis measurements which do not involve pulling a vacuum.

The results of the syneresis experiments and the statistical analysis are shown in Fig. 15, all ultrasound-homogenized groups (20/6, 40/6 and 100/6) showed significant difference from the control. The higher amplitude level, the less syneresis. The 100/6 treatment showed the best syneresis result. The 100/6 and 50/6 treatments had significantly less

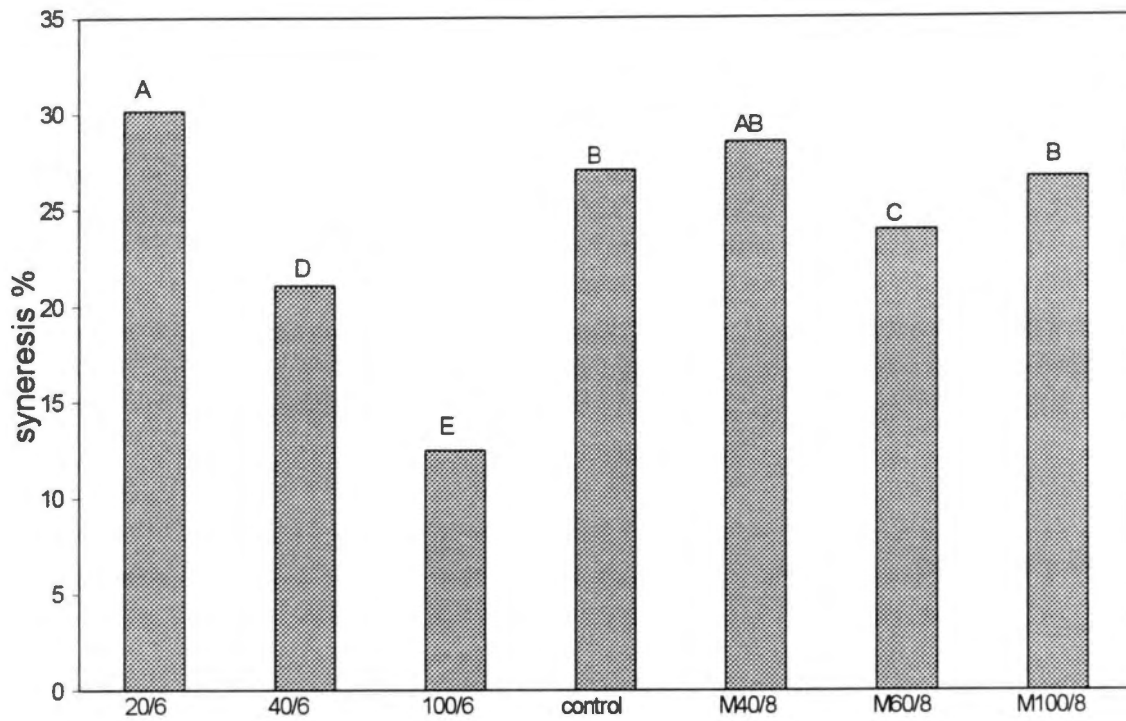


Fig.15 Syneresis of yogurt which was pretreated with ultrasound before (20/6, 50/6, 100/6) or after (M40/8, M60/8, M100/8) addition of starter culture

syneresis than the control. The 20/6 treatment resulted in greater syneresis than the control. Again, this was expected because the homogenization effect in the previous study was poor.

Sonicated inoculated mixtures, M100/8 and M40/8 had no significant effect on syneresis compared with the control, and the M60 had significantly less syneresis than the control.

Grigorov (1966) reported that homogenization causes a change in water holding capacity of the milk proteins, which tends to reduce syneresis. The results of the (20/6, 50/6 and 100/6) experiments correlate well with this assumption. However, the (M40/8, M60/8 and M100/8) treatments increased the water holding capacity without a beneficial syneresis effect. This may be due to a pH effect. The sonicated inoculated mixtures had a slightly lower pH than the control. Tammin et al. (1985) reported that lower pH induced more syneresis.

Therefore, the type of ultrasound treatment is very important. Treatment before inoculation results in an increase in water holding capacity and a decrease in syneresis. Treatment after inoculation results in a decreased processing time and increased water holding capacity but perhaps no beneficial syneresis effect. If pH is the main factor then stopping the fermentation at an earlier time would correct this problem.

Viscosity was increased in all cases. This may or may not be beneficial depending on the preferences of consumers.

CHAPTER V

SUMMARY

Yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of the appropriate bacteria.

Homogenization is a very important and necessary pretreatment for most dairy products, and particularly for yogurt. It can prevent creaming and wheying off during incubation and storage. Stability, consistency and body are enhanced by homogenization.

Based on the principles of sonication, ultrasound could be used for both milk homogenization and as a pretreatment to fermentation. Yogurt provided a good medium to study these effects. Ultrasonic processing could result in a reduction of processing time and an improvement in yogurt quality. The effect of ultrasound homogenization, the pH change during fermentation, the water holding capacity, the viscosity and the syneresis of yogurt product were studied.

In the homogenization experiments, all samples treated at amplitude level 100 had a good homogenizing effect. Compared with the control sample, samples treated at amplitude level 20 had a poor homogenizing effect. 50/1 had a poor effect, but 50/10 had a very good homogenizing

effect. 50/6 had a fair effect, between that of 50/1 and 50/10. For the fermentation experiments, 20/6, 50/6 and 100/6 were selected to produce samples with poor homogenization, fair homogenization and good homogenization, respectively.

