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To my husband, ***Ali İhsan***

INCREASING THE SHELF-LIFE OF PLAIN AND PEKMEZ ADDED YOGURTS

A Ph. D. THESIS

in

Food Engineering
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By
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Approval of the Graduate School of Natural and Applied Sciences

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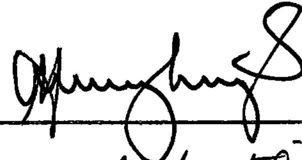
Prof. Dr. Hasan FENERCİOĞLU 

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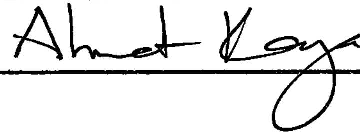
Assist. Prof. Dr. Ahmet KAYA 

ABSTRACT

INCREASING THE SHELF-LIFE OF PLAIN AND
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In this study four methods; pasteurization, nisin addition, lysozyme treatment and microwave treatment, were applied in order to increase the shelf-life of yogurt. Also a new type of fruit yogurt was produced by adding pekmez (concentrated grape juice) into milk, and nisin and microwave treatments were applied in order to increase the shelf-life of pekmez yogurt.

In the first part, yogurt prepared under laboratory conditions was pasteurized by applying different time/ temperature combinations (60-65 °C, 5-10-15 min). The second part was undertaken to observe the effect of nisin addition (50-100-150 RU/ml yogurt) on set yogurt. The third part was related with the use of lysozyme for preservation of yogurt. 0.3-0.6-1.2 g/l lysozyme containing yogurt samples were compared with the control samples during storage. The forth part involved microwave treatment at three different power settings and four different times (5-10-20-40 sec), combinations in a home scale microwave oven. The objective of the fifth part was to develop a new type of fruit yogurt by using pekmez. Influence of pekmez on the overall quality and fermentation process of yogurt was evaluated. Effect of nisin addition (150 RU/ml yogurt) and microwave treatment (power setting P5 for 10 sec) on the quality of pekmez yogurt was followed during storage.

Yogurt samples were prepared under laboratory

conditions, stored at 4 °C for a month. Protein content, pH, titratable acidity, viscosity, whey syneresis, number of starter culture, number of total bacteria, yeast and mold were determined weekly during refrigerated storage.

Heat treatment at 60 and 65 °C increased the storage stability of yogurt by reducing the lactic acid production. pH and titratable acidities of pasteurized yogurt stayed within TSE limits (min. 3.8, max. 1.6 %). At the end of one month of storage none of the samples except heat treated ones at 65 °C for 15 min. had bacterial count of less than $20x10^6$ which is the lower limit of some international standards.

Nisin sufficiently diffused through the yogurt. Addition of low level of nisin (50 RU/ml) effectively improved the storage stability of set yogurt.

As the concentration of lysozyme increased lethality of starter culture increased. 0.6-1.2 g/l lysozyme was found to be appropriate for the preservation of yogurt. But nisin diffused faster than the lysozyme in the yogurt.

During microwave treatment, high power setting and longer treatment time P5 10 sec, P3 20 sec and P1 40 sec, slowed down the acid production. Higher temperatures were needed in conventional heat treatment comparing the microwave treatment to kill yogurt bacteria. This indicated that non-thermal effects of microwaves were also responsible for microbial inactivation. Additionally shorter come up period to reach the necessary temperature for preventing textural damage is an important advantage for microwave treatment of yogurt.

Addition of 10 % pekmez to milk gave the sufficient sweetness to yogurt. Pekmez addition increased the fermentation time of yogurt by slowing down the growth rate of starter bacteria and decreased the viscosity of yogurt significantly. Nisin treatment was more effective on storage stability than microwave treatment of pekmez yogurt. Throughout this work, in none of the samples mould and yeast were detected.

Key words: Yogurt, Storage stability, Post heat treatment, Microwave, Nisin, Lysozyme, Pekmez

ÖZET

SADE VE PEKMEZ EKLENEN YOĞURTLARIN RAF ÖMRÜNÜN UZATILMASI

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Bu çalışmada yoğurdun raf ömrünü uzatmak için dört farklı method; pastörizasyon, nisin eklenmesi, lysozyme eklenmesi ve mikrodalga tekniği, uygulandı. Ayrıca süte pekmez (koyulaştırılmış üzüm suyu) eklenerek yeni bir çeşit meyveli yoğurt üretildi ve pekmezli yoğurdun raf ömrünü artırmak için nisin ve mikrodalga tekniği uygulandı.

Birinci kısımda, laboratuar şartlarında hazırlanan yoğurtlar farklı sıcaklık-zaman kombinasyonları (60 ve 65 °C, 5-10-15 d.) uygulanarak pastörize edildi. İkinci kısım, nisin eklenmesinin (50-100-150 RU/ml yoğurt) set yoğurt üzerine etkisini gözlemek için yapıldı. Üçüncü kısım ise yoğurdun saklanmasında lysozyme kullanımı ile ilgilidir. 0.3-0.6-1.2 g/l lysozyme içeren yoğurtlar depolama süresince kontrol ile kıyaslandı. Dördüncü kısım, üç farklı güç seviyesi ve dört farklı zaman (5-10-20-40 s) kombinasyonunda, ev tipi mikrodalga fırında mikrodalga uygulamasını içermektedir. Beşinci kısmın amacı ise pekmez kullanarak yeni bir çeşit meyveli yoğurt üretmektir. Pekmezin yoğurt kalitesi ve fermentasyonu üzerine etkileri incelendi. Nisin eklenmesinin (150 RU/ml yoğurt) ve mikrodalga uygulamasının (P5 10 s) pekmezli yoğurdun kalitesi üzerine etkisi depolama süresince takip edildi.

Laboratuar şartlarında yapılan yoğurtlar 4°C' de bir ay boyunca muhafaza edildi. Protein miktarı, pH, titrasyon asitliği, vizkozite, serum ayrılması, toplam starter kültür

sayısı, toplam bakteri, küf ve maya sayısı buzdolabı sıcaklığında haftalık ölçümler yapılarak belirlendi.

60 ve 65 °C' de uygulanan ısıl işlem laktik asit üretimini azaltarak yoğurdun depolama kalitesini artırdı. Pastörize yoğurtların pH ve titrasyon asitlikleri TSE limitleri arasında kaldı (en az 3.8, en fazla % 1.6). Depolama sonunda hiçbir yoğurt örneği, 65 °C' de 15 dakika ısıl işlem dışında, bazı uluslararası standartlarda alt limit olan $20x10^{6}$' dan az bakteri sayısı içermedi.

Nisin, yoğurt içerisinde yeteri kadar difüz oldu. Az miktarda nisin eklenmesi (50 RU/ml), set yoğurdun depolama stabilitesini etkili bir şekilde artırdı.

Lysozyme konsantrasyonu arttıkça starter kültürün ölüm oranı arttı. Yoğurdun saklanmasında 0.6-1.2 g/l lysozyme konsantrasyonun uygun olduğu bulundu. Fakat nisin yoğurt içerisinde lysozyma göre daha hızlı difüz oldu.

Mikrodalga uygulaması sırasında, yüksek güç seviyesi ve uzun zaman uygulaması, P5 10s, P3 20 s, P1 40 s, asit üretimini yavaşlattı. Bakteri sayısını azaltmak için, ısıl işlem uygulamasında mikrodalga tekniğine kıyasla daha yüksek sıcaklık derecesine gereksinim duyuldu. Bu sonuç, mikrodalga uygulamasında bakteri inaktivasyonu için ısısal olmayan başka etkilerin de olduğunu gösterdi. Bununla beraber istenilen sıcaklık derecesine ulaşmanın mikrodalga uygulamasında, pastörizasyon işlemine göre daha kısa oluşu, yoğurtta yapısal bozulmaları önleme bakımından önemli bir avantajdır.

Süte % 10 pekmez eklenmesi, yoğurt için yeterli tatlılığı sağladı. Pekmez eklenmesi starter bakteri büyümesini azaltarak fermetasyon zamanını artırdı ve viskoziteyi belirgin şekilde azalttı. Nisin uygulaması, pekmezli yoğurdun depolama kalitesi üzerinde mikrodalga uygulamasından daha fazla etkili oldu. Çalışmalar süresince hiçbir yoğurt örneğinde küf ve maya belirlenmedi.

Anahtar Kelimeler: Yoğurt, Depolama kararlılığı, Pastörizasyon, Mikrodalga, Nisin, Lysozyme, Pekmez

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LIST OF ABBREVIATIONS

ATP :	Adenosine triphosphate
a_w :	Water activity
BHI :	Brain Heart Infusion
BSA :	Bovine serum albumin
β-gal:	β-galactosidase
cfu :	Colony forming unit
cp :	Centi poise
EDTA :	Ethylenediaminetetraacetic acid)
EMP :	Embden-Meyerhof Pathway
FAO :	Food Administration Office
g :	glucose
HFCS :	High fructose corn syrup
Hz :	Hertz
IU :	International Unit
L :	Lactobacillus
LAB :	Lactic acid bacteria
LA :	Lactalbumin
Lb :	Lactobacillus
LDH :	Lactate dehydrogenase
LG :	Lactoglobulin
MIC :	Minimum Inhibitory Concentration
MRS :	Man, Rogosa, Sharpe
P :	Pekmez
β-Pgal:	β-phosphogalactosidase

PVC	:	Polyvinylchloride
PDA	:	Potato dextrose agar
P+M	:	Pekmez+Microwave
P+N	:	Pekmez+ Nisin
rpm	:	Revolution per minute
RU	:	Reading Unit
S	:	Streptococcus
s	:	sucrose
ssp	:	Subspecies
SNF	:	Solid nonfat
Str	:	Streptococcus
SMA	:	Standard method agar
SH	:	Sulfhydryl
TS	:	Total solids
TSE	:	Turkish Standard Institute
U	:	Unit
UHT	:	Ultra high temperature
UP	:	Undenatured protein
VRBA	:	Violet red bile agar
WHO	:	World Health Organization

CHAPTER I
INTRODUCTION

Fermented foods may be defined, as foods that have been modified in a desired way, by the activity of microorganisms (m. o.) and/or enzymes. The main purpose of food fermentations dominated by yeast and moulds is to improve digestibility, nutritional value, texture and aroma. In contrast, lactic acid fermentation of foods probably evolved as a preservation method, taking advantage of the bacteria to form large amounts of lactic acid in a short time. However, it also permitted the production of a large variety of foods with different aromas, flavours and consistencies, and there is evidence that lactic acid fermentation of certain foods may also improve their wholesomeness [1].

All lactic acid fermented foods, dairy products are the most important worldwide, by both weight and value. Acidification of milk by fermentation is one of the oldest methods of preserving milk. There are many different methods of carrying out this fermentation in various parts of the world and these give rise to a range of fermented milk products, including kumiss, kefir [2], acidophilus milk and yogurt. These products vary considerably in composition, flavour and texture according to the nature of fermenting organisms, the type of milk, and the manufacturing process used [3]. In Europe, more than 50 % of the total milk is processed into sour milks, sour cream, butter and cheese. All dairy fermentations include an initial lactic acid fermentation, only in rare cases accompanied by yeast [1].

Fermented dairy products are broadly subdivided into, sour milk products and cheeses. Preferences differ between consumers in various countries; for example, the Finnish and Swedish populations rank first in consumption of sour

milk fermented by strains having a lower temperature optimum (Lactococcus, Leuconostoc) while traditional yogurt is most popular in Southeastern Europe and Turkey. In the countries of Western Europe, traditional plain yogurt had only a small market share until yogurt-based sweetened convenience products such as fruit yogurt were developed and marketed and starter producing milder varieties of yogurt were introduced [1, 4].

Yogurt is a dairy product manufactured from milk fermentation with mixed starter culture composed of thermophilic bacteria mainly S. thermophilus and L. bulgaricus [5, 6, 7].

1. 1. History of Yogurt Making

Although there are no records available regarding the origin of yogurt, the belief in its beneficial effects influence on human health and nutrition has existing in many civilisations for a long time. It is likely, however origin of yogurt was the Middle East and evaluation of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world [8, 9]. Thus, the production of milk in the Middle East has always been seasonal. The main reason for the limited availability of milk is that intensive animal production has never really exist. Another major factor is that the Middle East area has a subtropical climate and summer temperatures can reach as high as 40°C. In such a climate, milk turns sour and coagulates within a short time after milking. However it may well have been evident at early stage that the souring of milk by no means a uniform process. Thus the fermentation brought about by non-lactic acid bacteria gives rise to a product which is insipid and stale and furthermore the coagulum is irregular, filled with gas holes and shows extreme whey syneresis. The lactic acid bacteria, however, act on the milk to produce a fermented product which is pleasant to eat or drink, thus latter product was usually referred as "sour milk" [3].

The production of sour milk soon became an established pattern of preservation. Gradually other communities learnt of this simple preservation treatment for milk and one such product became known as "yogurt" from the Turkish word "jugurt". Yogurt is known by different names according to the place where it is made [3, 10, 11]. Table 1 shows the variety of names by which this product is known in different countries.

Table 1. Yogurt and yogurt-like products known worldwide [3].

Traditional Name	Country
Jugurt/ Eyran/ Ayran	Turkey
Busa	Turkestan
Leban/ Laban	Lebanon-Arab countries
Dahi/ Dadhi/ Dahee	India
Tarho	Hungary
Iogurte	Brazil and Portugal
Kissel Mleka	Balkans
Roba	Irak
Yoghurt/Yogurt/Yaort/Yourt/ Yaourti/Yahourth/Yogur/ Yaghourt	Rest of the World (Y is replaced by J in some cases).

Yogurt is a traditional food and beverages in the Balkans and the Middle East. However its popularity has now spread to Europe and to many other parts of the world. Yogurts are usually eaten as it is but can be used as ingredients in dessert products [12], candy bars, snack products in meal and as a frozen soft-serve products [6]. Defreitas and Molis (1988) developed meat snack dips by using unflavoured yogurt as an ingredient [7]. Table 2. shows consumption of yogurt at different countries.

Table 2. Yogurt consumption at various countries [13].

Country	Consumption (kg/head/year)
England	1.5
Switzerland	7.5
USA	2.3
West Germany	4.8
Finland	6.3
Sweden	2.3
France	8.6
Holland	13.7
Netherlands	17.6
Bulgaria	30.0
Turkey	25-30

1. 2. Processing and Manufacture of Yogurt

The microorganisms and their enzymes in the yogurt starter cultures, play an important role during the production of yogurt,eg. the development of acid and flavour and their classification. In order to understand the principles of yogurt making it will be useful to describe separately the various stages of manufacture and their consequent effects on the quality of yogurt [3]. Figure 1 summarizes the process for the production of yogurt.

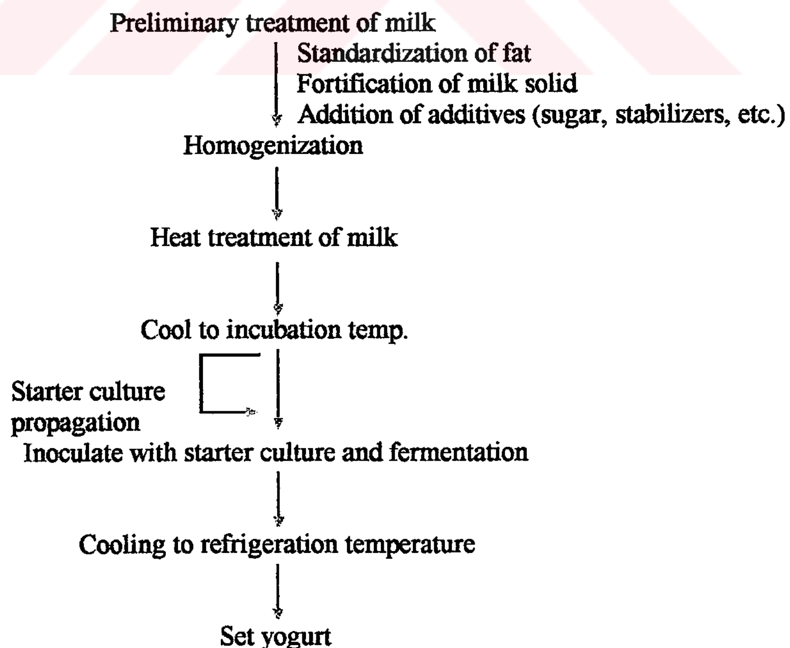

Figure 1. Improved process for the yogurt production [3].

1. 2. 1. Preliminary Treatment of Milk

1. 2. 1. 1. Standardization of Fat Content

The fat content of yogurt manufactured in different parts of the world can vary from as low as 0.1 % to as high as 10 % and in order to meet existing or proposed compositional standard of yogurt it is necessary to standardise the milk. The methods employed for standardization are as follows [14];

a) removal of part of the fat content from milk.

b) mixing full cream milk with skim milk.

c) addition of cream to full fat milk or skim milk.

d) a process which combines methods a and c i.e. use of standardising centrifuges.

1. 2. 1. 2. Standardization of Solid Non-fat Content of Milk.

The level of total solids in the milk is significant for both the consistency and aroma of the manufactured yogurt. The total solid level in the milk for yogurt manufacture can vary from as low as 9 % in skim milk yogurt to over 20 % in other types of yogurt. The best yogurt is made from milk containing 15.5-16 % total solids [15].

An increase in the total solids in the milk can be achieved by many methods [16, 17, 18, 19]; such as:

a) milk powder addition

b) evaporation

c) whey powder addition

e) ultrafiltration and reverse osmosis

1. 2. 1. 3. Addition of Preservatives

Preservatives are widely used in food industry and in particular for the preservation of fruit. However it is reasonably safe to assume that use of preservatives is prohibited in the yogurt industry although use of fruit in flavoured yogurt may carry some preservatives into it. FAO/WHO has suggested that preservatives like sorbic acid and its salts (potassium, sodium and calcium), sulphur dioxide and benzoic acid can be used at levels of 50 mg/kg

in the final product, if they come exclusively from fruit flavouring substances. Such a proposal is only applicable to fruit flavoured yogurt and not to the natural flavour.

The presence of preservatives (sodium sorbate, potassium sorbate or sodium dehydroacetate at levels as low as 0.1 % (w/v) in the yogurt can effect growth of the starter culture and cause the acetaldehyde level to decrease more rapidly during storage [3].

1. 2. 1. 4. Homogenization

Homogenization is an integral part of the yogurt manufacturing process. It is usually carried out before the heat treatment. It is carried out chiefly to effect a homogenous dispersion of the milk mix constituents and to increase the viscosity and coagulum stability of the yogurt. It also improves the mouth-feel of the product and increase the organoleptic quality. Homogenization is particularly important in manufacturing yogurt from milk containing fat (either low 1-2 % or full cream milk). Homogenization process splits the fat globules into smaller globules which become coated with a new membrane comprised largely of casein submicelles. This process increase density of fat globules and reduce their tendency to agglutinate [20]. In this way fat becomes evenly and permanently dispersed throughout the liquid and does not separate out during incubation in yogurt manufacture. Also homogenization process increases the viscosity and reduces the whey syneresis. Homogenization is essential during the manufacture of the yogurt [21].

1. 2. 1. 5. Heat Treatment

In commercial procedure, milk for yogurt manufacture is pre-heated at 85°C for 30 min or 90-95°C for 5-10 min [20]. This type of heat in dairy processing is unique to yogurt manufacture.

Effects of heat treatment can be broadly summarized as [10];

a) destruction and/or eliminating of pathogens and

other undesirable microorganisms

b) production of factors stimulatory/inhibitory to the yogurt starter cultures

c) changes in the physicochemical properties of the milk constituents

d) increase the concentration of milk solids in the basic mix

Mottar et al. reported that heating denature and associate the whey protein, β-LG and α-LA, on the micelle surface and this affect the rheological properties of yogurt [22].

1. 2. 1. 6. Fermentation Process

During the manufacture of yogurt, the heat treated milk is cooled to incubation temperature of the starter culture (S. thermophilus and L. bulgaricus) and in general the milk is fermented at 40-45°C for 3-5 hours, the optimum growth condition for the mixed culture, the short incubation method [21]. However, in longer incubation method the incubation conditions are 30°C for around 18 hours or until desired acidity is reached.

Briefly the formation of the yogurt gel is the result of the following biological and physical actions on milk:

a) The yogurt starter culture utilises the lactose in milk for its energy requirements and as a result lactic acid is produced

b) The development of lactic acid starts to destabilise the casein micelle [13, 20]

c) Casein micelles aggregate and partially coalesce as the pH approaches the isoelectric point pH 4.6-4.7

d) α-La/ β-Lg interact with the k-casein and this partially protects the micelles against complete destabilisation or disruption and as a result the gel network or matrix consist of a regular structure which entraps within it all the other constituents of the basic mix.

Vaitheeswamrn and Bhat [23] observed that production of lactic acid during fermentation causes the denaturation

of whey protein. The extend of denaturation increased with the increase in acidity and was completed as the acidity reached to 0.95-1 % lactic acid.

1. 2. 1. 7. Cooling

Cooling is a critical step in yogurt production. It is carried out directly after product reaches the desired acidity. The objective is to reduce the metabolic activity of the starter culture and hence to control the acidity of yogurt. Since yogurt organisms show limited activity around 10°C, the primary objective of cooling is to drop the temperature of the coagulum from 30-45°C to lower than 10°C (best around 5°C) as quickly as possible to control the final acidity of the product [24].

1. 3. Microbiology of Yogurt

There are four genera of lactic acid bacteria: Lactobacillus, Streptococcus, Leuconstoc, Pediococcus. LAB are gram (+), nonmotile, nonsporing, rods or cocci. Usually they can be characterized by homofermentation or heterofermentation of carbohydrates. Since these organisms are deficient in cytochromes and catalase, many are microaerophilic [25].

Lactic acid bacteria used in milk fermentation fall into two general categories, mesophilic organisms with optimum growth temperature less than 30 °C, and thermophilic ones with growth optima at higher than 37 °C. The thermophilic organisms also fall into two genera: Lactobacillus and Streptococcus [26].

1. 3. 1. Lactobacillus

This group belongs to the family Lactobacillaceae and contains some 15 species. They are long, gram positive, nonsporing rods, catalase negative. They often occur in long chains when viewed under the microscope. Most are microaerophilic or anaerobic while both homofermentative and heterofermentative species exist among them. Homofermentative lactobacilli produce primarily lactic acid

(L. helveticus, L. bulgaricus, L. lactis, L. acidophilus). Heterofermentative lactobacilli produce CO_2, ethanol, volatile compounds besides lactic acid, (L. casei, L. plantarum) [25]. They are widely distributed among plants and in dairy products. Some are employed in the production of fermented milks such as Acidophilus and Bulgaricus milks. Some are important in cheese making. Many are used in the microbiological assay of B-vitamins and amino acids due to their exacting growth requirements. One species, L. thermophilus, survives milk pasteurization temperatures. They are common in and on cured and processed meat products [25].

Characteristics that make the lactobacilli important in foods are,

1. their ability to ferment sugars with the considerable amount of lactic acid
2. production of gas and volatile compounds
3. their ability to synthesize most of the vitamins that they require
4. heat resistance of most of the high-temperature lactobacilli [27].

Lactobacillus bulgaricus is the member of Genus lactobacillus. The species of this genus are non spore forming, gram positive rods. They are non-motile, catalase negative, microaerophilic [28]. Its length and thickness are between 10-30 micron and 0.7-2 micron respectively. It can produce 1.7 % lactic acid [25].

1. 3. 2. Streptococcus

This genus belongs to the family Lactobacillaceae as do the lactobacilli and consists of 19 species. They are gram positive, catalase negative, cocci that often appear as spherical to void forms. All produce small colonies when growing on culture media as do the lactobacilli. They are nonpigmented and microaerophilic in nature. Some are associated with the upper respiratory tract of man and other animals where they may cause diseases such as scarlet fever, septic tore throat, etc. Others are found in the

intestinal tract of man and animals and tend to be rather widespread on plants and plant parts, and in dairy products. While most are mesophilic, some grow within the psychrophilic range. Some cause mastitis in cattle while others are important in dairy starter cultures. One species, S. lactis, is the most common cause of sour milk, and is important in the manufacture of cheese. Some produce a food-poisoning syndrome in man. The presence of some species in foods in large numbers may indicate fecal contamination [29].

S. thermophilus is the member of Genus streptococcus. Species of this genus are gram positive, catalase negative, cocci, form long or short chains in liquid medium. They are all homofermentative. Determination of the ability of the organisms to grow at 10 °C and 45 °C, to grow in the presence of 6.5 % NaCl or at pH 9.6, to survive heating at 60 °C for 30 min. [30] is used in their identifications.

Radius of S. thermophilus is 0.7-0.9 micron and it can produce 1 % lactic acid.

1. 3. 3. Microbiology of Yogurt Fermentation

Although definition of yogurt includes L. bulgaricus and S. thermophilus, recent developments showed that L. acidophilus [31] and Bifidobacteria [32, 33] were also used in the production of yogurt [17, 34]. Cultures can vary from country to country [6]. Table 3. shows the starter culture used for yogurt production.

The growth associated between two organisms of the yogurt starter culture is termed as symbiosis [20]. Rate of acid development was greater when mixed yogurt cultures of S. thermophilus and L. bulgaricus were used as compared with the single strains. Release of stimulatory factors by yogurt cultures takes place during the incubation period and while L. bulgaricus provides the essential nutrients, i.e. amino acids for S. thermophilus, the later produces a formic acid-like compounds which promote the growth of L. bulgaricus [20, 35].

Table 3. Starter cultures for yogurt [1].

Product Type	Starter composition	Main desired metabolic activity
Yogurt	Str. salivarius ssp. thermophilus; Lb. delbrueckii ssp. bulgaricus	Lactic acid from lactose acetaldehyde from pyruvate or threonine
Yogurt (mild)	Str. salivarius ssp. thermophilus and strains of Lb. delbrueckii partially or completely replaced by Lb. acidophilus	Same

1. 3. 4. Metabolically Injured Organisms

When microorganisms are subjected to environmental stresses as sublethal heat and freezing, many of the individual cells undergo metabolic injury resulting in their inability to form colonies on selective media that uninjured cells can tolerate. Whether or not a culture has suffered metabolic injury can be determined by plating aliquots separately on a nonselective and a selective medium and enumerating the colonies that developed after suitable incubation. The colonies that develop on the nonselective medium represent both injured and uninjured cells, while only the uninjured cells develop on selective medium. The difference between the number of colonies on the two media is a measure of the number of injured cells in the original culture or population.

1. 4. Biochemistry of yogurt

The fermentation of carbohydrates supplies energy to the lactic acid bacteria. Lactose is the only sugar present in milk and the yogurt organisms utilize it for this purpose. The catabolism of lactose by S. thermophilus and L. bulgaricus takes place inside the cell, hence the initial step is to transport the lactose molecule across the cell wall membrane. It is most likely that the transport of lactose across the cell membrane of S.

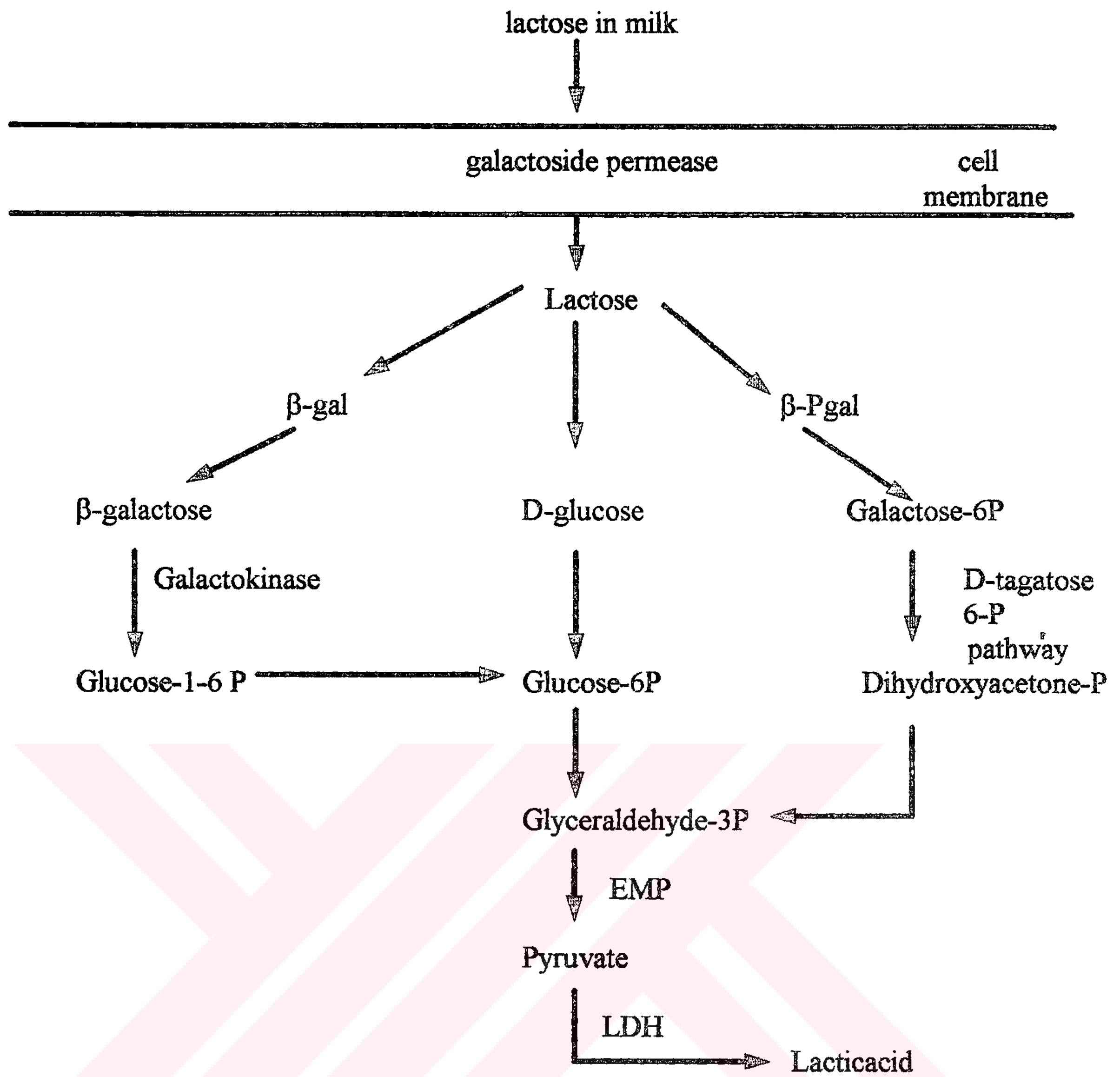

Figure 2. Possible pathways of lactose utilization by the yogurt bacteria [3].

thermophilus and L. bulgaricus mediated by the action of the enzyme galactose permease. These organisms possess the enzyme β-D-galactosidase which hydrolyses the lactose inside the cell to D-glucose and β-D-galactose [10]. The former monosaccharide is metabolised in both S. thermophilus and L. bulgaricus, to lactic acid, but the catabolism of galactose is not well established. Evidence of galactose accumulation in yogurt indicates the monosaccharide is not metabolised to any greater extend and permeates out through the cell membrane. In the presence

of β-gal and β-Pgal enzymes in the yogurt organisms, the intracellular hydrolysis of lactose yields D-glucose, β-D-galactose and/or galactose-6P. In view of the fact that some of yogurt bacteria are capable of fermenting galactose, it is possible that the galactose-6P is catabolized to lactic acid via same D-tagatose-6P pathway [18]. However in commercial practice the production of lactic acid is more likely to occur via the glycolysis of glucose [7] and to a lesser degree via the D-tagatose-6P pathway of galactose utilisation. The possible pathways of lactose utilisation by the yogurt starter bacteria is illustrated in Figure. 2.

1. 5. Types of Yogurt

Yogurt differs according to their chemical composition, their method of production, their flavour and the nature of post incubation processing.

Yogurt can be classified according to fat content (full, medium and low).

Set yogurt is the product formed when the fermentation/coagulation of milk is carried out in the retail container, and the yogurt produced is in a continuous semi-solid mass.

Stirred yogurt results when the coagulum is produced in bulk and gel structure is broken before cooling and packaging.

Fluid yogurt may be considered as stirred yogurt of low viscosity.

Fruit yogurts are made by addition of fruit, usually in the form of fruit preserves, puree or jam to yogurt [36].

Flavoured yogurts are prepared by adding sugar or other sweetening agents such as aspartame [37], sucrose, sorbitol, HFCS, calcium or sodium saccharin [38] and synthetic flavourings and colouring, fruit concentrates [39], flavoured syrups to the cultured milk before or after incubation process. Addition of flavoring agents decrease

the consistency of product so stabilizer or viscosity modifiers are added (starches, pectin etc.)

Pasteurized/UHT yogurt is heat treated after incubation, a process which leads to destruction of the yogurt starter bacteria and a reduction in the level of volatile compounds which are associated with the flavour of yogurt [40].

Partial separation of the liquid phase from yogurt leads to the production of **concentrated/condensed** yogurt with rheological properties and characteristics considerably different from those normally associated with yogurt [40, 41].

Frozen yogurt which can be either soft or hard, is a product whose physical state resembles ice-cream rather than yogurt, but which is similar to yogurt in chemical composition and manufacturing detail up to freezing stage [40].

Dried yogurt can be produced by sun drying, spray drying or freeze drying. Sun dried yogurt is produced in many rural areas in the Middle East for winter consumption. Industrial production of dried yogurt is achieved by either spray drying or freeze drying [42].

Low-calorie yogurt

The manufacture of low calorie yogurt can be achieved by;

a) reducing the fat content in the milk

b) replacing the sucrose with a low-caloric sweetener, e.g aspartame and/or saccharin (in flavoured yogurt)

c) replacing the fat with low-calorie products which have fat- like properties and are known as fat-substitutes [43].

Plain yogurt contains about 250-335 kJ/100 g. Fruit yogurt contains about 420 kJ/100 g. Low calorie yogurt has a colorific value of about 170 kJ/100 g.

Low lactose yogurt produced by the use of enzyme β-D-galactosidase. In this process, hydrolysis of the lactose leads to a sweeter product without adding of sugar. Low lactose yogurt is more digestable for the lactose

intolerant people [44, 45, 46, 47].

A new method of manufacturing lactose-hydrolysed fermented milk involving the process of sonication during fermentation [48].

It is evident that there are many different types of yogurt products, which may differ in chemical composition, microbiological status, biochemical characteristics or organoleptic properties. Thus it is extremely difficult to find a common definition for yogurt, which adequately covers all of these types [3].

1. 6. Production and Preservation of Starter Culture

Yogurt cultures consist of two species, i.e. S. thermophilus and L. bulgaricus, and as these organisms are mainly grown and propagated together, they are referred as mixed strain starter cultures. The culture organisms are preserved in small quantities known as **stock cultures.** When these cultures are reactivated for use in the dairy, scale-up system of propagation is employed to supply to required volume.

An active bulk starter culture must have the following characteristics;

a) It must contain the maximum number of viable cells;

b) It must be free form any contaminants; e. g. coliforms or yeasts and molds;

c) It must be active under processing conditions in the dairy, and hence maintenance of the intermediate and other cultures is extremely impertinent [49].

1. 6. 1. Preparation of Starter Cultures

The preparation of the starter culture in the production of yogurt demands maximum accuracy and hygiene. The ratio of cocci to bacilli in the culture and in the yogurt is usually about 1:1 or 2:1. However, the balance can easily be distributed unless all variables such as inoculation quantities, times and temperatures, are kept under close control. The cultures must be replaced at regular intervals, since repeated transfer will alter the

ratio. *Lactobacillus bulgaricus* will then often dominate, and this will lead to a sharp taste by excessive acid and acetaldehyde being formed [3].

Aseptic production of the culture eliminates the risk of infection by the bacteria and yeast. In conventional starter preparation, the culture can for example be infected by spore-forming bacteria. These are heat-resistant types which often survive the treatment of the milk. They impart a bitter taste and aroma to the yogurt [50].

1. 6. 1. 1. Simple Microbiological Techniques

In this system the equipment/materials used are basically laboratory utensils and may consist of glass test tubes, flask, pipettes.

Reconstituted skim milk powder (10- 12 % TS) is used as the growth medium and glassware containing the milk is plugged with cotton wool. The whole is sterilised in an autoclave. The reactivation and subculturing procedures must be carried under extremely hygienic conditions. The freeze drying ampoule is wiped with alcohol before breaking the glass or alternatively, if a liquid stock culture is used, the lip of the test tube or bottle must be flamed over a bunsen burner when the cottonwool or the screw cap is removed, and again immediately before replacing it. It also recommended that the starter working area and atmosphere should be clean, i. e. the air must be filtered and if possible, the whole starter laboratory should be under positive pressure; unfiltered air does not enter the room whenever the door is opened. Alternatively, subculturing can be carried out in a laminar-flow hood or cabinet to reduce the possibility of airborne contamination [49].

1. 6. 2. Methods of Preservation

Cultures can be preserved in the following forms:

a) Liquid starter

b) Dried starter

1. Vacuum drying
2. Spray drying
3. Freeze drying or lyophilisation [51]
4. Freeze drying of concentrated cultures to give concentrated freeze-dried cultures (CFDC)

c) Frozen Starters,
1. Deep or sub-zerofreezing (-30 to -40 °C) [50]
2. Ultra low temperature freezing (-196 °C) in liquid nitrogen

1. 6. 2. 1. Deep or Sub-Zero Freezing

Sterile liquid milk freshly inoculated with an active starter culture is deep frozen at -30 °C to -40°C for the preservation of a mother or feeder culture. Such frozen cultures can retain their activity of several months when stored at -40°C and this method of culture preservation become popular in the dairy industry because deep frozen cultures produced in centralised laboratories could be dispatched to a dairy in dry-ice whenever required. The reactivation procedure for the deep frozen cultures is as follows;

1. remove starter from freezer;
2. thaw the starter very quickly in water bath at 20°C;
3. incubate at 42°C until desired acidity is reached;
4. cool and store overnight in the refrigerator;
5. Subculture for the propagation of feeder or bulk starter.

The activity of deep frozen starter cultures tends to deteriorate after a certain time in storage due to several factors:

i) freezing can cause cell damage, in particular to L. bulgaricus [49]. However improved medium for frozen cultures held at -30°C, contains 10 % skim milk, 5 % sucrose, fresh cream and 0.9 % NaCl or 1 % gelatin, can prevent this damage. Other cryogenic compounds recommended for the preservation of concentrated culture, glycerol, or sodium citrates, glycerol, or sodium β-glycerophosphate.

Wright and Klaenhammer [52] demonstrated that presence of calcium in MRS medium protected L. bulgaricus from freezing death and injury.

ii) The use of mechanical separators for the concentration of the bacterial cells may cause some injury, so that the starter becomes sensitive to freezing.

iii) The destruction of bacterial cells during freezing is mainly due to an increased concentration of electrolytes and other solutes both inside the cell and in the suspending medium, rather than to mechanical damage as the result of ice crystals formation. The former situation results in the denaturation of protein components and enzymes of the bacterial cell, while the concentration of electrolytes outside the cell results in the dehydration of the protoplasm due to diffusion of water through the cell wall membrane [49].