From the homogenization results, as expected, it was found that ultrasound has a very good homogenization effect at high amplitude levels compared with conventional homogenization. Low amplitude levels did not have efficient homogenization effects. Longer exposure time could improve the ultrasound homogenization effect. If enough exposure time and amplitude level are provided, extremely small fat globules could be produced. The results imply that the ultrasound-homogenizing effect increases with increasing amplitude level, but energy consumption should also be considered.

During the entire fermentation, all groups of sonicated mixture (M40/8, M60/8 and M100/8) were significantly different than the control in terms of acid development. This method could reduce the total fermentation time by 0.5 hours.

The results showed that ultrasound can significantly change the water holding capacity when it is applied either before or after inoculation with starter culture. Ultrasound cavitation produced sized-reduced fat globules, hence total fat membrane surface area increased. The increased amount

of membrane included a lot of new-bonded casein, which would be hydrophilic. Water holding capacity was improved due to the ability of the membrane proteins to retain water within the yogurt structure. This was why all the high-amplitude ultrasound treatments increased the water holding capacity.

Ultrasound can significantly change the viscosity of yogurt whether it is applied before or after inoculation as long as a high enough amplitude is used. The viscosity increases with the viscosity of continuous liquid (whey) and the volume fraction of gel fragments. The volume fraction of casein particles increases with the use of ultrasound, so the viscosity of yogurt increases.

All ultrasound-homogenized groups (20/6, 40/6 and 100/6) showed significant difference from the control. The higher amplitude level, the less syneresis. Grigorov (1966) reported that homogenization causes a change in water holding capacity of the milk proteins, which tends to reduce syneresis. However, sonication after inoculation did not decrease the syneresis effect. This may have been due to the lower pH of those samples.

Therefore, the type of ultrasound treatment is very important. Treatment before inoculation results in an increase in water holding capacity and a decrease in syneresis. Treatment after inoculation results

in a decreased processing time and increased water holding capacity but perhaps no beneficial syneresis effect. If pH is the main factor then stopping the fermentation at an earlier time would correct this problem. Viscosity was increased in all cases. This may or may not be beneficial depending on the preferences of consumers.

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APPENDICES

APPENDIX A

Collected data for the fermentation experiments

Table 2. Raw data of pH measurements for milk undergoing ultrasonic treatment before addition of starter culture (20/6, 50/6, 100/6) or after addition of starter culture (M40/8, M60/8, M100/8)

0.0 h	20/6	50/6	100/6	Control	M40/8	M60/8	M100/8
Trial 1	5.74	5.71	5.73	5.74	5.74	5.73	5.74
Trial 2	5.75	5.74	5.74	5.76	5.74	5.75	5.75
Trial 3	5.72	5.73	5.73	5.74	5.76	5.73	5.76
0.5 h							
Trial 1	5.37	5.34	5.34	5.38	5.29	5.21	5.19
Trial 2	5.36	5.34	5.33	5.36	5.25	5.19	5.21
Trial 3	5.38	5.35	5.34	5.36	5.25	5.18	5.18
1.0 h							
Trial 1	5.14	5.1	5.11	5.09	5.04	4.95	4.99
Trial 2	5.11	5.1	5.09	5.11	5.04	4.9	4.89
Trial 3	5.12	5.09	5.08	5.05	4.98	4.92	4.96
1.5 h							
Trial 1	4.96	4.93	4.94	4.9	4.83	4.71	4.76
Trial 2	4.9	4.87	4.87	4.89	4.86	4.67	4.77
Trial 3	4.86	4.91	4.89	4.89	4.83	4.74	4.75
2.0 h							
Trial 1	4.73	4.73	4.71	4.7	4.62	4.54	4.6
Trial 2	4.73	4.71	4.72	4.72	4.65	4.56	4.61
Trial 3	4.74	4.69	4.71	4.69	4.67	4.55	4.59
2.5 h							
Trial 1	4.57	4.55	4.56	4.51	4.51	4.45	4.49
Trial 2	4.6	4.59	4.6	4.6	4.5	4.43	4.46
Trial 3	4.62	4.56	4.55	4.56	4.49	4.4	4.45
3.0 h							
Trial 1	4.45	4.42	4.4	4.43	4.38	4.36	4.39
Trial 2	4.49	4.43	4.43	4.49	4.4	4.35	4.39
Trial 3	4.45	4.47	4.47	4.47	4.39	4.36	4.37
3.5 h							
Trial 1	4.35	4.33	4.32	4.35	4.29	4.29	4.3
Trial 2	4.37	4.33	4.35	4.39	4.31	4.29	4.31
Trial 3	4.39	4.37	4.37	4.38	4.33	4.3	4.29
4.0 h							
Trial 1	4.29	4.26	4.24	4.29	4.26	4.23	4.25
Trial 2	4.33	4.29	4.26	4.35	4.27	4.22	4.27
Trial 3	4.27	4.32	4.29	4.33	4.27	4.24	4.22
4.5 h							
Trial 1	4.19	4.2	4.17	4.2	4.2	4.15	4.19
Trial 2	4.24	4.17	4.23	4.27	4.21	4.12	4.14
Trial 3	4.19	4.23	4.19	4.27	4.19	4.17	4.14

Table 3. The recorded pellet weights (g) for water holding capacity of yogurt which has pretreated with ultrasound before (20/6, 50/6, 100/6) or after addition of starter culture (M40/8, M60/8, M100/8)

Treat	20/6	50/6	100/6	Control	M40/8	M60/8	M100/8
Trial1	1.77	2.64	2.64	2.01	2.32	2.68	2.89
Trial2	1.86	2.67	2.81	2.05	2.22	2.44	2.65
Trial3	1.61	2.64	2.83	1.98	2.2	2.57	2.61

Table 4. Flow time (seconds) for the viscosity of yogurt which has pretreated with ultrasound before (20/6, 50/6, 100/6) or after addition of starter culture (M40/8, M60/8, M100/8)