1. 7. Methods and Media for the Enumeration of Yogurt Culture

Enumeration of yogurt starter bacteria on solid media is based on the morphology of the colony and/or selectivity of the medium. Selective media can be prepared by incorporation of antibiotics. However, with yogurt organisms this has proved to be difficult because of their high and strain variable sensitivity to these substances.

The starter strains are either monitored microscopically or examined after culturing on a solid medium [53].

While some countries do not regulate the levels of live bacterial cultures of S. thermophilus and L. bulgaricus in yogurt, some countries proposed standards of quality for yogurt products. Such standards may include the requirement that a minimum number of viable yogurt organisms, Lactobacillus bulgaricus and Streptococcus thermophilus, must be present [54].

According to Turkish standards there is no standard about the number of viable yogurt organism. The ratio of

yogurt organisms is also important in determining the quality of yogurt. However no such regulations is present [55].

1. 7. 1. Breed Smear Technique

Bread smear can easily be adapted for examining yogurt starters. The mechanical preparation of the smear and the possibility of sampling error make the technique inaccurate. The procedure involves spreading 0,01 ml of the sample over an area of 1 cm^2 on glass microscopic slide and drying quickly to overcome bacterial multiplication. The highest concentration of organisms tends to be in the center of the square because drying starts at the periphery of the smear and progresses gradually toward the center. For enumeration 10 fields could be counted to compensate for the uneven spreading [3, 28].

Another problem associated with microscopic counting of the starter is the high bacterial load, as it is impossible to count only cells, clumps or chains in an undiluted sample. However, upon dilution the chains are broken, particularly those of the Streptococcus. Therefore the estimates of the chain:chain ratio is not reliable and the ratio is usually based on cell numbers [3].

1. 7. 2. Culture Media for Enumeration of Starter Bacteria in Yogurt

Plate count agar (PCA) [56] (also referred to as Standard Methods Agar) and Elliker's Lactic agar are currently being used for an aerobic plate count of yogurt bacteria, but these media do not differentiate between two microorganisms [57].

1. 7. 2. 1. MRS Agar

The MRS formulation was developed by Man, Rogosa and Sharpe to replace a variable product and at the same time to provide a medium which would support good growth of Lactobacilli in general, even those strains which showed poor growth in other media. MRS medium is superior to the

tomato juice medium and the meat extract tomato juice medium. MRS culture media contain polysorbate, acetate, known to act as special growth factors for LAB. It gives more profuse growth of all strains of lactobacilli, especially for the difficult and slow growing strains of L. brevis and L. fermenti.

MRS agar and broth were designed to encourage the growth of the lactic acid bacteria which includes species of the following genera: Lactobacillus, Streptococcus, Pedicoccus and Leuconostoc. All these species can produce Lactic acid in considerable amounts. They are gram positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by micro-aerophilic conditions. Generally the lactic acid bacteria showed delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if 2-4 days of incubation is required.

Selection can be made by pH adjustment, thus lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range. The lactobacilli are micro-aerophilic and generally require layer plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery and are small, opaque and white [56].

1. 7. 2. 1. 1. Inoculation Technique

Products to be examined for lactobacilli content are diluted in a diluent. 0.1 ml volumes of the diluted samples are added to sterile petri dishes and molten MRS Agar (45°C) is poured into the dish and mixed thoroughly. The presence of carbondioxide stimulates growth and plates should be incubated in an atmosphere of 5 % CO_2 [58]. Also incubation can be carried out under anaerobic conditions.

1. 7. 2. 2. M17 Agar

It is used for improved growth of lactic streptococci

and their bacteriophages and selective enumeration of Streptococcus thermophilus from yogurt.

Lactic streptococci are nutritionally fastidious and require complex media for optimal growth. Their homofermentative and acid producing nature requires that the medium is well buffered so that the culture pH is maintained above 5.7 during active growth [56].

M17 agar contains di-sodium-glycerophosphate which has sufficient buffering capacity to maintain the pH above 5.7 of actively growing cultures even after 24 hr at 30 °C. M17 Agar is suitable for the isolation and enumeration of S. thermophilus from yogurt as the high concentration of di-sodium-glycerophosphate resulted in suppression of L. bulgaricus [59].

1. 7. 2. 3. L-S Differential Medium

It is medium for the differentiation and enumeration of lactobacilli and streptococci in yogurt. It allows maximal growth of lactobacilli and streptococci. The differentiation of the organisms is based on colonial morphology [56].

Table 4. Colony apperance on L-S differential medium [56].

Organisms	Colony Appearance
Lactobacillus species	irregular red, rhizoidal colonies, 1.0-1.5 mm diameter, surrounded by a white opaque zone
Streptococcus species	round or oval red colonies, 0.2-0.5 mm diameter, surrounded by a small clear zone

1. 7. 2. 4. Rogosa Agar

It is medium for the selective isolation and enumeration of Lactobacilli. The medium has given excellent results when used in quantitative and qualitative studies of lactobacilli in saliva and mouth rinses and in dairy

products.

Table 5. Examination of milk and dairy products [60].

Microorganisms	Culture media	Application
Total bacteria	Plate count agar Plate count skim milk agar TGE Agar Milk Agar Standard count agar China-blue lactose agar	Enumeration
Lactobacillus	MRS Agar	Isolation-selective
Streptococci	M17 broth M17 agar	Enrichment-isolation

Some other differential media have been used in research. Three of these, Hansen's Yogurt Agar, Modified Hansen's Yogurt agar and LAB medium differentiate the two bacteria on the basis of colonial morphology. S.thermophilus forms small, smooth colonies, whereas L. bulgaricus forms large, irregular colonies. Later LEE's agar which contains a pH indicator, brom cresol purple and appropriate combination of sucrose and lactose was developed. S. thermophilus, which can use both sugars, produces a substantial amount of acid, whereas L. bulgaricus, which can use lactose but not sucrose, produces less acid. The two organisms can then be distinguished by the intense yellow zone which only surrounds S. thermophilus colonies [61].

Other than these media, $LAPT_{g10}$ and $LAPT_{s4}$, contain peptone, tryptone, yeast extract and tween, have been used for the differential enumeration of yogurt culture [62]. Berkman et al. used tripticase soy agar for the enumeration of S. thermophilus and acidified lactic agar (pH 5.25) for L. bulgaricus. Lactic agar was used for total enumeration of mixed culture [63].

1. 8. Quality Control of Yogurt

If the essential requirements for manufacturing a high quality yogurt were to be tabulated, then the list might look rather like this [21],

1. milk of good quality and adequate SNF
2. correct heat treatment
3. an active, well-balanced, contaminant free starter culture
4. a clean and well-maintained plant
5. correct inoculation rate
6. correct incubation times and temperatures
7. an avoidance of rough handling of coagulum, particularly during cooling
8. correct storage of retail product preferably below 5 °C

1. 8. 1. A) Physical Characteristic of Set Yogurt

The essential gel structure of set yogurt means that the assessment of the product must be approached in a somewhat different manner, for any technique that destroys the delicate coagulum is of little value. The falling sphere technique can be adopted but the most appropriate technique makes use of a conventional penetrometer. The only special requirement is the spindle and cone. A perspex cone, 2.5 cm in diameter and with an apical angle of 100°, was used to examine the consistency of set yogurt in retail pots of 135-140 g capacity. The cone, after being located centrally over the pot covered around 50 % of the exposed surface of the yogurt and hence the risk of edge effects from the carton was minimised. The weight of the spindle was chosen in relation to product, e.g a light spindle (13,4 g) for examination at 42 °C immediately after incubation and a heavier spindle (47,4 g) for assessment of firm coagulum found in yogurt held subsequently at 7 °C for 24 hrs. These changes in spindle weight were necessary to distinguish at a given temperature, between samples of different gel strength, but the fact that comparisons were possible at 4 °C of the retail product prior to final cooling [49].

The technique is therefore, both reliable and versatile, and hence standardising the rheological properties of set yogurt becomes, a straightforward exercise. Other physical features or faults, for example lumpiness usually become apparent during sensory analysis [49].

1. 8. 2. B) Microbiological Analysis

The microbiological examination of the finished product may include checks on the survival of the starter organisms, as well as for the presence of undesirable spoilage of the pathogenic organisms.

Yogurt should contain abundant and viable organisms (Table 6) and there is a general feeling that yogurt should contain live bacteria unless specifically designated as pasteurized or heat-treated.

Table 6. Suggested advisory standards of yogurt [49].

Microorganisms	Satisfactory	Doubtful	Unsatisfactory
S. thermophilus*10^6/ml	>100	100-10	<10
L. bulgaricus*10^6/ml	>100	100-10	<10
Coliforms/ml	<1	1-10	>10
Yeasts/ml	<10	10-100	>100
Moulds/ml	<1	1-10	>10

As far as pathogens are concerned, yogurt with an acidity of around 1 % lactic acid is a fairly inhospitable medium and some pathogens like Salmonella ssp. will be incapable of growth. Coliforms will also be inactivated by the low pH. Staphylococcus ssp., and in particular coagulase-positive strains, can survive in acidic media is a matter of some dispute, but to the date there have no records of staphylococcal food poisoning being associated with the consumption of yogurt in the U. K. for this reason, an examination for staphylococci is not normally required for yogurt and even the test for coliforms is probably of more valuable as an indicator for plant hygiene than a warning that the product may constitute a health

risk.

Table 7. Turkish standards of yogurt [55].

Microorganisms	Limits
S. thermophilus	Should be alive (no count
L. bulgaricus	should be alive (no count)
Coliforms/g	>10 unsatisfactory
Yeast and mould/g	>100 unsatisfactory
E. coli	should not be found

Table 8. Some standard methods for examining the microbiological quality control of yogurt [49].

Country	Colony count	Yeast & mould	Coliform
U. K	Nutrient agar	Malt agar Salt dextrose agar	MC Concey broth, VRBA
U. S	SMA Lactic agar of Elliker	Acidified PDA	VRBA Deoxycholate lactose agar Brillant green lactose bile broth
IDF	Culture medium free from carbohydrates	Agar medium containing yeast extract, glucose oxytetracycline	Brillant green lactose bile broth
Czechoslovakia	-	Malt agar	Meat peptone, lactose and bromthymolblue
West Germany	-	Wort Agar	-
Turkey	-	Acidified PDA	VRBA

More significant procedure from the producer's stand point is the examination for yeast and moulds. Many fungi are little affected by low pH. Yeasts, whether lactose-utilisers like K. fragilis and K. lactis or more cosmopolitan species such as S. cerevisiae, are major concern, and to avoid in carton fermentation (often manifest by a doming of the lid of the carton). Moulds tend to develop more slowly than the yeast and hence should rarely be visible in retail products. Nevertheless, genera such as Mucor, Rhizopus, Aspergillus, Penicillium or

Alternaria can produce unsightly superficial growths of mycelium if cartons remain undistributed for any length of time [34].

1. 8. 3. Chemical Analysis

Many countries have legal standards or at least provisional regulations, covering the composition of yogurt. Overall measurement of total solids and SNF be of value as a check that concentration or fortification has been carried out correctly.

The gravimetric methods of determining fat in yogurt are regarded as most accurate but for routine purposes the normal Gerber method is totally appropriate [28].

The production of lactic acid beyond the point of coagulation is monitored principally in relation to consumer preference. Although the relationship between titratable acidity and pH is not straightforward in a highly buffered system like yogurt, the direct electrometric determination of pH is extremely convenient [49].

Table 9. Chemical composition of yogurt according to Turkish standard [55].

SNF % (w/w)	TS %	Fat content % (w/w)		
min. 12	13.5-15	full fat min. 3.8	medium min. 1.5	non-fat <1.5

Dye binding method can be used successfully to estimate the undenatured whey protein content of skim milk cultured with lactic cultures [23].

The method relys on the ability of dyes to combine with polar groups of proteins of opposite ionic charge. The insoluble complex is then removed by centrifugation or filtration and concentration of unbound dye is assessed from a spectrophotometric curve relating optical density.

1. 8. 4. Organoleptic Characteristics

To some extent, the chemical and physical analyses

will provide a reasonable indication that the normal standards have been achieved, but the use of some form of taste panel to perform a final check is a usual practice. The composition of such panels can rage from one man and a plastic teaspoon through to a full panel of trained testers selected. Organoleptic properties of yogurt can be evaluated interms of appearance and colour, body and texture, and flavour [64].

Table 10. Some term to express possible defects and expected characters of yogurt [49, 64].

Appearance and colour	-Extraneous matter -Lack of uniformity -Unnatural colour -Surface discolouration -wheyying-of, no wheying off, -free whey -fat separation -gassiness -Jelly-like coagulum -Slimy
Body and texture	-Curdy -Gassy -ropy -Too thin -Grainy -Gelatinous, weak/soft -Chalky, stringy -Lumpy or granular -Slimy -rubbery -thin body -wheyed-off
Flavour	-Miscellaneous -bitter, rancid, metallic -Excess acid, acid -Excess sugar -Excess stabilizer -Excess milk powder -Yeasty, cheesy -Unclean, coarse, flat, undeveloped -Typical pleasant yogurt tast -Mild to slight acidic
Aroma	-not too sour -aromatic

1. 9. Yogurt: Nutritive and Therapeutic Aspects

1. 9. 1. Yogurt and Health

It was suggested that one aspect of approaching senility in humans involved an undesirable passage of noxious compounds from the intestine to the blood stream, and that these chemicals arose from the action of putrefactive bacteria in the lower ileum and colon if the activity of these bacteria could be suppressed then, so it was argued, the adverse effects of their metabolic products would no longer be manifest and individual might anticipate longer and healthier life. Matta et al. tested the survival rate of Salmonella typhimurium, Y. enteracolitica and Campylobacter jejuni in yogurt and none of the pathogens survived in yogurt stored at 37 °C for more than 25 hrs [65]. Such a hypothesis sounded perfectly reasonable and the role of yogurt in curtailing the putrefactive bacterial action was readily explained as follows (65):

a) lactic acid bacteria in yogurt are tolerant of a low pH, whereas most bacteria shows optimum growth and metabolism around neutrality. Therefore, as the acidic yogurt passed along the intestine, the lactic acid in the food and perhaps, that still being secreted by the bacteria, would kill the undesirable microflora. Bacteria in the large intestine produce a range of phenolic compounds, such as sketol and indole, which could damage living tissue. Also there is a definite concern over their possible involvement in the initiation of cancer in the lower intestine. Lactic acid bacteria suppress the production of these compounds [18].

b) It was further suggested that this effect of yogurt was enhanced by the ability of L. bulgaricus to become established in the intestine in a viable state and to gradually dominate the resident microfolora. This latter changed ensured the continued absence of putrefactive organisms even during periods of reduced yogurt availability and hence the vitality of the consumer would maintained (66).

1. 9. 2. Therapeutic Effects

1. 9. 2. 2. Gastrointestinal Disorderness

Yogurt has been used for and believed to be effective in the prevention and treatment of illness in both animal and man. Its most common use has been in gastrointestinal disorders such as diarrhoea, particularly infantile gastroenteritis and constipation [66].

Yogurt itself has been reported to have antibacterial effects on pathogens that resist to standard antibiotics [67].

Pulusani et al. reported that S. thermophilus produces a potent antimicrobial compound(s) with a wide spectrum of antimicrobial activity against Bacillus sp., Salmonella typhimurium shigella sp., Pseudomonas sp., Escherichia coli and various strains of S. lactis [68].

Abdel-Bar et al. isolated antibiotic substances from the media of L. bulgaricus and this substance active against Pseudomonas sp. and Staphylococcus aureus [69].

1. 9. 2. 2. Coronary Heart Disease

In view of the current interest in the relationship between diet, blood cholesterol levels and coronary heart disease, reports of the hypocholesterolemic effects of yogurt are noteworthy. They have been several suggestions regarding of the reason for the hypocholesteromic effects of yogurt. It was shown that conversion of acetate to cholesterol was inhibited and presence of hydroxymethylglutarate in yogurt may inhibit cholesterol synthesis. Including yogurt daily in the diet significantly reduced fasting total serumcholesterol 10- 12 % in human adult males [70]. Calcium, orotic acid, lactose and casein have all been suggested as possible hypocholesterolemic factors [71].

Yogurt has been useful for individuals with allergies to milk proteins since yogurt proteins have a reduced allergenicity. Other interesting therapeutic uses of yogurt include its use in the treatment of non-specific vaginal discharge and inhibitory effect on tumor cells (65).

Yogurt is particularly rich source of calcium. Suffers of lactose intolerance who cannot drink milk often develop osteoporosis as a result of deficiency of dietary calcium. Since lactose intolerance can tolerate yogurt, this product is useful for alleviating deficiency [13]. It is also useful source of calcium for middle aged women who suffer bone deformity during and after menopause due to calcium deficiency [72].

Partial digestion of protein in yogurt may have beneficial effects for individuals with poor digestive capacity.

1. 10. Preservation Techniques of Yogurt

In general, the keeping quality of yogurt is limited because of the growth of yeasts and molds. This can even occur at cold (5 to 7 °C) storage temperature. If yogurt is not contaminated, it will become too acid and acquire a bitter taste after a certain time because of further acidification and a possible protein hydrolysis caused by L. bulgaricus.

Yogurt is a perishable fermented dairy product which has a shelf-life of up to 3 weeks under refrigeration, depending on the standard of hygiene during manufacturing and packaging. Shelf-life of yogurt is 10 days at refrigeration temperature in Turkey [75]. The keeping quality of yogurt can be improved by various methods. These are; gas flushing, use of preservatives, concentration, drying, freezing, use of aseptic equipment, post heat treatment, microwave treatment, sterilization.

Another approach is the genetic manipulation of yogurt cultures to overcome the problems both over-acidification and maintenance of viability to yield yogurt with desired characteristics [74].

1. 10. 1. Preservatives

Preservatives are widely used in the food industry and in particular for preservation of fruit. However, it is

reasonably safe to assume that the use of preservatives is prohibited in the yogurt industry although use of fruit to flavour yogurt may carry some preservatives into it. WHO has suggested that preservatives like sorbic acid and its salts (potassium, sodium and calcium), sulfurdioxide and benzoic acid can be used at levels of 50 mg/kg (singly in fruit or in combination) in final product, if they come exclusively from the flavouring substances. Such a proposal is only applicable to fruit-flavoured yogurt and not the natural flavour (49).

The presence of preservatives, sodium sorbate, potassium sorbate or sodium dehydroacetate at levels as low as 0.1 % (w/v) in yogurt can effect the growth of the starter culture and cause the acetaldehyde level to decrease more rapidly during storage (42).

1. 10. 2. Solute Addition

Lactic acid development can be reduced by solute addition. This inhibition can be partially explained by the decrease of the amount of water available for microorganisms. Water activity can influence enzyme action not only by acting directly on enzyme kinetic parameter but also by inducing conformational changes in the substrate (50). Larsen and Anon searched the effect of a_w of milk on acid production by S. thermophilus and L. bulgaricus. They adjusted the a_w of milk between 0.992-0.943 with glycerol. Decrease of a_w inhibited acid production by both organisms. Minimum a_w for acid production was always lower when a_w of milk was adjusted with glucose or sucrose than with glycerol. The effect was more important on Lactobacillus bulgaricus, than S. thermophilus. Result showed that water activity was an important parameter for the fermentation of milk for yogurt production. Modification of a_w occurred during conditioning of raw material for fermentation, especially by the addition of natural sweeteners [75].

Lacroix and Lachance prepared samples by adding humectants, NaCl, sorbitol, and sucrose. Water activity ranged from 0.974-0.908. Proteolysis of samples remained

limited and lag time for yeast and mold growth was largely increased by humectant addition. A 1% increase in salt, sucrose and sorbitol content of yogurt resulted in about 7.7, 1.5, 1.3 days increase in shelf-life respectively. Similarly, a 0.01 decrease in a_w increased shelf-life by 11.5 days [76].