Treat	20/6	50/6	100/6	Control	M40/8	M60/8	M100/8
Trial 1	7.88	37.16	49.69	7.65	19.5	41	51.63
Trial 2	7.38	59.03	59.88	9.11	27.44	62.38	80.62
Trial 3	9.38	42.78	65.75	11.82	26.56	58.97	66.46

Table 5. The syneresis weights (g) of yogurt which has pretreated with ultrasound before (20/6, 50/6, 100/6) or after addition of starter culture (M40/8, M60/8, M100/8)

Treat	20/6	50/6	100/6	Control	M40/8	M60/8	M100/8
Trial 1	5.78	4.11	2.7	5.66	5.86	5.19	5.26
Trial 2	6.06	4.2	2.46	5.34	5.53	4.39	5.61
Trial 3	6.25	4.32	2.32	5.23	5.72	4.78	5.11

APPENDIX B

Statistical analysis for the fermentation experiments

B-1. Statistical analysis for the pH changes during fermentation

The MIXED Procedure

Class Level Information

Class	Levels	Values
HOURS	10	0 1 2 3 4 0.5 1.5 2.5 3.5 4.5
TREAT	7	100/6 20/6 50/6 Control M100/8 M40/8 M60/8

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
Residual	0.00053381

Model Fitting Information for FERMENT

Description	Value
Observations	210.0000
Res Log Likelihood	290.3802
Akaike's Information Criterion	289.3802
Schwarz's Bayesian Criterion	287.9094
-2 Res Log Likelihood	-580.760

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
HOURS	9	140	9809.17	0.0001
TREAT	6	140	135.13	0.0001
HOURS*TREAT	54	140	5.29	0.0001

Mixed RBD ANOVA for the pH changes								
----- _ADJUST_ =LSD(.05) BYGROUP=1 Effect=HOURS -----								
OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
1	0		5.73952381	0.00504178	140	1138.4	0.0001	A
2	1		5.04095238	0.00504178	140	999.84	0.0001	C
3	2		4.66523810	0.00504178	140	925.32	0.0001	E
4	3		4.41857143	0.00504178	140	876.39	0.0001	G
5	4		4.27380952	0.00504178	140	847.68	0.0001	I
6	0.5		5.29523810	0.00504178	140	1050.3	0.0001	B
7	1.5		4.84428571	0.00504178	140	960.83	0.0001	D
8	2.5		4.52619048	0.00504178	140	897.74	0.0001	F
9	3.5		4.33380952	0.00504178	140	859.58	0.0001	H
10	4.5		4.19333333	0.00504178	140	831.72	0.0001	J

----- _ADJUST_ =LSD(.05) BYGROUP=2 Effect=TREAT -----								
OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
11		100/6	4.75866667	0.00421825	140	1128.1	0.0001	B
12		20/6	4.78033333	0.00421825	140	1133.2	0.0001	A
13		50/6	4.76200000	0.00421825	140	1128.9	0.0001	B
14		Control	4.77566667	0.00421825	140	1132.1	0.0001	A
15		M100/8	4.68033333	0.00421825	140	1109.5	0.0001	D
16		M40/8	4.71833333	0.00421825	140	1118.6	0.0001	C
17		M60/8	4.65633333	0.00421825	140	1103.9	0.0001	E

----- _ADJUST_ =LSD(.05) BYGROUP=3 Effect=HOURS*TREAT -----								
OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
18	0	100/6	5.73333333	0.01333928	140	429.81	0.0001	A
19	0	20/6	5.73666667	0.01333928	140	430.06	0.0001	A
20	0	50/6	5.72666667	0.01333928	140	429.31	0.0001	A
21	0	Control	5.74666667	0.01333928	140	430.81	0.0001	A
22	0	M100/8	5.75000000	0.01333928	140	431.06	0.0001	A
23	0	M40/8	5.74666667	0.01333928	140	430.81	0.0001	A
24	0	M60/8	5.73666667	0.01333928	140	430.06	0.0001	A
25	1	100/6	5.09333333	0.01333928	140	381.83	0.0001	EF
26	1	20/6	5.12333333	0.01333928	140	384.08	0.0001	E
27	1	50/6	5.09666667	0.01333928	140	382.08	0.0001	EF
28	1	Control	5.08333333	0.01333928	140	381.08	0.0001	F
29	1	M100/8	4.94666667	0.01333928	140	370.83	0.0001	H
30	1	M40/8	5.02000000	0.01333928	140	376.33	0.0001	G
31	1	M60/8	4.92333333	0.01333928	140	369.09	0.0001	HI
32	2	100/6	4.71333333	0.01333928	140	353.34	0.0001	L
33	2	20/6	4.73333333	0.01333928	140	354.84	0.0001	KL
34	2	50/6	4.71000000	0.01333928	140	353.09	0.0001	L
35	2	Control	4.70333333	0.01333928	140	352.59	0.0001	L