1. 10. 3. Gas-flushing

An alternative method for prolonging the keeping quality of yogurt, in particular the fruit-flavoured types, is the use of gas flushing (carbondioxide or nitrogen). The purpose of such process is to restrict the growth of yeasts and moulds in the product. Corbondioxide flushing was not suitable if yogurt was placed in PVC containers sealed with an aluminium laminate because such containers are permeable to carbondioxide and oxygen. Furthermore nitrogen flushing was effective in suppression Oidium ssp. but ineffective against facultative anaerobic yeasts. However, Braun claimed that carbondioxide improved the shelf-life of yogurt for several months, but there is no indication of the type of packaging material used. The combination of post incubation heat treatment and gas flushing with carbondioxide improved the keeping quality of yogurt for more than 4 weeks at 15 °C [3, 49].

Although application of gas-flushing can improve the keeping quality of yogurt, the feasibility of using such a process in the yogurt industry is mainly governed by economic factors and depends on use of packaging materials which are impermeable to gasses used (49).

1. 10. 4. Food Preservation by Use of High Temperatures

The use of high temperatures to preserve food is based on their destructive effects on microorganisms. By high temperatures are meant any and all temperatures above ambient. Some factors or parameters of microorganisms and their environment affects the destruction rate of m.o's. These factors are presented below: [27, 29]

1. 10. 4. 1. Effect of Water

Heat resistance of microbial cells increases with decreasing humidity or moisture. Dried microbial cells placed into test tubes and then heated in a water bath are considerably more heat resistant than moist cells of the same type, because it is well established that the protein denaturation occurs at a faster rate when heated in water than in air. Heating of wet proteins causes the formation of free -SH groups with a consequent increase in the water-binding capacity of proteins. The presence of water allows for thermal breaking of peptide bonds, and consequently confers a greater refractivity to heat.

1. 10. 4. 2. Effect of Fat

In the presence of fats, there is a general increase in the heat resistance of some microorganisms. This is some times referred to as fat protection and is presumed to increase heat resistance by directly affecting cell moisture. Long chain fatty acids are better protectors than short-chain acids (27).

1. 10. 4. 3. Effect of Salts

The effect of salt on the heat resistance of m. o's is variable and dependent upon the kind of salt, concentration employed and other factors. It has been observed that some salts have a protective effect upon microorganisms while others tend to make cells more heat sensitive. It has been suggested that some salts may decrease water activity and thereby increase heat resistance by a mechanism similar to that of drying, while other may increase water activity (e.g Ca^{+2} and Mg^{+2}) and consequently increase sensitivity to heat (29).

1. 10. 4. 4. Effect of Carbohydrates

The presence of sugars causes an increase in the heat resistance of microorganisms. This effect is most likely due to decrease in water activity that is caused by high concentrations of sugar (49).

1. 10. 4. 5. Effect of pH

Microorganisms are most resistant to heat at their optimum pH of growth. As pH is lowered or raised from this optimum value, there is a consequent increase in heat sensitivity. Advantage is taken of this fact in the processing of high acid foods where considerably less heat is applied to achieve sterilization (27).

1. 10. 4. 6. Effect of Proteins and Other Substances

It is well known that proteins in the heating medium have a protective effect upon microorganisms. Consequently high protein content foods must be heat processed to a greater degree than low protein content food in order to achieve same end results.

1. 10. 4. 7. Effect of Number of Organisms

The larger the number of organisms, the higher is the degree of heat resistance. It has been assumed that the mechanism of heat protection by large microbial populations was due to the production of protective substances excreted by the cells. Since proteins are known to offer some protection against heat, many of the extracelluar compounds in a culture would be expected to be protein in nature and consequently capable of affording some protection.

1. 10. 4. 8. Effect of Age of Organisms

Bacterial cells tend to be most resistant to heat while in the stationary phase of growth (old cells) and less resistant during the logarithmic phase. Heat resistance has been reported to be high also at the beginning of the lag phase but decreases to a minimum as the cells enter the log phase (27, 49).

1. 10. 4. 9. Effect of Temperature of Growth

The heat resistance of microorganisms tends to increase as the temperature of incubation increases. Although precise mechanism of this effect is unclear, it is conceivable that the genetic selection favours the growth

of the more heat resistant strains at successively high temperatures.

1. 10. 4. 10. Effect of Inhibitory Compounds

As might be expected, a decrease in heat resistance of most microorganisms occurs when heating takes place in the presence of microbial inhibitors such as heat resistant antibiotics, SO_2 and others. The use of heat+ antibiotics and heat+nitrate has been found to be more effective in controlling the spoilage of certain foods. The practical effect of adding inhibitors to foods prior to heat treatment is to reduce the amount of heat that would be necessary if used alone.

1. 10. 4. 11. Effect of Time and Temperature

One would expect that the longer the time of heating, the greater is the killing effect of heat. As temperature increases time necessary to achieve the same effect decreases. Also size of the heating vessel or container and its composition (glass, metal, plastic etc) are important. It should be obvious that it would take longer to affect pasteurization in large containers than in smaller ones. The same would be true of containers with walls that did not conduct heat as readily as others [26].

The resistance of cells of bacteria varies widely with the species. A few general statements can be made about heat resistance of vegetative cells of bacteria;

1. Cocci usually are more resistant than rods, although there are many notable exceptions.
2. The higher the optimal and maximal temperatures for growth, the greater the resistance to heat is likely to be.
3. Bacteria that clump considerably or form capsules are more difficult to kill than those which do not.
4. Cells high in lipid content are harder to kill than other cells.

A few examples of thermal death times of bacterial cells were shown in Table 11.

Table 11. Thermal death times of bacterial cells [29].

Bacterium	Time (min.)	Temperature, °C
Staphylococcus aureus	18.8	60
E. coli	20-30	57.3
S. thermophilus	15	70-75
L. bulgaricus	30	71
Salmonella typhose	4.3	60

It should be kept in mind that these thermal death times are for various concentrations of cells, heated in different substrate, and might be higher or lower under other conditions.

1. 10. 5. Post Production Heat Treatment of Yogurt

Application of heat after incubation is now being used in same instances as a preservation method and the product resulting after the heat treatment is referred to as **pasteurized** or **UHT yogurt**.

Effect of heat treatment on quality and extend of survival or destruction of lactic acid bacteria during pasteurization may depend upon the heat resistance of bacterial species, time-temperature combinations encountered, initial number of starter culture present in yogurt, initial pH value of yogurt, application of heat treatment.

Pasteurization conditions similar to those used for milk are suitable for yogurt. However it has been reported that a shorter time of heat treatment may be required because of a lower pH of yogurt as compared to milk.

The main problems associated with pasteurized yogurt are loss of flavour (less significant in fruit-flavoured yogurt) and whey syneresis, but if set yogurt is kept perfectly still during pasteurization, there is no need for addition of stabilizers. Another recommended method to overcome whey syneresis is to heat yogurt at 70 °C for 30-40 sec and pack at 55-60 °C [3].

Although post-production heat treatment of yogurt prolongs the shelf-life of product, it raises the question

of the definition of yogurt since most existing standards stipulate that yogurt must contain an abundant viable count of S. thermophilus and L. bulgaricus. However, it appears reasonable to reserve the term "yogurt" for the traditional product and to designate the new product "pasteurized" or "UHT" yogurt.

El- Abboudi et al. studied on the heat-shocking of lactobacilli. They established adequate conditions, for heat shocking cells of lactobacilli, to sufficiently suppress lactic acid production without damaging the proteolytic enzyme system important for cheese maturation. In this study bacterial suspension, pH 6.5, was pumped through a sterilized steel coiled tube immersed in water bath at 65-78 °C with a flow rate 125 ml/min., by the use of peristaltic pump. The best combination for maximum retardation of lactic acid production and minimum damage to proteolytic system was obtained by treating cells at 67 °C for 22 sec. Following such treatment, lactic acid production was retarded by 24 hr. They reported that there is a reduction in the cell viability. Cell mortality did not mean the cells were dead but they may have been injured in the cell wall or membrane to the extend that they could no longer grow [77].

Partially inactivated culture by heat shocking treatment can be adopted to stirred yogurt production, after fermentation was completed. Application of such treatment to set yogurt processing can disturb the texture.

Speck and Geoffrion do research on heating yogurt. Heat treatment was carried out in water bath shaker and agitated at 250-300 rpm. Although time required to reach the test temperature was short, this process disturb the texture during stirring. They applied heat treatment to yogurt samples with different initial pH and concluded that heat was more damaging to starter in yogurt at pH 4.2 than in yogurt at pH 4.6. [40].

Another study by Waes on the heat shock treatment of set yogurt, prepared by 5 different yogurt culture,

indicated that sensitivity of cultures to heat treatment was different. Some cultures survived easily at heat treatment but some could not and failed the test if the requirement "10^6 m.o's should be present per ml yogurt" taken into consideration [78].

According to TSE, microorganisms S. thermophilus and L. bulgaricus in final product, yogurt, must be viable and abundant. Viable cell number is a good index for evaluating degree of heat damage exerted during pasteurization of yogurt as well as for optimizing processing conditions.

1. 10. 6. Microwave Treatment

Microwave ovens have become a common place tool in both home and workplace. This devices provide convenience, speed and economy in food preparation especially in large institutional facilities [79].

An increasing consumer demand for foods that offer greater convenience and time savings in preparation has led to a home microwave oven saturation level of more than 70 %. This demand is providing a strong marketing incentive to the food manufacturing and foodservices industries to consider innovative approaches in food formulation and packaging design to develop new and improved microwavable consumer products for home oven use [80].

Recent improvements in the design of high-powered microwave ovens offered rapid and economic methods for manufacturing food products of high organoleptic and nutritional value. However, the development of industrial processes has been rather disappointing. With a few notable exceptions, e.g., tempering of frozen foods [79], precooking of poultry and pork products [81, 82, 83] and drying of pasta [84] and onions, there are not many large scale microwave installations in food industry.

1. 10. 6. 1. Electrical and Physical Properties of Microwave

Microwaves are generated by a magnetron-a device that converts electrical energy at low frequencies, e.g. 60

cycles/sec,Hz, into an electro-magnetic field with centers of positive and negative charge that change direction billions of times each second. Penetration and heating of foods by microwave energy sources are instantaneous. In contrast, conventional heating methods transfer thermal energy from products surfaces toward their center 10-20 times more slowly. As the microwave enter the product, they interact with the regions of positive and negative charges on water molecules, that rotate the molecules in the electrical field and the dipoles. This results in the disruption of hydrogen bonds between neighbouring water molecules and generates heat by molecular friction [83].

Positive and negative ions of dissolved salts in foods, such as common table salt, sodium chloride, also interact with an electrical field by migrating toward oppositely charged regions of the electrical field and disrupt hydrogen bonds with water to generate additional heat [86].

1. 6. 10. 2. Microbial Inactivation

Microwave energy is believed to inactivate microbes by conventional thermal mechanisms, e.g., thermal denaturation of proteins and nucleic acids. This is supported by experiments comparing the survival of vegetative cells and spores exposed to microwaves with survival under conventional heating methods [87].

There was a limited study comparing microwave cooking and conventional cooking with respect to survival of bacteria in the foods. There is no easy way to compare the two methods, because they operate on different principles [88]. The time used for microwave cooking was that recommended by manufacturer. The time used for conventional cooking depended on the subjective quality of "doneness" of the foods involved. It is readily apparent that microwaves will kill bacteria faster than conventional methods on the same time scale [89]. Wrapping foods during microwave cooking also effectively reduced bacteria. This was due to prevention of internally generated heat from escaping to

the cool environment [81].

Effects of microwaves on microorganisms in foods are influenced by the intrinsic characteristics of foods (pH, moisture level, oxidation-reduction potential, nutrient content, antimicrobial constituents, biological structures, chemical composition of the food and shape or size of the food) and extrinsic characteristics (temperature, humidity and gases of the environment, frequency and intensity of radiation, length of time of exposure, position of the food in the effective field and other factors) [90, 91]. Furthermore, the physical and chemical composition of the organisms being irradiated, their stage of existence (vegetative cell, spore and phase of growth, moist or wet etc.) and numbers present are also important factors [87]. Table 12 indicate that bacterial species differ in their susceptibility to microwave inactivation.

Table 12. Survival of bacteria in tomato soup after 2 min of microwave exposure [87].

Organisms	Percent survival
Streptococcus faecalis	7.9
Escherichia coli	0.93
Salmonella typhimurium	0.87
Staphylococcus aureus	0.46
Pseudomonas fluorescens	0.41
Alcaligenes viscolactis	0.0070
Salmonella pullorum	0.046
Micrococcus rhodochrous	0.0036
Shigella flexneri	0.0029
Proteus vulgaris	0.018

The major observations related to food spoilage and food safety concerning microwave cooking of foods are summarized below:

1. Microwave-heating of food is more food dependent than conventional heating because of the mechanism of generation of heat by microwaves.

2. The manufacturer-recommended microwave-treatment time for some food products may not be adequate for destroying high levels of bacteria in foods. It is

important that food to be irradiated has acceptable microbiological quality (i.e., counts as low as possible before irradiation).

3. Use of microwaves in combination with conventional heating methods will result in more uniform heating of foods and destruction of bacteria.

4. Heat generated by microwaves in food will kill naturally-occurring mo's as long as the size and type of food are carefully correlated with exposure time.

5. Microwaves exert different killing effects on individual bacterial species. Spores are more resistant than vegetative cells and different species have different survival ability under microwave irradiation [87, 91].

1. 6. 10. 2. Applications in Food Processing

Microwave process are slowly gaining acceptance by the food industry for various processing operations such as blanching, cooking, drying, pasteurization, sterilization and thawing of bulk food products [92]. Perhaps the most widely accepted microwave food processing are the precooking of some meat products and tempering or thawing of frozen foods [93, 94]. Two new applications which also seem promising for future commercial process development are aseptic packaging and flexible pouch sterilization process. Such processes offer a significant potential for rapid and uniform heating of food products, reduced thermal inactivation of essential nutrients and increased retention of food quality factors compared to conventional thermal processes [95]. Industrial food processes may be classified in six major unit operations, as shown in Table 13.

Microwave of food processes has been generally successful for low and intermediate- moisture products and for high moisture solid food when combined with convection heating methods. But successful development of new industrial microwave will require additional research. It is important to recognize that the microwave heating characteristics of food products may vary considerably with the processing frequency and with the temperature, chemical

composition and physical state of the product [96].

Table 13. Major Unit operations in Microwave Food Processing [80]

Unit operation	Major objective
Blanching	Inactivate spoilage enzymes
Cooking	Modify flavour and texture
Dehydration	Reduce moisture content
Pasteurization	Inactivate vegetative microbes
Sterilization	Inactivate microbial spores
Tempering	Raise temperature below freezing

Decareau reported some 250 microwave installations in operation at that time, about 200 for tempering of frozen meats and fish, 16 for precooking bacon, poultry and patties, 30 for drying pasta, onions, and snack foods, 5 for vacuum concentration of fruit juices and 3 for pasteurizing bread and yogurt [92].

Pasteurization of dairy product especially milk by microwave radiation should be of interest to food scientist and processors, yet not many reports could be found on this subject. Chernova reported that pasteurization of milk could be achieved using continuous (2450 MHz) microwave radiation for 15 sec with a resultant temperature of 72 °C. Taste panel members found no significant difference between microwave-pasteurized milk and conventionally pasteurized milk (62.8 °C for 30 min.) [87].

Microwave vacuum drying which seems a promising alternative to the traditional process with respect to cost as well as quality has been applied to produce yogurt by Kim and Bhowmik [42].

An alternative method, which could be used to pasteurise yogurt is the application of the multiple-frequency or microwave technique. The principle of this method consist of a two stage, rapid dielectric heating of yogurt in plastic cups.

The first stage is applied horizontally (low frequency microwaves with high penetration), while the second stage is applied vertically (high frequency microwaves with low penetration). The actual pasteurization is at a lower

temperature than required for a conventional process and the treatment takes place during the passage of the yogurt cups through a water bath. The two stages are complementary to each other and need to achieve adequate pasteurization. This system results in the destruction of yeast and moulds, but no adverse effect on the milk proteins or starter bacteria [49].

1. 10. 7. Nisin

Nisin is a polypeptide antimicrobial substance or bacteriocin produced by the fermentation of a modified milk medium by certain strains of the lactic acid bacterium, Lactococcus lactis [97]. It shows antibacterial activity against a range of gram positive bacteria and also limit sporulation of a range of Bacilli and Clostridia. This property has been exploited by the food industry and it is very widely employed as a preservative for processed foods particularly milk products [98]. Nisin is composed of 34 amino acids arranged in a five-ring structure and includes the unusual a.a lanthionine, β-methyl-lanthionine, dehydroalanine and dehydrobutyrine (Figure 3) [99].

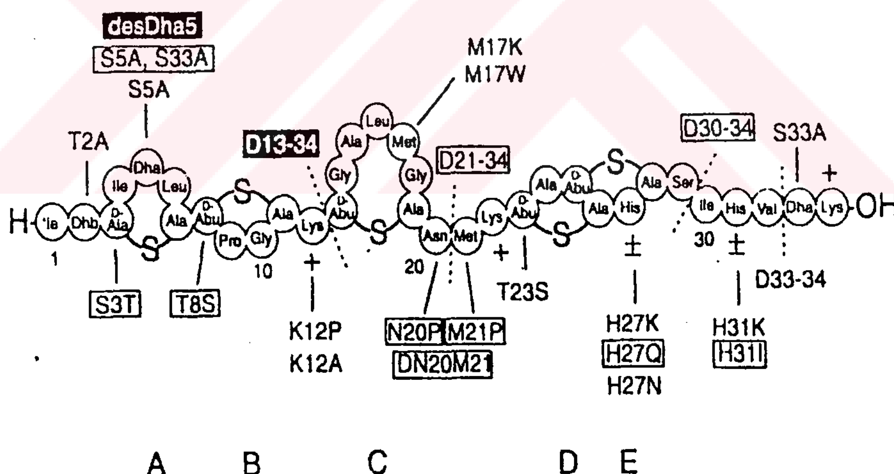

Figure 3. Schematic structure of nisin A. When indicated boxed, alteration of the molecule resulted in a dramatic loss of activity. Modifications indicated in black boxes caused an almost complete loss of antimicrobial activity. Fragmentation of nisin molecule by proteolysis is indicated by dotted lines. Dhb, dehydrubutyrine; Dha, dehydroalanine; D-Ala, alanine moiety of lanthionine or 3 methyllanthionine D-Abu, α-aminobutyric acid moiety of 3-methyllanthionine [99].

1. 10. 7. 1. Stability

The nisin molecule is acidic in nature and exhibits greatest stability under acidic conditions. It is also more soluble at lower pH. It has low solubility in body liquids, it is unstable at physiological pH (pH 7-7,5) [97].

Large molecules such as those in milk or broth had a protective effects so that the degree of inactivation is less drastic in foods than in buffer. Such protection of nisin in various foods during heat treatment was confirmed. Loss of nisin activity occurs during storage of foods. Losses are more pronounced at high pH and high temperature [98].

In one study [100], factors assumed to be responsible for the loss of activity of nisin in Camembert cheese during the first week of ripening reported as;

1. inappropriate conditions for pH and temperature for nisin activity
2. adsorption of nisin on cheese components, fat, proteins, lactic acid bacteria
3. reduced nisin diffusion in the curd matrix
4. resistance of target bacteria:
   a. loss of sensitivity due to inappropriate physiological conditions
   b. occurrence of naturally resistant cells in the population
   c. acquired resistance in presence of nisin
5. combination of the above factors.