----- _ADJUST_ =LSD(.05) BYGROUP=3 Effect=HOURS*TREAT -----								
OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
36	2	M100/8	4.60000000	0.01333928	140	344.85	0.0001	N
37	2	M40/8	4.64666667	0.01333928	140	348.34	0.0001	M
38	2	M60/8	4.55000000	0.01333928	140	341.10	0.0001	O
39	3	100/6	4.43333333	0.01333928	140	332.35	0.0001	QR
40	3	20/6	4.46333333	0.01333928	140	334.60	0.0001	PQR
41	3	50/6	4.44000000	0.01333928	140	332.85	0.0001	QR
42	3	Control	4.46333333	0.01333928	140	334.60	0.0001	PQR
43	3	M100/8	4.38333333	0.01333928	140	328.60	0.0001	TU
44	3	M40/8	4.39000000	0.01333928	140	329.10	0.0001	ST
45	3	M60/8	4.35666667	0.01333928	140	326.60	0.0001	TUVW
46	4	100/6	4.26333333	0.01333928	140	319.61	0.0001	Z (2)A
47	4	20/6	4.29666667	0.01333928	140	322.11	0.0001	YZ
48	4	50/6	4.29000000	0.01333928	140	321.61	0.0001	YZ
49	4	Control	4.32333333	0.01333928	140	324.11	0.0001	WXY
50	4	M100/8	4.24666667	0.01333928	140	318.36	0.0001	(2)A
51	4	M40/8	4.26666667	0.01333928	140	319.86	0.0001	Z (2)A
52	4	M60/8	4.23000000	0.01333928	140	317.11	0.0001	(2)AB

(continued)

53	0.5	100/6	5.33666667	0.01333928	140	400.07	0.0001	B
54	0.5	20/6	5.37000000	0.01333928	140	402.57	0.0001	B
55	0.5	50/6	5.34333333	0.01333928	140	400.57	0.0001	B
56	0.5	Control	5.36666667	0.01333928	140	402.32	0.0001	B
57	0.5	M100/8	5.19333333	0.01333928	140	389.33	0.0001	D
58	0.5	M40/8	5.26333333	0.01333928	140	394.57	0.0001	C
59	0.5	M60/8	5.19333333	0.01333928	140	389.33	0.0001	D
60	1.5	100/6	4.90000000	0.01333928	140	367.34	0.0001	I
61	1.5	20/6	4.90666667	0.01333928	140	367.84	0.0001	I
62	1.5	50/6	4.90333333	0.01333928	140	367.59	0.0001	I
63	1.5	Control	4.89333333	0.01333928	140	366.84	0.0001	I
64	1.5	M100/8	4.76000000	0.01333928	140	356.84	0.0001	K
65	1.5	M40/8	4.84000000	0.01333928	140	362.84	0.0001	J
66	1.5	M60/8	4.70666667	0.01333928	140	352.84	0.0001	L
67	2.5	100/6	4.57000000	0.01333928	140	342.60	0.0001	NO
68	2.5	20/6	4.59666667	0.01333928	140	344.60	0.0001	N
69	2.5	50/6	4.56666667	0.01333928	140	342.35	0.0001	NO
70	2.5	Control	4.55666667	0.01333928	140	341.60	0.0001	O
71	2.5	M100/8	4.46666667	0.01333928	140	334.85	0.0001	PQ
72	2.5	M40/8	4.50000000	0.01333928	140	337.35	0.0001	P
73	2.5	M60/8	4.42666667	0.01333928	140	331.85	0.0001	RS
74	3.5	100/6	4.34666667	0.01333928	140	325.85	0.0001	UVWX
75	3.5	20/6	4.37000000	0.01333928	140	327.60	0.0001	TUV
76	3.5	50/6	4.34333333	0.01333928	140	325.60	0.0001	VWX
77	3.5	Control	4.37333333	0.01333928	140	327.85	0.0001	TUV
78	3.5	M100/8	4.30000000	0.01333928	140	322.36	0.0001	YZ
79	3.5	M40/8	4.31000000	0.01333928	140	323.11	0.0001	XY
80	3.5	M60/8	4.29333333	0.01333928	140	321.86	0.0001	YZ
81	4.5	100/6	4.19666667	0.01333928	140	314.61	0.0001	(2)B

----- _ADJUST_=LSD(.05) BYGROUP=3 Effect=HOURS*TREAT -----
(continued)

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
82	4.5	20/6	4.20666667	0.01333928	140	315.36	0.0001	(2)B
83	4.5	50/6	4.20000000	0.01333928	140	314.86	0.0001	(2)B
84	4.5	Control	4.24666667	0.01333928	140	318.36	0.0001	(2)A
85	4.5	M100/8	4.15666667	0.01333928	140	311.61	0.0001	(2)C
86	4.5	M40/8	4.20000000	0.01333928	140	314.86	0.0001	(2)B
87	4.5	M60/8	4.14666667	0.01333928	140	310.86	0.0001	(2)C

Univariate Procedure

Extremes

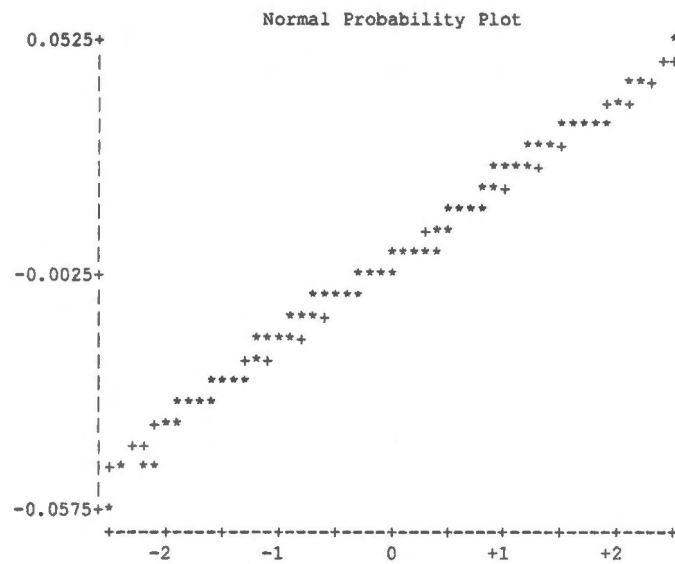
Univariate Procedure

80

Univariate Procedure

Variable=_RESID_

Residual



Check equal variance normality for the pH changes during fermentation
 Check for for the pH changes