1. 10. 7. 2. Antimicrobial Activity

Nisin like other bacteriocins, possess antimicrobial activity against a limited range of microorganisms. It does not inhibit Gram negative bacteria, yeasts or fungi. Gram negative bacteria such as E. coli are affected by nisin when the outer membrane is disrupted. Nisin inhibit a wide range of gram positive bacteria, particularly those that produce spores. Thus within the gram positive bacteria, nisin inhibits certain strains of species of

Staphylococcus, Streptococcus, Micrococcus and Lactobacillus and the majority of spore forming species of Clostridium, Salmonella [101, 102, 103] and Bacillus with the spores being more sensitive than the vegetative cells [97].

1. 10. 7. 3. Mode of Action

Cytoplasmic membrane is the site at which nisin activity is believed to occur and loss of cell viability may involve interaction of dehydroalanine residues in nisin with membrane sulfhydrly groups. Cell inactivation is a result of cellular damage, which can range from disruption of proton motive force to loss membrane integrity [100].

The outer membrane of gram-negative bacteria act as a permeability barrier for the cell. It is responsible for preventing molecules such as antibiotics, detergents and dyes from reaching the cytoplasmic membrane. Gram negative bacteria are not generally sensitive to nisin. Although the cytoplasmic membrane should be susceptible, the outer membrane protects the cell by excluding nisin. Magnesium ions serve to stabilize the lipopolysaccharide layer of outer membrane. Chelating agents such as EDTA, bind magnesium ions in the lipopolysaccharide layer and produce cells with increased susceptibility to antibiotics and detergent. So nisin treatments, applied in combination with EDTA were effective against several gram negative bacterial species. Such as E. coli is only affected by nisin when the outer membrane is disrupted [104].

Nisin cause disruption either resulting in leakage of essential cellular material such as ATP from cell or in more severe cases lysis. Disruption is caused by nisin inactivating sulphydryl groups in the cytoplasmic membrane.

The overall results is the formation of large pores in the cytoplasmic membrane which allow the efflux of ions and small intracellular molecules (potassium, amino acids, ATP) finally resulting in cell death and possibly lysis (Figure 4).

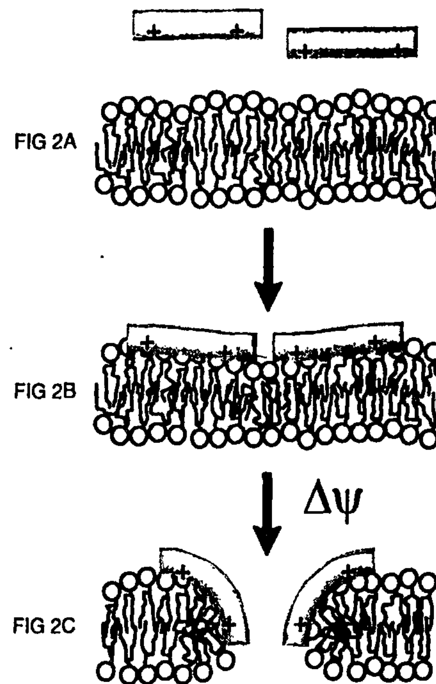

Figure 4. Wedge-model of pore-formation. At first nisin is in solution (A). Then bound nisin disturbs the lipid dynamics (B). Above the threshold level, nisin insert. Coinsertion of bound, anionic phospholipids results in bending of the lipid surface giving rise to a wedge-like nonspecific pore (104).

In addition to the pore forming activity, nisin is known to inhibit the outgrowth of bacterial spores and it might interfere with some enzymatic step in the biosynthesis of the cell wall. Nisin also affects the activity of autolytic enzymes [105].

1. 10. 7. 3. Use of Nisin

Because of low toxicity use of nisin as a food preservative has attached a number of researches to look for potential applications.

Nisin has provided to be a most effective preservative in pasteurized processed cheese [106, 107]. One application of nisin in dairy products include the extension of shelf-life of dairy desserts. Such products cannot be subjected

to full sterilization without damaging the appearance, taste, or texture of the product. Pasteurized products have a limited shelf-life but the addition of nisin can give a significant increase. While nisin additions to milk are not permitted in the UK and other countries with temperate climates, it is permitted in some Middle East countries where special shelf-life problems are experienced in supplying good quality pasteurized milk to customers. Such problems can be attributed to warm climate, the need to transport the milk from production areas over long distances to the centre of population and inadequate refrigeration facilities. Due to the limited refrigeration facilities, deterioration of the finished fermented products starts quickly during storage interms of growth of the mo's (lactic and non-lactic) present naturally or as contaminants.

The shelf-life of milk is relatively short and nisin has been demonstrated to have a beneficial effect more than doubling the shelf-life at chilled, ambient and elevated temperatures at nisin levels of 30 to 50 IU/ml.

Nisin can be used also in canned foods, cured meat, poultry products, chocolate and sterilized, evaporated and condensed, fluid milk, kleer, cream [108].

Nisin has potential in controlling spoilage lactic acid bacteria mainly lactobacilli and pediococci in beer and wine.

In the US nisin is generally recognized as safe and is approved for use in some pasteurized cheese spreads to prevent Clostridium botulinum. There are numerous application of nisin as a food preservative including shelf-life extension of dairy products and spoilage prevention in canned foods.

Use of nisin is allowed in various products in 47 countries including USA and UK. Table 14 summarize the use of nisin in various products.

Table 14. Countries Permitting the Use of Nisin [97].

Country	Food in which nisin is permitted	Max. level IU nisin/g food product
US	Certain pasteurized process cheese	10,000
Turkey	Various cheeses	4,000
UK	Cheese Canned foods, clotted cream	No limit
Saudi Arabia	Some foods&dairy products	No limit
Qatar	Milk Milk products	500 No limit
Peru	Nisin is permitted additive	No limit
Italy	Cheese Canned vegetables Confectionary creams	500 100
Abu Dhabi	Pasteurized milk Flavoured milk Long life milk Processed cheese Cheese Other dairy products Canned vegetables	No limit
Australia	Cheese Canned tomatoes Canned tomato puree&paste Canned soups	No limit
Bolivia	Use not prohibited in foods	No limit
Cyprus	Cheese Clotted cream Canned vegetables	No limit
Czechoslovakia	Bakery products&fillings Mayonnaise Processed cheese Prepared foods Semi-prepared foods Canned vegetables Babyfoods-dairy&vegetables	500

1. 10. 7. 5. Toxicological Studies

The fact that nisin is produced by lactic streptococci which occur naturally in raw milk is an indication of its low toxicity. A total 251 raw milk samples were examined from 9 countries spread over 3 continents. 109 of the samples were found to contain nisin producing m.o's (97).

Nisin is rapidly inactivated in the intestine by digestive enzymes and it cannot be detected in the saliva

of humans ten minutes after consuming liquid containing nisin [97].

1. 10. 7. 6. Recent Studies About Nisin

There are numerous studies about the nisin in the preservation of foods.

Kumar and Parasad [109] studied the inhibitory effect of nisin on various lactic and non-lactic microorganisms. Sixteen strains belonging to the genera Lactobacillus and Bacillus exhibited wide difference in behaviour towards nisin. The MIC was shown in Table 15. This information on nisin sensitivity may be of great use in controlling the bacterial growth in industrial fermentation and preservation of fermented milk and milk products.

Table 15. Minimal inhibitory concentration of nisin against Lactobacillus and Bacillus sp. in MRS and peptone yeast extract broth, respectively, at optimum pH and temperature, after 24 h incubation [109].

Culture	MIC (RU/ml)
Lactobacillus sp.	
L. plantarum R	100
L. plantarum 89	20
L. acidophilus 1899	35
L. acidophilus R	25
L. acidophilus 447	50
L. delbruecki subsp. bulgaricus 1373	50
L. delbruecki subsp. bulgaricus RTS	50
L. delbruecki subsp. bulgaricus 1373	30
L. delbruecki subsp. bulgaricus W	15
Bacillus sp.	
B. cereus 430	45
B. cereus 10876	75
B. subtilis 9144	150
B. subtilis 6633	125
B. subtilis 441	135
B. stearothermophilis 953	45
B. stearothermophilis 37	0.5
B. stearothermophilis 38	1.0

They examine the nisin sensitivity of 10 strains of lactococcus ssp. (nisin producers and non-nisin producers) in Elliker broth and skim milk following Table 16 summarize this study.

Table 16. Comparative MIC of nisin against Lactococcus sp. in Elliker broth and skim milk at optimum temperature and pH [110].

Culture	MIC (RU/ml) Elliker broth	MIC(RU/ml) skim milk
Non-nisin producers:		
Lactococcus lactis subsp lactis (C_{10})	35	75
L. lactis subsp. lactis (ML8)	75	175
L. lactis subsp. diacetylactis (DRC1)	5	25
L. lactis subsp. diacetylactis (DRC2)	25	35
L. lactis subsp. ceremoris (C1)	35	100
L. lactis subsp. ceremoris (C3)	175	225
S. salivarus subsp. thermophilus(H)	75	100
S. salivarus subsp. thermophilus(I)	125	150
Nisin producers:		
L. lactis subsp. lactis (496)	13200	13500
L. lactis subsp. lactis (440)	1600	2000
	14000*	14200*
	2000*	2400*

* MIC after 18 hrs incubation

Stevens et al. inactivate Salmonella species, commonly associated with food borne illness, and other gram negative bacteria, by using nisin and chelating agents (EDTA, ethylenebis, tetraacetic acid, citric acid monohydrate, sodium phosphate dibasic). They also studied the effect of nisin concentration, incubation temperature and protein interference on the inactivation. The most effective treatment consisted of 50-100 micrograms/ml nisin applied in combination with 20 micrograms EDTA or citric acid monohydrate at a temperature range of 30 to 42 °C [111].

Stevens et al also searched the antimicrobial action of nisin against Salmonella typhimurium lipopolysaccharide mutants. Nisin sensitivity was associated with the extent of saccharide deletions from the outer membrane core oligosaccharide. The results indicated that the core oligosaccharide in lipopolysaccharide plays a role in nisin sensitivity [112, 104].

Shefet et al determine the efficiency of nisin containing preparations for reducing the population of Salmonella typhimurium NAR on broiler carcasses to extend shelf-life. They compared the results with the 20 ppm

chlorine solution. Numbers of survivors following a 30 min. dip ranged from <10 to $2,57*10^1$ organisms per ml of skin rince for nisin formulation versus $1,32*10^2$ organisms per ml on the chlorine-treated drumsticks [111].

Khattub et al. work on the sensitivity of five strains of Listeria monocytogenus to nisin at different pH either on BHI agar and in skim milk. MIC ranged between 4000-5000 IU of nisin/ml at pH 7 dropping to 500 IU/ml at pH 5.0. The strain was completely eliminated from the acidified milk at pH 4.5 containing 250-500 IU of nisin/ml respectively and at pH 5.0 and 500 IU nisin/ml after 6 hr at 37 °C [112].

Mahnoud and Khattab worked on stimulating the growth and nisin production by Lactococcus lactis subsp lactis in milk. Nisin producing strains Lactococcus lactis subsp lactic and L. lactic subsp. lactis grew slowly in milk with weak nisin production. Addition of 0.5 to 2 % tomato juice or 1% glucose to the medium or treating the milk with β-galactosidase enhanced both growth and nisin production.

They concluded that glucose and enzyme treatment caused about 3 to 5 fold increase in nisin production. On the other hand culturing these strains with other lactic acid bacteria had antagonistic effect on acid production by some of the cultures [113].

Khattab et al. used nisin producing lactic starters to control the spoilage and growth of some pathogenic m.o.'s in sour cream. Lactoccous lactis subsp. lactis in combination with Lactococcus lactis subsp. cremoris and Leuconostoc mesenteroides subsp. cremoris were used in sour cream manufacture. The presence of 190 microgram/g nisin inhibited the Listeria monocytogenes and Staphylococcus aureus within 3-7 days while control contained 52 and $9.2*10^2$ cfu/g after 15 days, but no clear effect was noticed on E. coli [114].

1. 10. 8. Lysozyme

Lysozyme is an enzyme present in many animal and human secretions. Human milk contains on average 390 mg/l while cow's milk contains only 130 microgram. The lysozyme

currently on the market is prepared from the whites of hen's eggs. It is a basic protein, stable at acidic pH even at comparatively high temperatures, but labile at alkaline pH values [115].

Lysozyme, which is also known as muramidase, catalyses the hydrolysis of β-1,4-glycosidic bonds between the residues of N-acetylmuramic acid and N-acetylglucosamine in peptidoglycon (murein), a compound found in the cell walls of most bacteria, but present particularly large quantities in gram positive bacteria. Its effect is to produce lysis of the bacterial cells in the culture, resulting in inhibition of growth and death of the cells (Figure 5) [79].

Egg-white lysozyme, which hydrolyses N-acetylmuramide linkages, is most often used to degrade bacterial cell walls. The activity of lysozyme is influenced by the pH and ionic strength. Hen egg white lysozyme has been shown to be active over a wide range pH range (pH 4-10), most active at pH range of 6.7-8.6. It requires an ionic strength of at least 0.01.

A number of variables determine the success of a lysis method. These are, strain differences, choice of growth media, whether the cells are processed immediately or frozen, the presence of protease inhibitors, the choice of buffers, growth phase at which the cells are harvested. Degradation of peptidoglycon in gram negative cells is more difficult by the presence of asymmetric lipid bilayer. Thus gram negative bacteria are less susceptible than gram positive bacteria to lysozyme [116].

In 1967 Pulay, by adding egg white to cheesemaking milk, observed that the lysozyme could prevent butyric blowing in cheese. The enzyme has no effect on the actual spores of C. tyrobutyricum even when they have germinated, but inhibition of growth from the spores or vegetative cells becomes apparent at 400 to 500 U. per ml (i.e. 20-25 microgram of lysozyme at 20,000 U per mg.) and only becomes total above 1000 to 2000 U per ml depending on the strains and amount of inoculum. At intermediate concentrations,

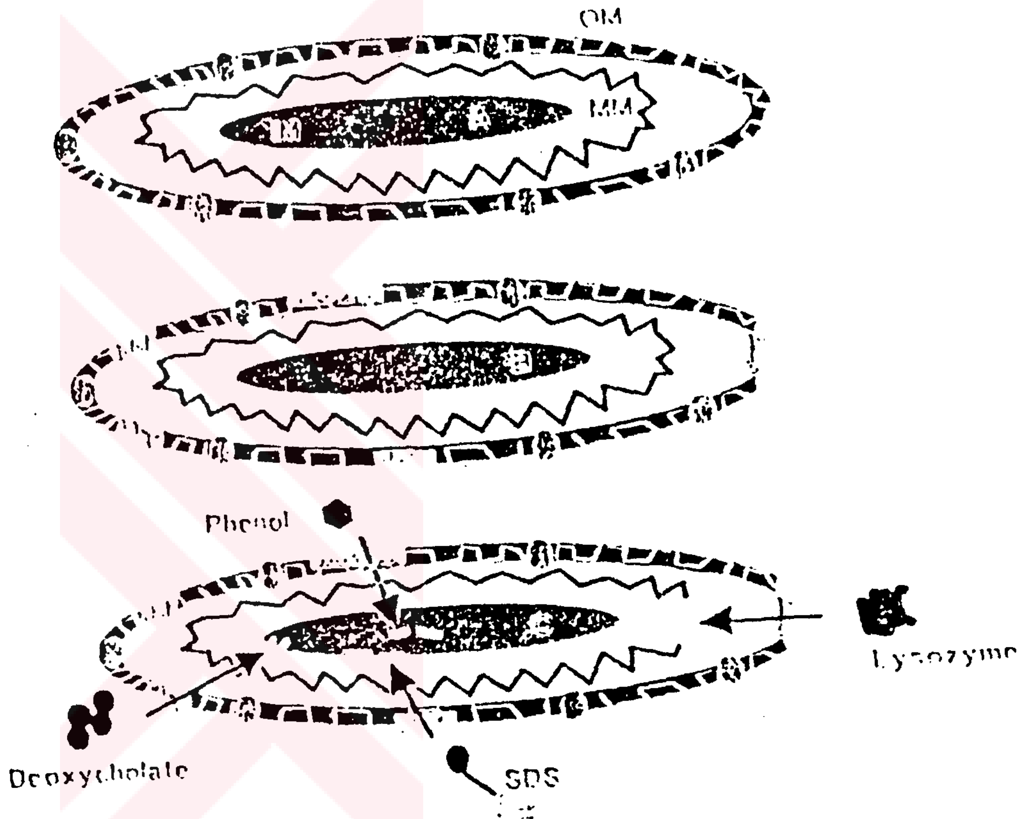

Fig.5 Bacterial cells, initially resistant to different chemicals, become sensitive to them following stress due to sublethal injury of the barriers.

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growth is merely retarded to a greater or lesser degree depending on the amount of lysozyme, but subsequently takes place at almost the same rate, due to fact that a proportion of the C. tyrobutyricum cells are resistant to the enzyme.

Acid production by the lactic acid bacteria used in cheesemaking usually begins to be appreciably affected only at lysozyme level above 1000 to 2000 U per ml of milk, but some lactobacilli may be inhibited at levels below 1000 U per ml. These should be taken into account when they are used in cheesemaking. Propionic acid bacteria are not affected by amounts of 2000 to 5000 U per ml. Experiments in hard cheese showed that lysozyme added to milk in amounts of 500 U per ml is an effective means of preventing the appearance of butyric blowing for milk contamination levels ranging from 160 to 5000 spores per liter. The use of this amount of lysozyme did not interfere with the manufacturing process and even though the coagulation times were slightly increased, the texture of the cheese did not seemed to be changed. It has been shown that 90-99 % of the lysozyme added to milk is retained in the curd, probably by fixation on the casein, but the enzyme keeps its activity [100].

When Emmental is considered, addition of 625 U per ml of lysozyme to milk does not change the manufacturing parameters, coagulation time, rate of hardening and curd behaviour. Neither does it have any appreciable effect either on the acidification rate or on the pH and solid content at 24 h, especially if resistant starters are chosen.

In Japan lysozyme has been patented for use as a food preservative in all types of foods. It has been found to be an effective alternative for nitrate in semihard cheeses to prevent defects caused by Clostridium tyrobutyricum. Metal chelator such as lactoferrin or conalbumin, EDTA enhanced inhibition by lysozyme, producing a bacteriostatic effect not present with lysozyme alone [117].

1. 10. 9. Pekmez

Turkey is an important grape producer country and it is the fifth biggest grape producer country in the world. Approximately 3,600,000 tons/year of grapes are produced in Turkey. That's why grapes and its products have significant effect on Turkish economy [118].

Maturation of grapes is a long time progress and consumption of grapes as fresh fruit is possible only between June-October. This is not a uniform maturation time for all types of grapes. Different types of grapes mature at different time intervals depending on climatic condition and type of the grapes. However during this time intervals large amount of fresh grape is supplied to the market for consumption. Since grapes have very short shelf-life, significant amount of grape loss occurs due to deterioration. This situation cause the fresh grape price to be too low. For that reason, it is required to process the grapes into a from that can be stored for a long time without any significant loss within nutritional value [119]. So that it is required to process the grapes. Some of that known processes are;

a) Drying of grapes to produce raisin

b) Production of grapes juice and concentrate

c) Production of alcoholic drinks by fermentation

Table 17. Composition of pekmez [120, 121].