OBS	TRTCOMB	_TYPE_	_FREQ_	STDDEV
1	0-100/6	0	3	0.005774
2	0-20/6	0	3	0.015275
3	0-50/6	0	3	0.015275
4	0-Control	0	3	0.011547
5	0-M100/8	0	3	0.010000
6	0-M40/8	0	3	0.011547
7	0-M60/8	0	3	0.011547
8	0.5-100/6	0	3	0.005774
9	0.5-20/6	0	3	0.010000
10	0.5-50/6	0	3	0.005774
11	0.5-Control	0	3	0.011547
12	0.5-M100/8	0	3	0.015275
13	0.5-M40/8	0	3	0.023094
14	0.5-M60/8	0	3	0.015275
15	1-100/6	0	3	0.015275
16	1-20/6	0	3	0.015275
17	1-50/6	0	3	0.005774
18	1-Control	0	3	0.030551
19	1-M100/8	0	3	0.051316
20	1-M40/8	0	3	0.034641
21	1-M60/8	0	3	0.025166
22	1.5-100/6	0	3	0.036056
23	1.5-20/6	0	3	0.050332
24	1.5-50/6	0	3	0.030551
25	1.5-Control	0	3	0.005774
26	1.5-M100/8	0	3	0.010000
27	1.5-M40/8	0	3	0.017321
28	1.5-M60/8	0	3	0.035119
29	2-100/6	0	3	0.005774
30	2-20/6	0	3	0.005774
31	2-50/6	0	3	0.020000
32	2-Control	0	3	0.015275
33	2-M100/8	0	3	0.010000
34	2-M40/8	0	3	0.025166
35	2-M60/8	0	3	0.010000
36	2.5-100/6	0	3	0.026458
37	2.5-20/6	0	3	0.025166
38	2.5-50/6	0	3	0.020817
39	2.5-Control	0	3	0.045092
40	2.5-M100/8	0	3	0.020817
41	2.5-M40/8	0	3	0.010000
42	2.5-M60/8	0	3	0.025166
43	3-100/6	0	3	0.035119
44	3-20/6	0	3	0.023094
45	3-50/6	0	3	0.026458
46	3-Control	0	3	0.030551
47	3-M100/8	0	3	0.011547
48	3-M40/8	0	3	0.010000
49	3-M60/8	0	3	0.005774
OBS	TRTCOMB	_TYPE_	_FREQ_	STDDEV
50	3.5-100/6	0	3	0.025166
51	3.5-20/6	0	3	0.020000
52	3.5-50/6	0	3	0.023094
53	3.5-Control	0	3	0.020817
54	3.5-M100/8	0	3	0.010000
55	3.5-M40/8	0	3	0.020000
56	3.5-M60/8	0	3	0.005774
57	4-100/6	0	3	0.025166
58	4-20/6	0	3	0.030551
59	4-50/6	0	3	0.030000
60	4-Control	0	3	0.030551
61	4-M100/8	0	3	0.025166
62	4-M40/8	0	3	0.005774
63	4-M60/8	0	3	0.010000
64	4.5-100/6	0	3	0.030551

65	4.5-20/6	0	3	0.028868
66	4.5-50/6	0	3	0.030000
67	4.5-Control	0	3	0.040415
68	4.5-M100/8	0	3	0.028868
69	4.5-M40/8	0	3	0.010000
70	4.5-M60/8	0	3	0.025166

Mixed RBD ANOVA for treatments' effect on the pH changes during fermentation

----- _ADJUST_=LSD(.05) BYGROUP=1 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
1	0	M100/8	5.75000000	0.01333929	14	431.06	0.0001	A
2	0	Control	5.74666667	0.01333929	14	430.81	0.0001	A
3	0	M40/8	5.74666667	0.01333929	14	430.81	0.0001	A
4	0	20/6	5.73666667	0.01333929	14	430.06	0.0001	A
5	0	M60/8	5.73666667	0.01333929	14	430.06	0.0001	A
6	0	100/6	5.73333333	0.01333929	14	429.81	0.0001	A
7	0	50/6	5.72666667	0.01333929	14	429.31	0.0001	A

----- _ADJUST_=LSD(.05) BYGROUP=2 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
8	0.5	20/6	5.37000000	0.01333929	14	402.57	0.0001	A
9	0.5	Control	5.36666667	0.01333929	14	402.32	0.0001	A
10	0.5	50/6	5.34333333	0.01333929	14	400.57	0.0001	A
11	0.5	100/6	5.33666667	0.01333929	14	400.07	0.0001	A
12	0.5	M40/8	5.26333333	0.01333929	14	394.57	0.0001	B
13	0.5	M60/8	5.19333333	0.01333929	14	389.33	0.0001	C
14	0.5	M100/8	5.19333333	0.01333929	14	389.33	0.0001	C

----- _ADJUST_=LSD(.05) BYGROUP=3 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
15	1	20/6	5.12333333	0.01333929	14	384.08	0.0001	A
16	1	50/6	5.09666667	0.01333929	14	382.08	0.0001	A
17	1	100/6	5.09333333	0.01333929	14	381.83	0.0001	A
18	1	Control	5.08333333	0.01333929	14	381.08	0.0001	A
19	1	M40/8	5.02000000	0.01333929	14	376.33	0.0001	B
20	1	M100/8	4.94666667	0.01333929	14	370.83	0.0001	C
21	1	M60/8	4.92333333	0.01333929	14	369.09	0.0001	C

----- _ADJUST_=LSD(.05) BYGROUP=4 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
22	1.5	20/6	4.90666667	0.01333929	14	367.84	0.0001	A
23	1.5	50/6	4.90333333	0.01333929	14	367.59	0.0001	A
24	1.5	100/6	4.90000000	0.01333929	14	367.34	0.0001	A
25	1.5	Control	4.89333333	0.01333929	14	366.84	0.0001	A
26	1.5	M40/8	4.84000000	0.01333929	14	362.84	0.0001	B
27	1.5	M100/8	4.76000000	0.01333929	14	356.84	0.0001	C
28	1.5	M60/8	4.70666667	0.01333929	14	352.84	0.0001	D

A-11. Statistical analysis on Mixed RBD ANOVA for treatments' effect on the pH changes during fermentation

----- _ADJUST_=LSD(.05) BYGROUP=5 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
29	2	20/6	4.73333333	0.01333929	14	354.84	0.0001	A
30	2	100/6	4.71333333	0.01333929	14	353.34	0.0001	A
31	2	50/6	4.71000000	0.01333929	14	353.09	0.0001	A
32	2	Control	4.70333333	0.01333929	14	352.59	0.0001	A
33	2	M40/8	4.64666667	0.01333929	14	348.34	0.0001	B
34	2	M100/8	4.60000000	0.01333929	14	344.85	0.0001	C
35	2	M60/8	4.55000000	0.01333929	14	341.10	0.0001	D

----- _ADJUST_=LSD(.05) BYGROUP=6 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
36	2.5	20/6	4.59666667	0.01333929	14	344.60	0.0001	A
37	2.5	100/6	4.57000000	0.01333929	14	342.60	0.0001	A

38	2.5	50/6	4.56666667	0.01333929	14	342.35	0.0001	A
39	2.5	Control	4.55666667	0.01333929	14	341.60	0.0001	A
40	2.5	M40/8	4.50000000	0.01333929	14	337.35	0.0001	B
41	2.5	M100/8	4.46666667	0.01333929	14	334.85	0.0001	BC
42	2.5	M60/8	4.42666667	0.01333929	14	331.85	0.0001	C

----- _ADJUST_=LSD(.05) BYGROUP=7 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
43	3	20/6	4.46333333	0.01333929	14	334.60	0.0001	A
44	3	Control	4.46333333	0.01333929	14	334.60	0.0001	A
45	3	50/6	4.44000000	0.01333929	14	332.85	0.0001	A
46	3	100/6	4.43333333	0.01333929	14	332.35	0.0001	A
47	3	M40/8	4.39000000	0.01333929	14	329.10	0.0001	B
48	3	M100/8	4.38333333	0.01333929	14	328.60	0.0001	B
49	3	M60/8	4.35666667	0.01333929	14	326.60	0.0001	B

----- _ADJUST_=LSD(.05) BYGROUP=8 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
50	3.5	Control	4.37333333	0.01333929	14	327.85	0.0001	A
51	3.5	20/6	4.37000000	0.01333929	14	327.60	0.0001	A
52	3.5	100/6	4.34666667	0.01333929	14	325.85	0.0001	AB
53	3.5	50/6	4.34333333	0.01333929	14	325.60	0.0001	AB
54	3.5	M40/8	4.31000000	0.01333929	14	323.11	0.0001	BC
55	3.5	M100/8	4.30000000	0.01333929	14	322.36	0.0001	C
56	3.5	M60/8	4.29333333	0.01333929	14	321.86	0.0001	C

----- _ADJUST_=LSD(.05) BYGROUP=9 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
57	4	Control	4.32333333	0.01333929	14	324.11	0.0001	A
58	4	20/6	4.29666667	0.01333929	14	322.11	0.0001	AB
59	4	50/6	4.29000000	0.01333929	14	321.61	0.0001	AB
60	4	M40/8	4.26666667	0.01333929	14	319.86	0.0001	BC
61	4	100/6	4.26333333	0.01333929	14	319.61	0.0001	BC
62	4	M100/8	4.24666667	0.01333929	14	318.36	0.0001	C
63	4	M60/8	4.23000000	0.01333929	14	317.11	0.0001	C

----- _ADJUST_=LSD(.05) BYGROUP=10 Effect=TREAT -----

OBS	HOURS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
64	4.5	Control	4.24666667	0.01333929	14	318.36	0.0001	A
65	4.5	20/6	4.20666667	0.01333929	14	315.36	0.0001	AB
66	4.5	50/6	4.20000000	0.01333929	14	314.86	0.0001	B
67	4.5	M40/8	4.20000000	0.01333929	14	314.86	0.0001	B
68	4.5	100/6	4.19666667	0.01333929	14	314.61	0.0001	BC
69	4.5	M100/8	4.15666667	0.01333929	14	311.61	0.0001	CD
70	4.5	M60/8	4.14666667	0.01333929	14	310.86	0.0001	D

B-2. Statistical analysis for variable water holding capacity

```

The MIXED Procedure

Class Level Information

Class      Levels  Values
TREAT      7      100/6 20/6 50/6 Control
              M100/8 M40/8 M60/8

Covariance Parameter Estimates (REML)

Cov Parm      Estimate
Residual      0.00999524

Model Fitting Information for WHC

Description                                Value
Observations                                21.0000
Res Log Likelihood                          8.5292
Akaike's Information Criterion              7.5292
Schwarz's Bayesian Criterion               7.2097
-2 Res Log Likelihood                      -17.0585

Tests of Fixed Effects

Source      NDF    DDF    Type III F    Pr > F
TREAT        6     14      45.89    0.0001

```

Mean Separation for water holding capacity

```

----- _ADJUST_=LSD(.05) BYGROUP=1 Effect=TREAT -----