Soluble solid	82.2 %
Moisture	28.0 %
Total acid	4.8 g/kg
pH	5.1
Reducing sugar	82.1 (dry base)
Total sugar	83.8 (dry base)
Saccharose	1.5 (dry base)
Glucose	43.2 (dry base)
Tannin	1952.8 mg/kg
Total ash	1.9 %
Protein	0.6 %
HMF	27.5 mg/kg

Concentrated grape juice produced by boiling without fortification is called as Pekmez. Pekmez is one of the

most common and known product of grapes in Turkey. The shelf-life of pekmez is extended by boiling without any further process or fortification [120, 122]. In Turkey 23 % of the grape produced is consumed as fresh grapes, 37 % dried, 3 % is processes for wine production and approximately 37 % is processed to pekmez. Above percentage related with processing types of grapes varies with different locations of Turkey. In the western part of Turkey most grapes are dried or processed to wine or alcohol production. However in eastern part it is processed to pekmez. These variations in grape processing are due to

a) marketing opportunities

b) grape produced

c) traditional and food consumption trend

d) availability of other foods.

In the less developed parts such as eastern and middle part of Turkey people is supplied mostly with pekmez rather than saccharose for their sugar requirement [120].

Most of the pekmez is produced by traditional methods without any technological and scientific consideration on it. Generally the farmers produce their own requirement and supply small amount of the market. Beside these farmers few local producers produce pekmez for markets directly. Some of these producers produce pekmez during the grape harvesting season from the low quality grape that cannot be marketed as fresh grape but some of them dry grapes under sun and later produce pekmez from that dried grapes during winter time when fresh grape is not available.

Pekmez is the concentrated and shelf-life extended form of grape juice by only boiling without any addition of sugar or any other food additives. Therefore it can assumed as a natural food containing natural sugars such as glucose, galactose and minerals [122].

Grape juice is concentrated by evaporation. After crushing and pressing the grape juice is boiled to concentrate. The purpose of the concentration is extending the shelf-life of the grape juice by reducing the water activity and pasteurizing the grape Juice [118].

1. 10 .9. 1. The Role of Pekmez in Our Diet

Pekmez at 15 °C has density of 1300 g/L water content of 36.5 % carbohydrate of 60 %, acidity 8 % and ash content is 3.5 %. Most of the carbohydrate is natural fruit sugar of glucose and fructose. Pekmez also contains some important minerals for the diet. Such as iron, magnesium and calcium. Beside these minerals it contains small amount of carotene and B-vitamins [123]. Since glucose in pekmez pass to the blood easily and quickly, it may be used in our urgent energy requirement. When it is mixed with tahin its nutritional value increases. Sometimes it is mixed with yogurt and this mix is called as fakıbeyni.

Two spoons (approximately 20 g) of pekmez contain 1-2 mg iron, 50-80 mg calcium, has 58 calories. That's why children, sportsmen, workers, sucker mother can be supplied with pekmez for urgent energy requirements and in the case of anaemia [124].

In the organism due to lack of some nutrients various type of anaemia presents. Some of these nutrients are metal ions such as iron, copper, cobalt [125], some vitamins such as folic acid, vitamin-B_{12} and vitamin-C. Anaemia due to lock of iron is seen throughout the world but especially in developing countries. In our country anaemia due to lock of iron is common but effect mostly babies, child, young and sucker mothers [126]. If the anaemia which is due to lock of iron in the diet, it can be treated by feeding with pekmez because of its high natural iron content, low cost and availability. Supply of anaemic patient with pekmez as a good iron source food substance increase Hb value of that patient. The problem in immune system due to lock of iron can also be solved by intake of this minerals with pekmez feed and risks of infectious disease is decreased. Due to its low protein content it is useful for the treatment of protein metabolism disorderness [127].

1. 11. Aim of the Research

Yogurt has become a highly popular food product over the world. The increased popularity is largely due to yogurt's organoleptic, nutritional and therapeutic properties. Yogurt can also serve as a food ingredient to be used in various food formulations.

However yogurt is not a very stable product. Shelf life of yogurt is limited to about 3 weeks in cold storage and 2-3 days at room temperature [49].

Various factors are responsible for the limited shelf-life of yogurt.

Post-acidification leading to excessive acidity arises from the metabolic activity of yogurt microorganisms which are considerably reduced by cooling after incubation but not completely stopped. Although the growth of bacteria is stopped, the organisms continue to slowly produce acid even at cold. This acid production eventually limits the shelf-life of yogurt [127].

Microbial spoilage is largely related to yeast and mould contamination since these organisms can grow at low temperatures in the acidic product. Yeasts can produce colourless, flat, moist colonies and mould results in white or blue colonies with the formation of film and overgrowth of whole surfaces. Surface growth of these microorganisms results in enzymatic activity that leads such sensory defects as yeasty, mouldy, cheesy, rancid and/or bitter off-flavours, gas formation and associated carton swelling and possible whey separation in set yogurts [128].

Proteolysis in stored yogurt arises from the proteinase and peptidase activity of the starter organisms, i.e. Streptococcus salavarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus [128]. The degree of proteolysis during manufacturing and storage of yogurt depends on the balance between amino acids liberated by L. bulgaricus and utilization by S. thermophilus. An extensive

amount of proteolysis during incubation may result in syneresis. In addition, free amino acids formed from excessive proteolysis may serve as precursors for the production of undesirable flavours such as bitterness [129].

This characteristic involves high product returns from distribution which are costly for the dairy industry. Since whey is removed from returned product and drained yogurt is produced. Production of drained yogurt requires additional costs such as transportation, labour, loss of whey and weight, new packaging, marketing etc. Moreover, deterioration of quality and functionality of the product during storage creates an obstacle for the use of yogurt as a food ingredient.

Both distributors and consumer are looking for a yogurt with good keeping quality, which also contains a sufficiently high number of living bacteria.

The consumption of yogurt over the world has increased. Part of this growth is due to new marketing strategies, yet most of it is a response to the incorporation of fruit flavourings accompanied by additional sweetener.

The aim of this PhD thesis is to increase the shelf-life of set yogurt during refrigerated storage. For this purpose several treatments will be experinced:

In the first part, post heat treatment with different time-temperature combination, 60 °C and 65 °C for 5-10-15 minutes, will be applied to increase the shelf-life of yogurt and results will be compared with the microwave treatment. Possible impacts on the optimization of commercial yogurt shelf-life and the development of stable yogurt for industrial uses will be discussed.

The second part deals with nisin treatment at various concentration, 50-100-150 RU/ml yogurt. Stability of nisin is high at acidic conditions. It's solubility is high at lower pH and large molecules had a protective effect on nisin. From these points, yogurt is a good medium for nisin addition.

The third part involved lysozyme addition at three different concentration, 0.3-0.6-1.2 g/l. Similar to nisin, lysozyme is stable at acidic conditions and gram positive bacteria are susceptible to its activity.

The fourth part investigates the effects of microwave treatment on shelf-life of yogurt in relation to radiation time and power level and examine the relationship between time of heating and final temperature of yogurt.

In addition to these, new type of a flavoured yogurt, with high iron content, (trace amount of iron present in yogurt) will be produced by the addition of pekmez before and after fermentation. By this way market samples of yogurt will be enriched. Effect of pekmez addition on the overall quality and fermentation process will be evaluated.

Yogurt will be stored at refrigeration temperature (6-8 °C) and physical properties (whey syneresis, viscosity), chemical properties (pH, titratable acidity, protein content), microbiological analysis (total count, enumeration of S. thermophilus and L. bulgaricus, yeast and mould determination), statistical analysis and sensory properties will be analyzed periodically.

CHAPTER II
MATERIAL AND METHODS

2. 1. Culture and Other Materials

Commercial freeze dried yogurt culture (Jogurt series 500) was provided by the Wiesby starter culture and media laboratory. The culture consisted of 1:1 ratio of L. bulgaricus and S. thermophilus. It was maintained in a 20 ml sterile solution (12 % w/v) of skim milk powder (Pınar A. Ş.) autoclaved at 121 °C for 5 min. The culture was inoculated in skim milk until pH reached 4.62 in 6 hours. Activated lactic culture was used to inoculate yogurt mix.

BSA and nisin were obtained from Sigma Chemical Company. Lysozyme was provided by Mis A. Ş.; citric acid from Carlo Erba; M17, lactose and MRS from Oxoid, SMA and PDA from Acumedia, tartaric acid, HCl, orange G from Merck, phenolphthalein and NaOH from Riedel-de Haen were used.

Solutions were prepared by using distilled water.

2. 2. Preparation of yogurt

Yogurt was prepared from skim milk powder, a gift from the dairy company Pınar A. Ş. Turkey. Skim milk with 16 % total solid was sterilized in an autoclave at 121 °C for 5 min. Then the temperature was reduced to 43 °C under tap water and 3 % (v/v) active yogurt culture was added to milk and stirred gently. Milk was transferred into a 100 ml plastic jars (10 cm in height, 6 cm in diameter) and covered with aluminum foil. Both glass and aluminum foil were previously sterilized with alcohol, 80 % (v/v). Then milk was incubated at 43 °C in Nuve EN 500 model incubator until pH of 4.4 was obtained (3 hr). At that moment either cooling at 4°C for control samples or heat treatment, nisin and lysozyme addition, microwave treatment were applied.

2. 3. Post Heat Treatment

After the desired pH was reached, control samples were removed from the incubator, transferred to refrigerator and temperature of the incubator was increased to heat shock temperature (60-65 °C). When the desired temperature was reached (at the center of the sample), this moment was considered to be the beginning of the heating period. After heating was completed, jars were transferred to refrigerator. Experiments were carried out for temperature-time combinations of 60, 65 °C for 5, 10, 15 minutes. Fig. 6 shows schematic presentation of post heat treatment.

2. 4. Nisin Application

Nisin was stored at 4 °C in a desiccator. It was prepared in 0.02 N HCl. 0.1 g nisin was suspended in 10 ml 0.02 N HCl (10,000 RU). 1 ml of nisin solution was spreaded on the surface of 100 ml yogurt so that final concentration of nisin in yogurt was 50-100-150 RU. Fig. 7 shows schematic presentation of nisin application.

2. 5. Lysozyme Treatment

Lysozyme was stored at 4 °C in desiccator until use. 0.6-1.2- 2.4 g lysozyme was dissolved in 20 ml distilled water. 1 ml of lysozyme solution was added to the surface of the 100 ml yogurt and final concentration of the lysozyme was 0.3- 0.6- 1.2 g/l yogurt. Fig. 8 shows schematic presentation of lysozyme treatment.

2. 6. Microwave Treatment

Microwave oven was purchased from Arçelik Company. It's frequency is 2450 MHz, power is 700 W and has 5 power setting. During microwave treatment, jars were covered with microwavable foil. After fermentation was completed jars were placed in the microwave oven at 3 different power settings and two different time combinations. P_1 10-40 sec, P_3 10-20 sec, P_5 5-10 sec. Fig. 9 shows schematic presentation of microwave treatment.

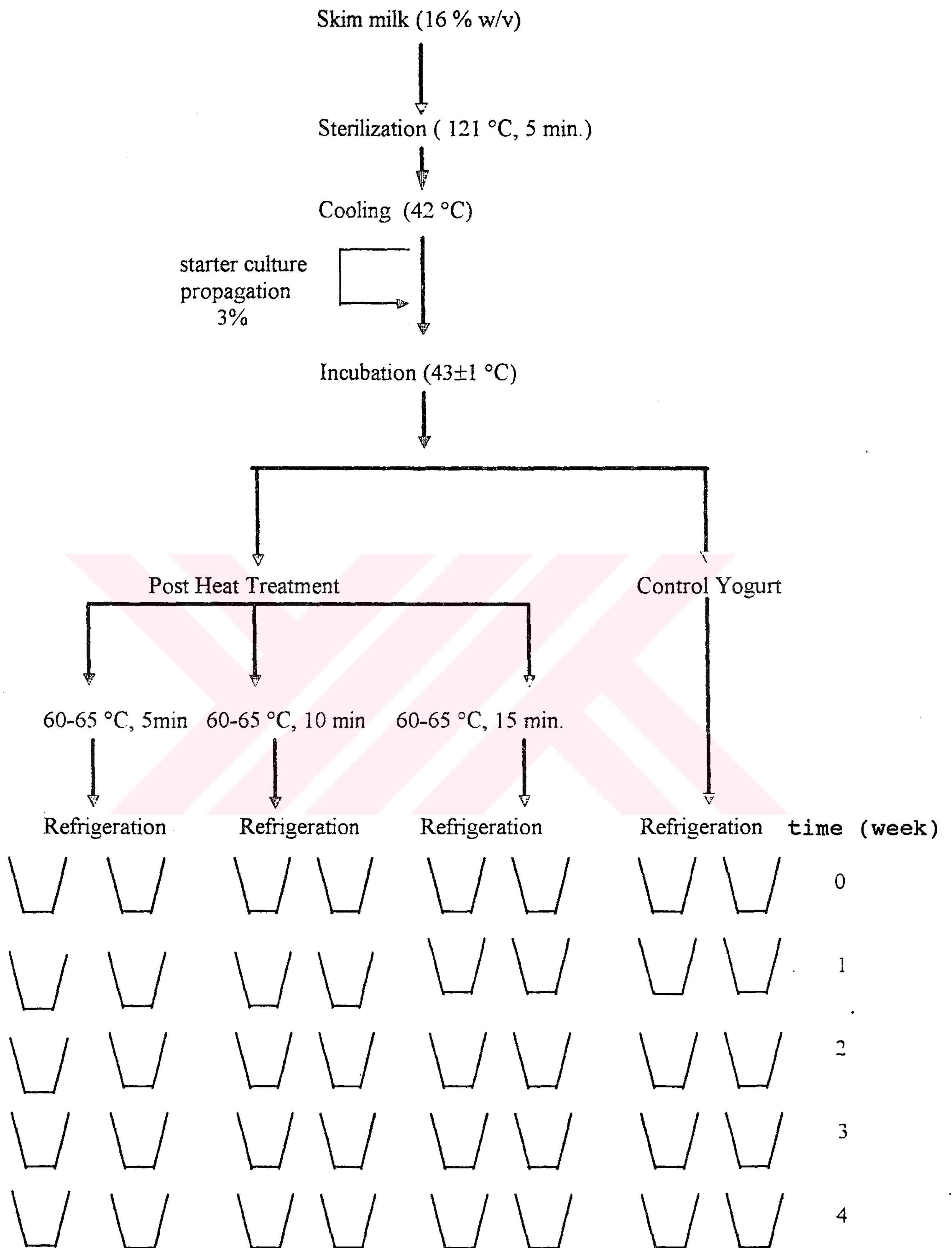

Figure 6. Schematic presentation of Post Heat treatment.
* Each jar contains 100 ml yogurt.

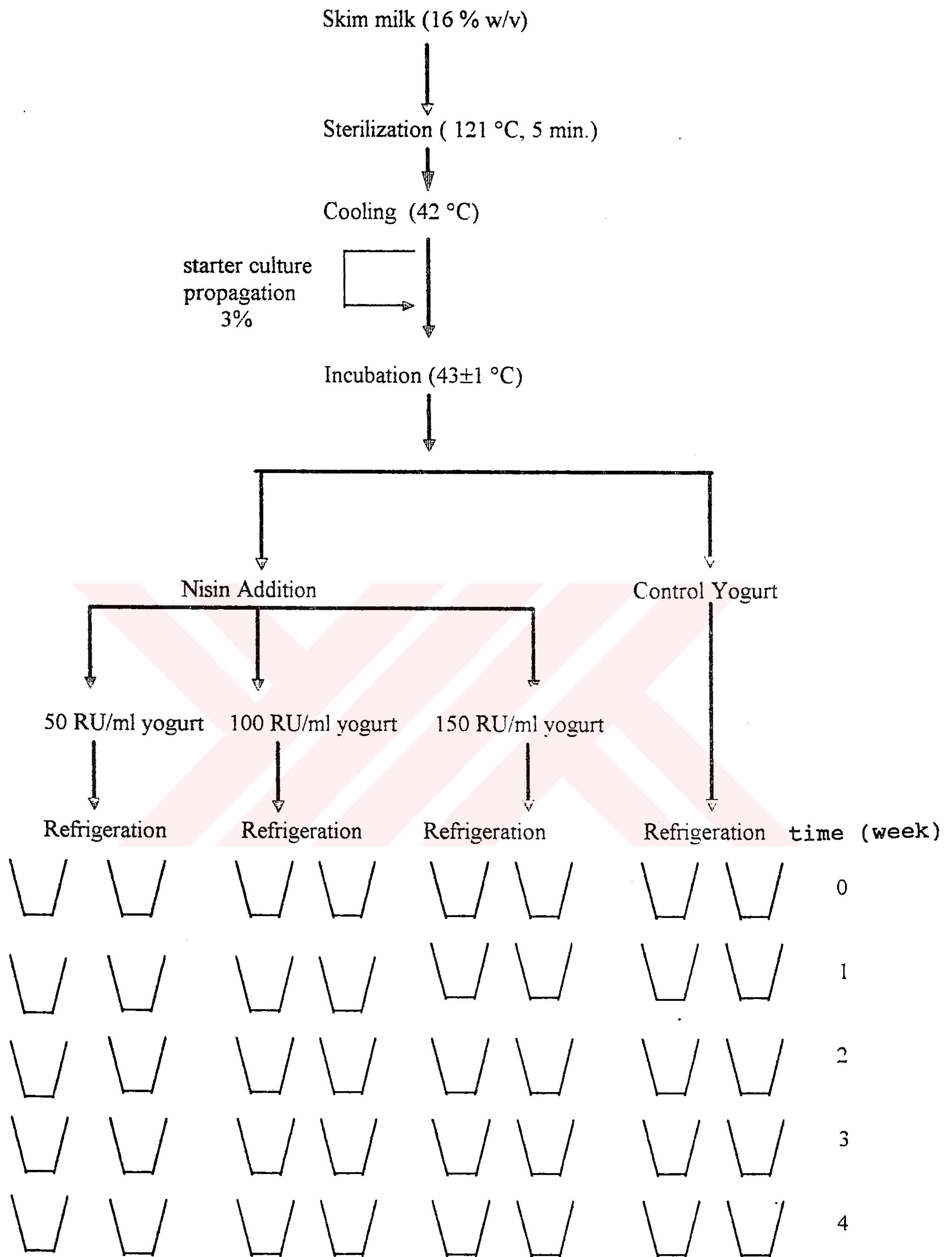

Figure 7. Schematic presentation of nisin treatment.
* Each jar contains 100 ml yogurt.