```

OBS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
1	100/6	2.76000000	0.05772128	14	47.82	0.0001	A
2	20/6	1.74666667	0.05772128	14	30.26	0.0001	E
3	50/6	2.65000000	0.05772128	14	45.91	0.0001	AB
4	Control	2.01333333	0.05772128	14	34.88	0.0001	D
5	M100/8	2.71666667	0.05772128	14	47.07	0.0001	AB
6	M40/8	2.24666667	0.05772128	14	38.92	0.0001	C
7	M60/8	2.56333333	0.05772128	14	44.41	0.0001	B

Check on normality for water holding capacity

Univariate Procedure

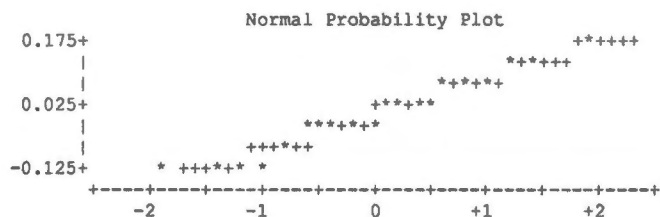
Variable=_RESID_ Residual

Moments				Quantiles (Def=5)			
N	21	Sum Wgts	21	100% Max	0.173333	99%	0.173333
Mean	0	Sum	0	75% Q3	0.05	95%	0.116667
Std Dev	0.083646	Variance	0.006997	50% Med	-0.00333	90%	0.113333
Skewness	0.128212	Kurtosis	-0.39374	25% Q1	-0.04667	10%	-0.12
USS	0.139933	CSS	0.139933	0% Min	-0.13667	5%	-0.12333
CV	.	Std Mean	0.018253			1%	-0.13667
T:Mean=0	0	Pr> T	1.0000	Range	0.31		
Num ^= 0	21	Num > 0	10	Q3-Q1	0.096667		
M(Sign)	-0.5	Pr>= M	1.0000	Mode	-0.01		
Sgn Rank	-1.5	Pr>= S	0.9599				
W:Normal	0.976292	Pr<W	0.8460				

Extremes

Lowest	Obs	Highest	Obs
-0.13667(	3)	0.07(	9)
-0.12333(	17)	0.073333(	13)
-0.12(	7)	0.113333(	2)
-0.10667(	21)	0.116667(	16)
-0.06667(	20)	0.173333(	19)

Stem	Leaf	#	Boxplot
1	7	1	
1	12	2	
0	577	3	+-----+
0	1224	4	+
-0	33110	5	*-----*
-0	75	2	+-----+
-1	4221	4	
-----+-----+-----+			
Multiply Stem.Leaf by 10**--1			



Check on equal variance for water holding capacity

OBS	TREAT	_TYPE_	_FREQ_	RAWMEAN	STDDEV
1	100/6	0	3	2.76000	0.10440
2	20/6	0	3	1.74667	0.12662
3	50/6	0	3	2.65000	0.01732
4	Control	0	3	2.01333	0.03512
5	M100/8	0	3	2.71667	0.15144
6	M40/8	0	3	2.24667	0.06429
7	M60/8	0	3	2.56333	0.12014

B-3. Statistical analysis for variable viscosity

The MIXED Procedure

Class Level Information

Class	Levels	Values
TREAT	7	100/6 20/6 50/6 Control M100/8 M40/8 M60/8

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
Residual	80.23082381

Model Fitting Information for VIS

Description	Value
Observations	21.0000
Res Log Likelihood	-54.4046
Akaike's Information Criterion	-55.4046
Schwarz's Bayesian Criterion	-55.7242
-2 Res Log Likelihood	108.8093

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	6	14	21.34	0.0001

Mean Separation for Viscosity

```
----- _ADJUST_=LSD(.05) BYGROUP=1 Effect=TREAT -----
```

OBS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
1	100/6	58.44000000	5.17142224	14	11.30	0.0001	AB
2	20/6	8.21333333	5.17142224	14	1.59	0.1346	D
3	50/6	46.32333333	5.17142224	14	8.96	0.0001	B
4	Control	9.52666667	5.17142224	14	1.84	0.0867	CD
5	M100/8	66.23666667	5.17142224	14	12.81	0.0001	A
6	M40/8	24.50000000	5.17142224	14	4.74	0.0003	C
7	M60/8	54.11666667	5.17142224	14	10.46	0.0001	AB

check on normality for viscosity

Univariate Procedure

Variable=_RESID_ Residual

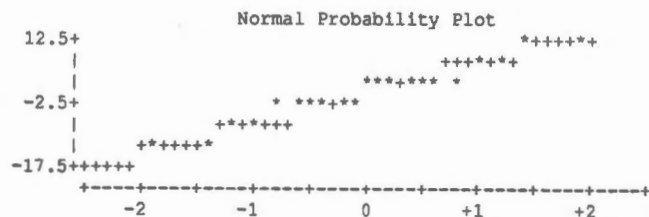
Moments				Quantiles (Def=5)			
N	21	Sum Wgts	21	100% Max	14.38333	99%	14.38333
Mean	0	Sum	0	75% Q3	2.94	95%	12.70667
Std Dev	7.494103	Variance	56.16158	50% Med	0.223333	90%	8.263333
Skewness	-0.11056	Kurtosis	0.043804	25% Q1	-3.54333	10%	-9.16333
USS	1123.232	CSS	1123.232	0% Min	-14.6067	5%	-13.1167
CV	.	Std Mean	1.635347			1%	-14.6067
T:Mean=0	0	Pr> T	1.0000	Range	28.99		
Num ^= 0	21	Num > 0	11	Q3-Q1	6.483333		
M(Sign)	0.5	Pr>= M	1.0000	Mode	-14.6067		
Sgn Rank	2.5	Pr>= S	0.9333				
W:Normal	0.970957	Pr<W	0.7373				