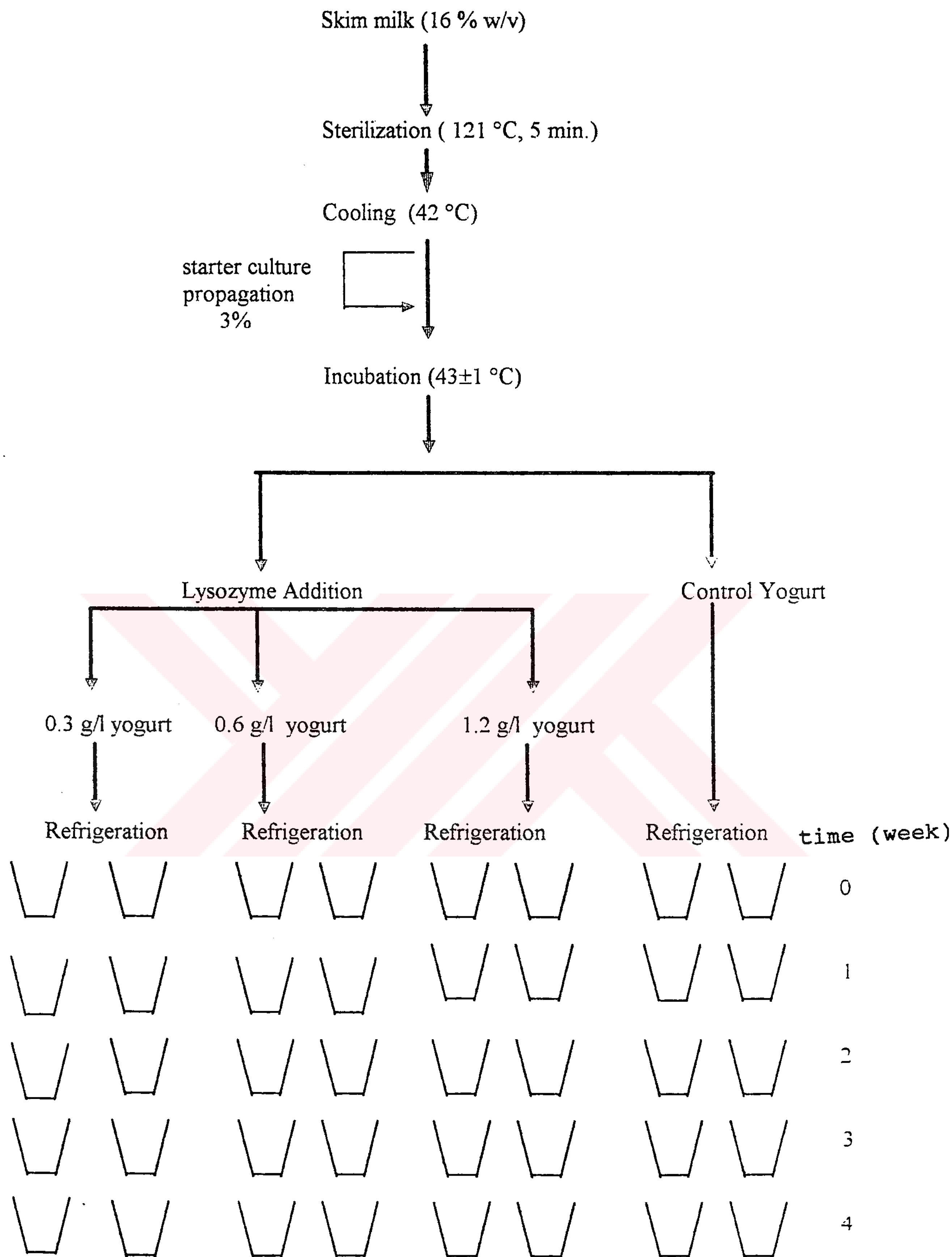

Figure 8. Schematic presentation of lysozyme treatment.
* Each jar contains 100 ml yogurt.

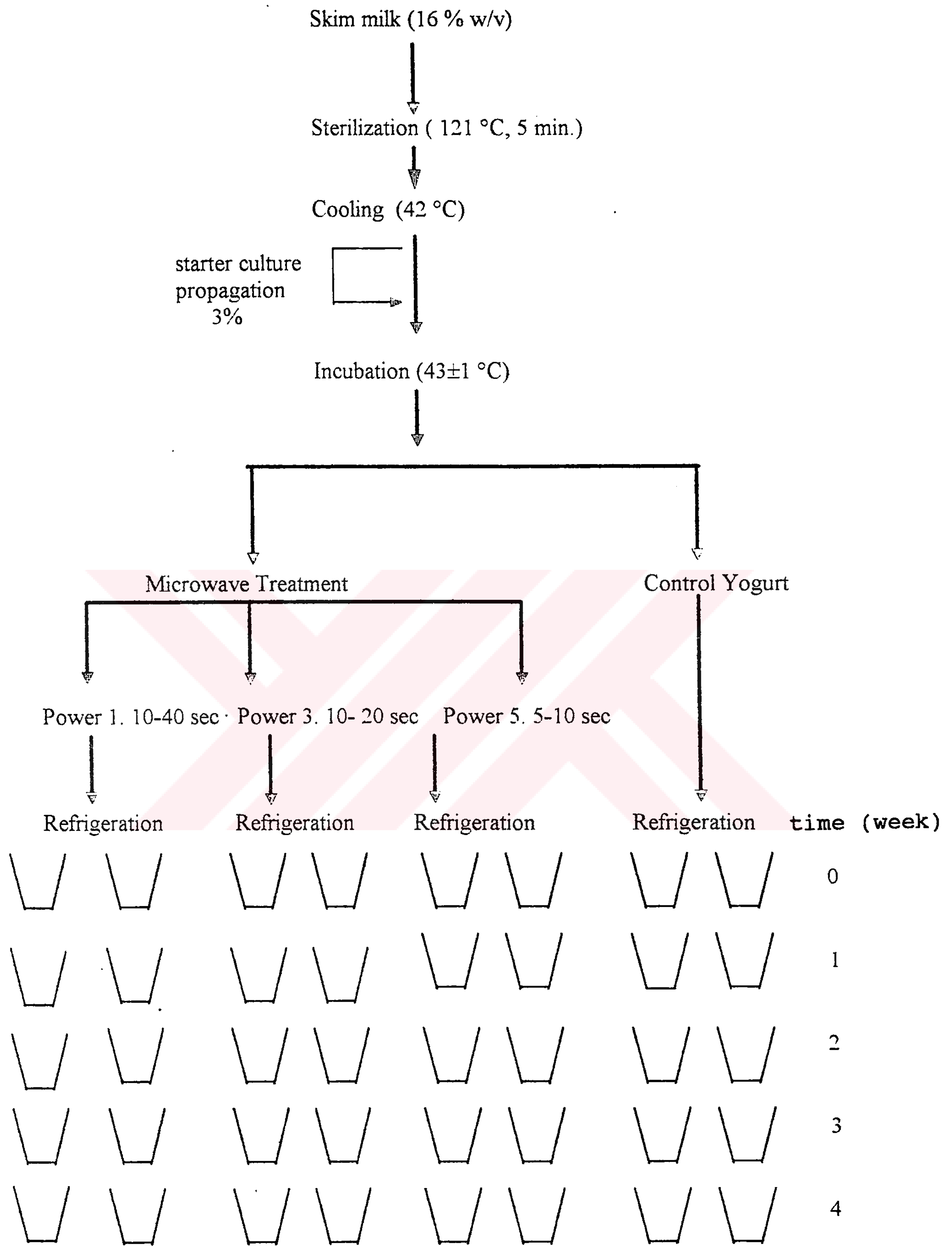

Figure 9. Schematic presentation of microwave treatment.
* Each jar contains 100 ml yogurt.

2. 7. Pekmez Yogurt

In order to determine the optimum pekmez concentration for pekmez yogurt production, 5-10-15 % pekmez was (pH 4.96, Brix 72°) added to milk and it was inoculated with starter culture (3 % v/v). After determination of optimum pekmez concentration (10 %, result of sensory analysis) nisin addition (150 RU/ml yogurt) and microwave treatment at power setting 5 for 10 sec. were applied to pekmez yogurt.

2. 8. Storage of Yogurt

Samples were stored at 4 °C and following parameters were analyzed weekly. Before analysis yogurt samples were homogenized by gently stirring with a sterilized glass rod under flame. All determinations were carried out weekly in duplicates.

2. 9. Analysis of Physical Properties

2. 9. 1. Whey Syneresis

Syneresis was determined as follows: 5 ml yogurt was centrifuged in Hettich model centrifuge (5700 rpm or 3400g, for 20 minute) and the volume of whey drained in 1 min was measured. % syneresis was expressed as volume of drained whey per total volume of yogurt [130].

2. 9. 2. Viscosity

Apparent viscosity was determined by using RV model Brookfield viscometer on 100 ml yogurt at room temperature which was stirred for 40 sec. Spindle number 4 was used for the measurement with rotation of 10 rpm. Readings were taken at 20 °C after 10 minute spindle rotation. Viscometer reading was converted to centipoise units multiplying by 200 [128].

2. 10. Analysis of Chemical Properties

After fermentation and post-heat treatment and during storage pH and titratable acidity were measured. pH was determined on a 10 ml yogurt sample, diluted to 30 ml,

using a Jenway 3010 model pH-meter. Acidity was evaluated, in terms of percent lactic acid (g/100g yogurt), on the same sample by titration with 0.1 N NaOH. Phenolphthalein was used as an indicator [131]. Acid production rate was expressed as the % lactic acid produced per unit time.

2. 10. 1. Protein content

Protein content of samples were determined with dye binding method. 1 ml samples were diluted to 20 ml with distilled water. 2 ml of this solution was added to 4 ml dye solution (1 g orange G per liter of 0.3 M citric acid) in a centrifuge tube and mixed. Similarly blank was set up containing 2 ml water and 4 ml dye solution. After standing for 10 minute, tubes were centrifuged at 2500 rpm for 5 min. 0.5 ml supernatant liquid was diluted to 25 ml and optical density was measured at 485 nm against water by using Novespec II model spectrophotometer [129, 131]. The protein content was assessed from the difference between optical density of sample and blank when compared with the standard graph, prepared by using BSA as the standard.

2. 11. Analysis of microbiological properties

Samples were homogenized by using NM 110 Nuve model vortex. Samples were diluted up to $1/10^6$ dilution with sterile peptonized water.

2. 11. 1. Total Starter culture

Total number of starter bacteria (L. bulgaricus and S. thermophilus) were enumerated on MRS agar [2, 56, 77]. The media were autoclaved in Armfield model portable autoclave at 121 °C for 15 min. All plates were incubated for 3 days at 37 °C and counted with the aid of a Bilser BC 4005 model colony counter.

2. 11. 1. 1. Preparation of MRS Agar

70 g of MRS medium was mixed in a liter of distilled water until evenly dispersed. With repeated stirring it was

boiled to dissolve completely and autoclaved at 121 °C for 15 minutes.

2. 11. 2. Count of S. thermophilus

S. thermophilus were counted by spreading the diluted yogurt on M17 agar. 10 % lactose solution was sterilized separately and was added to M17 agar at around 50 °C. Plates were incubated aerobically at 37 °C [2, 34].

2. 11. 2. 1. Preparation of M17 Agar

48.25 g M17 agar was suspended in 950 ml of distilled water and brought gently to boil. It was sterilized by autoclaving at 121 °C for 15 minute. After cooling to 50 °C 50 ml of sterile lactose solution (10 % w/v) was added.

2. 11. 3. Count of L. bulgaricus

The difference between the total number of colony forming units and the number of S. thermophilus colonies was regarded as the number of L. bulgaricus colony forming units. All counts were an average of duplicate plates [98].

2. 11. 4. Total viable organisms

Total bacterial counts were determined by plating diluted yogurt samples on plate count agar by pour plate count. Plates were incubated at 30 °C for 72 hr [7, 60].

2. 11. 4. 1. Preparation of SMA

23.7 g of SMA medium was dissolved completely by heating in a liter of distilled water. It was autoclaved at 121 °C for 15 minutes.

2. 11. 5. Count of Yeast and Moulds

Samples were plated for yeast and molds on PDA acidified to 3.5 with 10 % tartaric acid. Counts were conducted in duplicates and two weeks interval period. Incubation time was four days at 21 °C [60, 132, 133].

2. 11. 5. 1. Preparation of PDA Agar

39 g of PDA medium was mixed in a liter of distilled water until evenly dispersed. After boiling for one minute to dissolve completely, medium was autoclaved at 121 °C for 15 minute. pH of the medium was adjusted by the addition of 14 ml of sterile 10 % tartaric acid solution prior to pouring into plates.

2. 11. 6. Microscopic Count

0.01 ml of the sample from 10^{-1} dilution was spreaded over an area of 1 cm^2 on glass microscopic slide. After drying and heat fixation, sample was stained with methylene blue for 1 min. Slide was examined under microscope [56, 134, 135].

2. 12. Statistical Analysis

To estimate the effect of each parameter; heating time, heating temperature, microwave power level and time, lysozyme concentration, nisin concentration, storage time on pH, titratable acidity, protein content, viscosity and syneresis and starter bacteria count 3-way and 2-way ANOVA were done at α=0.05 level. Also interactions between each other were interpreted.

CHAPTER III
RESULTS AND DISCUSSION

Skim milk, with 0.27 % (w/v) lactic acid, 3.58 % (w/v) protein content, 6. 43 pH and 16 % (w/v) total solids was used for yogurt making.

Stock culture was prepared by adding 0.0245 g of lyophilized culture to 150 ml skim milk. Microscopic observation showed that ratio of Lactobacillus and Streptococcus was 1:1. Fermentation time was 6 hrs at 42 °C and pH of the stock culture was 4.62 at the end of fermentation. Variation in pH, during fermentation period, is shown in Figure 10.

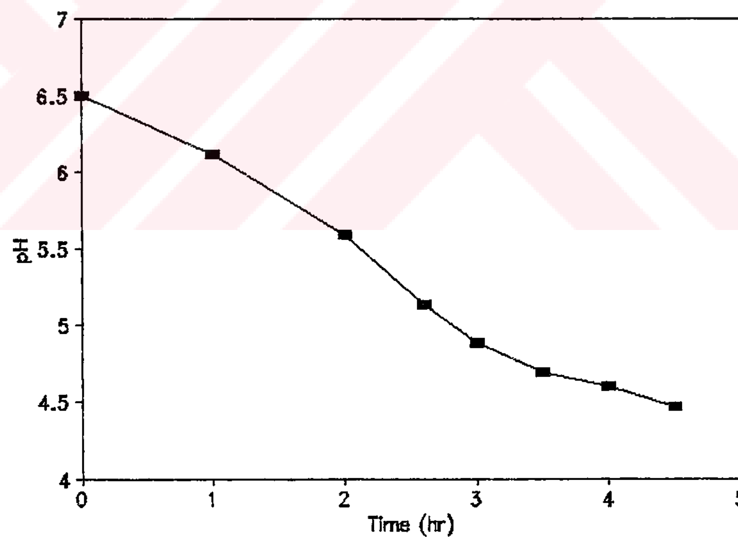

Figure 10. pH versus incubation time profile.

3. 1. Post Heat Treatment at 60 °C for 5-10-15 Minutes.

Yogurt was prepared by adding 3 % (v/v) stock culture (refrigerated overnight) to sterilized skim milk with 16 % total solid. Then it was incubated at 42 °C and pH was 4.48 at the end of fermentation. Fermentation time was 4 hrs. Unheated samples were refrigerated and temperature of the incubator was raised to 60 °C. Time required to reach the 60 °C (center of yogurt) was 20 min. As soon as 60 °C was reached, this moment was considered to be the beginning of the heating period. After a treatment of 5-10-15 min. at 60 °C, yogurts were refrigerated overnight for further observation. Lactic culture used gave a pH of 4.27 (for control) after overnight incubation at 4°C.

3. 1. 1. Effects of Post Heat Treatment on pH

After overnight storage of control and heat treated yogurts, final pH ranged between 4.29 to 4.20. The pH of the heat treated samples decreased slightly during post heat treatment comparing the pH of unheated sample. pH value of control was 4.29. During post heat treatment fermentation continued since metabolic activity of m.o. continued and microorganisms still produced lactic acid. But pH values of 5-10-15 min. heated samples showed very slight variation. Increasing the heating time from 5 to 10 min. decreased the pH from 4.23 to 4.20, further increasing to 15 min. increased the pH value to 4.24. Figure 2 shows the influence of heat treatment on pH values. Waes and Güldas et al. observed the same fluctuation during post heat treatment of yogurt at different temperatures [136].

Decline in pH was observed during storage for all samples (Fig. 11). Rate of this reduction was different for control and heated samples. Reduction in pH of 10 and 15 min. heated yogurt was slower than 5 min. heated yogurt and control. This was more pronounced during the last two weeks of storage period.

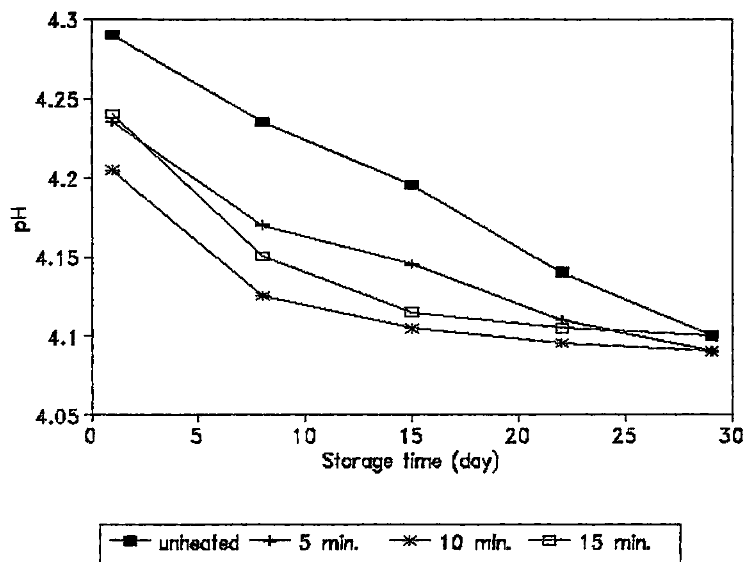

Figure 11. Changes in pH of unheated and heat treated yogurt samples during storage for four weeks at 60 °C.

When a standard of "pH should not be lower than 3.8" is requested post heat treatment for 5-10-15 min. at 60 °C can be applied. In this study, initial pH value of control was higher than that of heated yogurts. If it had the same pH value, pH of the control sample would have been reduced below 4. In that case, it cannot comply with the requirement at the end of storage.

Initial pH values of the samples were different after heat treatment. At the end of storage, they reached nearly the same pH value. But the rate in pH change was different for each sample. % pH change versus storage time graph was plotted to eliminate the difference in initial pH's. % pH change was expressed as follows;

$$\%pH=\frac{pH_0-pH_t}{pH_0}*100$$

From Figure 12, the lowest pH changes occurred in 15 minutes heated yogurts. Control had the highest pH change. Especially after one week of storage, pH change slowed down in 10 and 15 min. heated samples. Heat treatment reduced the rate of pH change. Effect of heat treatment on extending shelf-life of yogurt was significant.

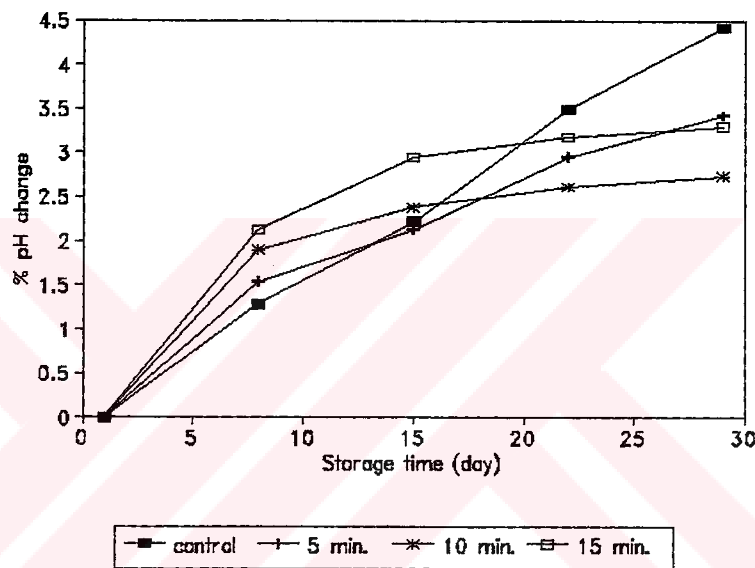

Figure 12. % pH change for unheated and 5-10-15 min. heated samples at 60 °C during storage.

To solve the over acidification problem, selection of compatible strains which would remain viable during storage and maintain higher pH is important. Selected strains of lactobacillus and streptococcus used can hold desirable pH in yogurt during storage at 4°C [52].