Extremes

Lowest	Obs	Highest	Obs
-14.6067(	19)	4.853333(	18)
-13.1167(	16)	7.31(	9)
-9.16333(	4)	8.263333(	17)
-8.75(	7)	12.70667(	5)
-5(	13)	14.38333(	20)

Stem Leaf	#	Boxplot
1 34	2	0
0 578	3	
0 011223	6	+---+---
-0 42100	5	+-----+
-0 995	3	
-1 3	1	
-1 5	1	0

Multiply Stem.Leaf by 10**+1



Check on equal variance for Viscosity

OBS	TREAT	_TYPE_	_FREQ_	RAWMEAN	STDDEV
1	100/6	0	3	58.4400	8.1263
2	20/6	0	3	8.2133	1.0408
3	50/6	0	3	46.3233	11.3574
4	Control	0	3	9.5267	2.1160
5	M100/8	0	3	66.2367	14.4963
6	M40/8	0	3	24.5000	4.3524
7	M60/8	0	3	54.1167	11.4866

B-4. Statistical analysis for variable syneresis

The MIXED Procedure

Class Level Information

Class	Levels	Values
TREAT	7	100/6 20/6 50/6 Control M100/8 M40/8 M60/8

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
Residual	1.45404762

Model Fitting Information for SYNER

Description	Value
Observations	21.0000
Res Log Likelihood	-26.3307
Akaike's Information Criterion	-27.3307
Schwarz's Bayesian Criterion	-27.6503
-2 Res Log Likelihood	52.6615

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	6	14	74.18	0.0001

Mean Separation for syneresis

----- _ADJUST_=LSD(.05) BYGROUP=1 Effect=TREAT -----

OBS	TREAT	LSMEAN	Std Error	DF	t	Pr > t	LetGrp
1	100/6	12.46666667	0.69619145	14	17.91	0.0001	E
2	20/6	30.15000000	0.69619145	14	43.31	0.0001	A
3	50/6	21.05000000	0.69619145	14	30.24	0.0001	D
4	Control	27.05000000	0.69619145	14	38.85	0.0001	B
5	M100/8	26.63333333	0.69619145	14	38.26	0.0001	B
6	M40/8	28.51666667	0.69619145	14	40.96	0.0001	AB
7	M60/8	23.93333333	0.69619145	14	34.38	0.0001	C

Check on normality for variable syneresis

Univariate Procedure

Variable=_RESID_ Residual

Moments				Quantiles(Def=5)			
N	21	Sum Wgts	21	100% Max	2.016667	99%	2.016667
Mean	0	Sum	0	75% Q3	0.783333	95%	1.416667
Std Dev	1.008877	Variance	1.017833	50% Med	-0.05	90%	1.25
Skewness	0.151576	Kurtosis	-0.42823	25% Q1	-0.86667	10%	-1.08333
USS	20.35667	CSS	20.35667	0% Min	-1.98333	5%	-1.25
CV	.	Std Mean	0.220155			1%	-1.98333
T:Mean=0	0	Pr> T	1.0000	Range	4		
Num ^= 0	21	Num > 0	9	Q3-Q1	1.65		
M(Sign)	-1.5	Pr>= M	0.6636	Mode	-1.98333		
Sgn Rank	-2	Pr>= S	0.9466				
W:Normal	0.982901	Pr<W	0.9480				

Extremes

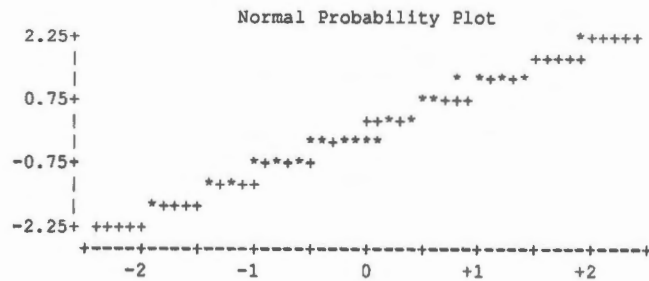
Lowest	Obs	Highest	Obs
-1.98333(	17)	1.033333(	7)
-1.25(	1)	1.1(	3)
-1.08333(	21)	1.25(	10)
-0.9(	12)	1.416667(	20)
-0.86667(	14)	2.016667(	16)

Stem	Leaf	#	Boxplot
2	0	1	
1			
1	0124	4	
0	68	2	+-----+
0	12	2	+
-0	43200	5	+-----+
-0	9995	4	+-----+
-1	21	2	
-1			
-2	0	1	
-----+-----+-----+-----+			

Check on normality for syneresis

Univariate Procedure

Variable=_RESID_ Residual



Check on equal variance for syneresis

OBS	TREAT	_TYPE_	_FREQ_	RAWMEAN	STDDEV
1	100/6	0	3	12.4667	0.96090
2	20/6	0	3	30.1500	1.18216
3	50/6	0	3	21.0500	0.52678
4	Control	0	3	27.0500	1.11692
5	M100/8	0	3	26.6333	1.28290
6	M40/8	0	3	28.5167	0.82815
7	M60/8	0	3	23.9333	2.00021

VITA

Hongyu Wu was born in P. R. China in October 1972. In 1996, he received a Bachelor degree in Food Engineering & Science from Beijing Agriculture University, Beijing, P. R. China. From 1994 to 1997, he was employed as a technical representative to provide technical support for two food companies in Beijing, P. R. China. In January 1998, he entered the Food Science and Technology Department with a Graduate Research Assistantship at The University of Tennessee, Knoxville.

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