Sinha et al. studied the changes in acidity in commercial yogurts during storage. They observed that some yogurt brands showed pH values higher than 4.0 and maintained this during storage at 4 °C for four weeks, but

some of them showed pH values below 3.90 during entire storage period [77]. For these strains, which cannot hold desirable pH in yogurt during storage, heat treatment can solve the over acidification problem.

3. 1. 2. Effects of Post Heat Treatment on Titratable Acidity

Lactic acid content was 1.02 % for control yogurt after overnight storage. During heating period, lactic acid content increased, because the starter culture was not completely inactivated and continued its metabolic activity. Lactic acid content of 5 min. heated yogurt was 1.125 %, 10 min. heated yogurt was 1.135 % and 15 min. heated yogurt was 1.172. Changes in titratable acidity of control and heat treated yogurts were shown in Fig. 13.

During storage, % increase in lactic acid content for control was higher than heated yogurt. Similar to pH data, there was no great difference in % lactic acid for 5-10-15 min. heated samples during the first day.

Unheated yogurts with low initial acidity showed relatively the highest increase in acidity (1.02 % to 1.3 %). Acidity changes were minimum during one month of storage of heat treated samples. Acidity increased from 1.125 % to 1.24 % for 5 minute heated yogurt, from 1.135 to 1.295 for 10 min. heated yogurt and 1.172 to 1.250 for 15 min. heated yogurt. When Turkish standards were considered, the maximum value for the titratable acidity was 1.6 %. All titratable acidities stayed below this limit.

% Lactic acid production rate versus time was plotted to observe the effect of heat treatment on acidity which was shown in Figure 14. Lactic acid production rate of 15 min. heated yogurt was lowest; it was maximum for control. Consequently heat treatment reduced the lactic acid production. Relationship between titratable acidity and pH is not straightforward in a highly buffered system like yogurt. But titratable acidity is a reasonable indication of the performance of the starter culture.

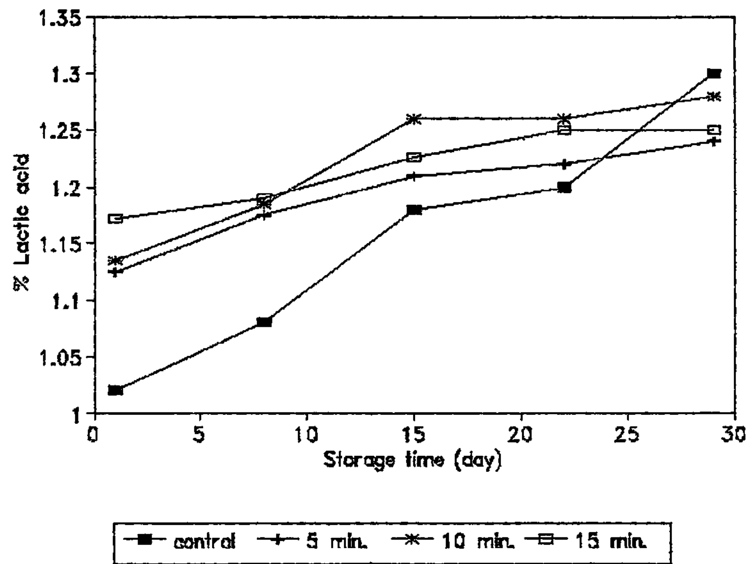

Figure 13. Changes in titratable acidity of control and heated yogurt at 60 °C during four weeks of storage.

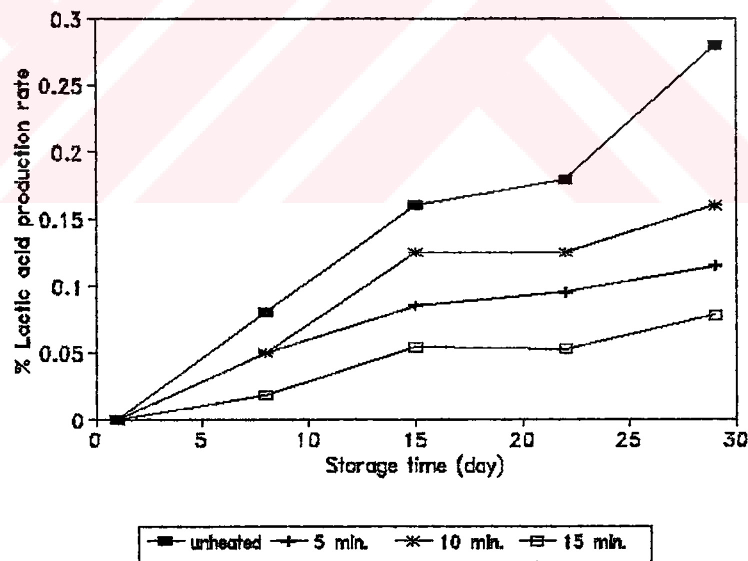

Figure 14. Lactic acid production rate of heated yogurt at 60 °C and control.

3. 1. 3. Effects of Post Heat Treatment on Protein Content

By using dye binding method undenatured protein content was measured. It was observed that there was reduction in protein content during fermentation and heat treatment. Table 18. summarizes the experimental observations;

Table 18. Variation in the undenatured protein content during fermentation and post heat treatment at 60 °C for 5-10-15 minutes prior to storage.

Heating Time (min.)	Protein content % (w/v)	Protein Content (Ave)	%Reduction
Milk	3.58		
Control	2.88 2.94	2.91	18.71
5	2.28 3.10	2.69	24.86
10	1.94 2.22	2.08	41.89
15	1.52 2.04	1.78	50.27

The undenatured protein (UP) content of milk decreased with increase in acidity during fermentation stage. At that stage, lactic cultures produce lactic acid. Increase in acidity decrease the undenatured protein content because of precipitation of proteins as the isoelectric point is reached. At that pH protein exhibits no net charge because the number of positively and negatively charged groups are equal [116]. It has been noted in the literature numerous times that reduction in undenatured protein content was not only due to the increase in lactic acid [23, 121].

Lactic culture hydrolyse whey proteins also, though their main attacks is on casein. Proteolytic action of lactic culture cause reduction in UP content of milk [3]. Vaitheeswaran and Bhat observed greater decrease in undenatured whey protein content in cultured skim milk than in the milk acidified to the same extent with lactic acid [23].

Experimental results demonstrated that reduction in UP content proceeds during heating period. This can be explained in two different way. Lactic acid bacteria

contain a wide range of proteinases and peptidases. Enzymes are intracellular and cell bound. Heat treatment damage the cell walls, it increases the rate of cell lysis permit release of enzyme system more rapidly causing more rapid proteolysis and peptidolysis compare to live cells [137]. Although heat treatment can cause reduction in the activity of peptidase, it was not completely inactivated. The effect of heat depend on the degree of heat employed and location of proteases in the cell. Proteases are located near the membrane and are therefore more sensitive to heat than the peptidases. Starter culture liberate high level of amino acids in acidic environment. The greater proteolysis may have stimulated acid production.

Secondly heat treatment of yogurt itself, causes additional denaturation of protein other than cell lysis. A rise in temperature can weaken the strength of dipolar interactions such as hydrogen bonds and can favour formation of hydroscopic interactions [138].

3. 1. 4. Effects of Post Heat Treatment on Syneresis

Whey syneresis of control was 44 %. After 5-10-15 min heat treatment, the levels of whey syneresis were lower than control. They reduced to 40 %, 41 % and 41 % respectively. These reductions were 4, 3 and 3 %. During heating period, fermentation process continues, starter culture produces lactic acid and lowers the pH. The lower the pH of the product the more resistant the casein particles were to syneresis [121]. As a result of this, whey syneresis decreased. Results were illustrated in Table 19.

After one week of storage, whey syneresis of all samples increased. Percent increases of whey syneresis were 2.5 % for unheated yogurt, 5 % for 5 min. heated yogurt, 4 % for 10 min. heated yogurt and 3 % for 15 min. heated yogurts. It was lowest for control sample. This indicated that heat treatment affected the yogurt texture negatively. Subsequently whey syneresis of all samples, except 15 min. heated yogurt, reduced. In general the application of heat

followed by separation of precipitated casein from whey. This effect was clearly observed for yogurt heated for 15 min. Increase in whey syneresis was highest for 15 min. heated yogurt during storage period. 5 and 10 min. heating gave less whey syneresis than 15 min. heating. It was important to note that level of syneresis depends on the degree of heat applied and generally some precautions will be advised if yogurt is heated to temperatures above 70 °C.

Table 19. Effect of post heat treatment at 60 °C on whey syneresis during storage.

Storage time (day)	% Whey Syneresis			
	control	5 min.	10 min.	15 min.
1	44.0	40.0	41.0	41.0
8	46.5	45.0	45.0	44.0
15	43.0	43.0	41.0	44.0
22	40.0	42.0	41.0	43.0
29	42.0	42.0	41.0	44.0

Langton searched microstructure of yogurt. The microstructure of yogurt is a coarse network believed to be composed of casein particles. The casein particles are linked together in clusters and/or chains, forming a network with void spaces or pores. The aqueous phase is kept in the pores and aqueous leakage gives rise to syneresis. Susceptibility to syneresis and firmness are important functional properties of yogurt. Structural parameters considered to be of importance are the size of casein particles, pore size, strength of linkages between the particles, the state of aggregation and interactions between different milk proteins or additives. The microstructure of yogurt influences the functional properties and may explain the susceptibility to syneresis viscosity, firmness, etc. Post heat treatment weaken the strength of linkages between the particles and interactions between proteins this cause a reduction in viscosity and increase in syneresis of whey as a result of fracture of the gel [139].

Although post heat treatment increased syneresis and

decreased viscosity, there are some solutions to prevent such adverse effects. Galvan et al. suggested to use polymer forming starter cultures in yogurt making. They observed that this types of culture reduce syneresis and increase viscosity. Since they produce threads of polysaccharides which linked both the cells of bacilli to each other and at the same time bacilli to the yogurt matrix of coagulated casein. It improved the viscosity of the product, prevented separation of the whey and made the yogurt more resistant to mechanical treatment [140].

Tamime recommended the addition of stabilisers, less than 1 %, to prevent whey syneresis and viscosity reduction. Commonly used stabilizers are gelatin 0.2-0.7 %, starches 0.2-4 %, agar 0.4-1 %, locust bean gum 0.2-0.6 %, quar gum 0.3-0.5 %, pectins 0.05-0.8 % [141]. Secondly, Tamime advised to cool yogurt first to 20 °C and then proceed with the heat treatment to overcome these adverse effects if yogurt is heated to temperatures above 70°C [49].

3. 1. 5. Effects of Post Heat Treatment on Viscosity

Viscosity of control was 3620 cp. During post heat treatment, parallel to results of whey syneresis, post heat treatment of 5 min. was increased the viscosity from 3620 cp to 3680 cp. Viscosities of 10 and 15 min. heated yogurt were 3600. After a week storage, reduction in viscosity was observed. This reduction was 200 cp for unheated sample, 380 cp for 5 minute heated sample, 400 cp for 10 min. heated sample and 280 cp for 15 min.heated sample. Viscosities of heat treated sample stayed nearly constant during 3 week storage period. Increase in storage time led to increased viscosity form 3620 cp to 3820 cp for unheated yogurt at the end of storage period. Table 20 gives the viscosity values obtained for heat treated samples.

A number of study showed that curd stability was positively influenced during storage due to the increase of acidity [139, 142].

Table 20. Influence of post heat treatment on viscosity during storage for four weeks.

Storage Time (day)	Viscosity (cp)			
	control	5 min.	10 min.	15 min.
1	3620	3680	3600	3600
8	3420	3300	3200	3020
15	3180	3060	2950	2900
22	3500	3020	3000	2860
29	3820	3100	3010	2940

3. 1. 6. Effects of Post Heat Treatment on Starter Culture Counts

Number of starter culture was 406*10^6 cfu om MRS media for control. Heat treatment reduced the number of viable starter culture. After heat treatment of 15 min. number of cfu decreased to below 100*10^6. During storage of four weeks, control maintained higher cell population. High lactic acid production rate and pH reduction of control could be explained with the higher cell population. Starter culture variations were presented in Table 21. Number of starter culture in control was high especially on second and third week. This was expected since no heat treatment was applied for control which means cells were not injured. But number of starter culture in heated sample showed reduction during storage, since they were injured.

Table 21. Influence of post heat treatment at 60 °C on the number of starter bacteria in yogurt during storage for four weeks.

Storage time (day)	Starter culture*10^6 (cfu)			
	control	5 min.	10 min.	15 min.
1	406	270	152	82
8	248	188	106	46
15	972	146	94	82
22	704	128	44	60
29	356	64	24	32

While Turkey do not regulate the levels of live bacterial cultures of S. thermophilus and L. bulgaricus in yogurt [55], some countries do have regulations; for

example France requires more than 100 millions total live lactic acid bacteria per ml of yogurt. Less than 10 million per ml is considered unsatisfactory [74]. The total number of starter bacteria of unheated sample at the beginning of storage was 406 millions per ml. Such a high starter bacteria count indicated that yogurt was not heat treated after manufacturing. Number of starter culture is a good criteria to understand whether yogurt is heated or not. The bacteria in unheated yogurt showed better stability during whole storage. 5 min. heated yogurt met this standard only for three weeks storage. 10 min. heated yogurt comply this standard only for two weeks. At the end of 4 weeks of storage, none of the sample showed bacterial counts less than 20 millions. It means, results were satisfactory.

If the cultures were judged against a requirement that $1*10^6$ starter bacteria should be present per ml yogurt, all the samples comply standards at the end of storage.

3. 1. 7. Total viable organisms

After heat treatments number of starter culture was determined by using PCA and MRS. PCA is the nonselective media for starter culture. But MRS media was selective for yogurt culture. Difference between them gave the number of injured starter culture. Cell injury is harmful for food products since pathogenic bacteria such as E. coli, S. aureus can repair themselves in non-selective media and become active during storage. But no such danger exist for desirable microorganisms. Table 22 gave the injury data at 60 °C.

Table 22. Number of starter culture on MRS and PCA media after heat treatment at 60 °C.

Culture Media	Starter culture (cfu)$*10^6$		
	5 min.	10 min.	15 min.
MRS	270	152	82
PCA	278	164	102
Difference	8	12	20

Number of injured culture increased as the heating time became longer. It was $8*10^6$ for 5 min. heated yogurt, $12*10^6$ for 10 min. heated yogurt, $20*10^6$ for 15 min. heated yogurt.

3. 1. 8. Microscopic Observation

Yogurts stored overnight were examined under microscope to determine the L:S ratio. Ratio was 1:1.08 for control. L:S ratio of 5 minutes sample was 1: 0.75, 10 minutes sample was 1:1.2 and 15 minutes sample was 1:1.5. It seemed that the S. thermophilus was more heat stable than L. bulgaricus for longer heat treatment.

3. 1. 9. Effects of Post Heat Treatment on Mold and Yeast

No yeast and mold were detected during entire storage. Waes examine the effect of heat shock treatment on count of yeast [78]. He inoculated 10^6 yeast per ml yogurt before heat shock treatment. After heating for 5 min. at 58 °C, no yeast were fond. This indicates clearly that such a treatment will eliminate yeasts that are normally present in fresh yogurt. Aziz emphasized that advantages of heated yogurt supersede its disadvantages when the production will be done under poor hygienic conditions [143]

3. 2. Post Heat Treatment at 65 °C for 5-10-15 Minutes.

Fermentation and post heat treatment procedure at 65 °C was the same as the post heat treatment at 60 °C for 5-10-15 minutes. Time required to reach 65 °C was 30 minutes. It was 10 minutes longer than reaching 60 °C. pH of the control was 4.47 at the end of fermentation.

3. 2. 1. Effects of Post Heat Treatment on pH

pH of the control yogurt was 4.32 after overnight storage. Similar to heat treatment data at 60 °C, pH of the heated yogurts reduced during heating period. pH of 5 min. heated yogurt was 4.24, 10 min. heated yogurt was 4.25, 15

min. heated yogurt was 4.24 after overnight storage. It can be seen that the initial pH values were approximately same.

When pH of 5-10-15 min. heated yogurts were considered, difference among them was lower at 65 °C than 60 °C. It was nearly 0.03 at 60 °C and 0.005 at 65 °C. The changes in pH with respect to storage time were illustrated in Figure 15.

When pH of heated yogurts compared with the pH of the control yogurt, reduction after heat treatment was higher at 65 °C than that of samples 60 °C. While pH reduced from 4.29 to 4.23 (1.28 % reduction) for 5 min.heating at 60 °C it reduced from 4.320 to 4.24 (1.736 % reduction) for the same heating time at 65 °C. Possible reason for the lower pH was due to higher heating time during come up period and degree of heat treatment. As a result m.o.'s produced more lactic acid and reduced pH to lower value.

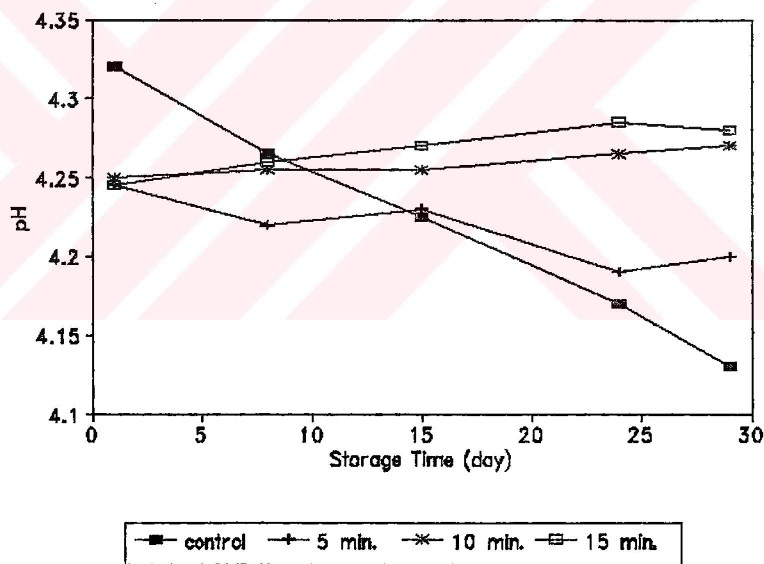

Figure 15. Changes in pH of unheated and heated yogurts during storage (65 °C).

It was important to indicate that heat treatment at 65 °C gave stability to pH especially for 10 and 15 minute heated yogurts during four weeks storage. Slight reduction

in pH was observed for 5 min. heated yogurt at 65 °C. But this reduction was lower than 5 min. heated yogurt at 60 °C.

To estimate the effect of heating time, heating temperature and storage time on pH and the interaction between each other 3-way ANOVA at α=0.05 level were done. The resultsof the test were shown in Table 23.

Table 23. Result of ANOVA for pH

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.0677910	3	.0225970	32.632	.0000
Heating time	.0011556	2	.0005778	.834	.4578
Heating temp.	.0600347	2	.0300174	43.348	.0000
Interactions					
S.time-H.temp.	.0031444	6	.0005241	.757	.6167
S.time-H.time.	.0374486	6	.0062414	9.013	.0007
H.time-H.temp.	.0146569	4	.0036642	5.291	.0108

Storage time and heating temperature significantly affected pH of yogurt. Interaction between storage time and heating temperature; heating time and heating temperature were also significant at α=0.05 level (Figure. 16, 17)

Since storage time and heating temperature had significant affect on pH of yogurt, Multiple Range analysis with Duncan Test of 95 % had been applied and the results were tabulated in Table 24 and 25.

Table 24. Multiple Range Analysis of pH for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	6	4.1377778	X
15	6	4.1833333	X
8	6	4.2205556	X
1	6	4.2544444	X

pH of 1, 8, 15, 29 days stored yogurts were significantly different from each other. Highest LS Mean (Least Squares Mean) was observed at storage time one day

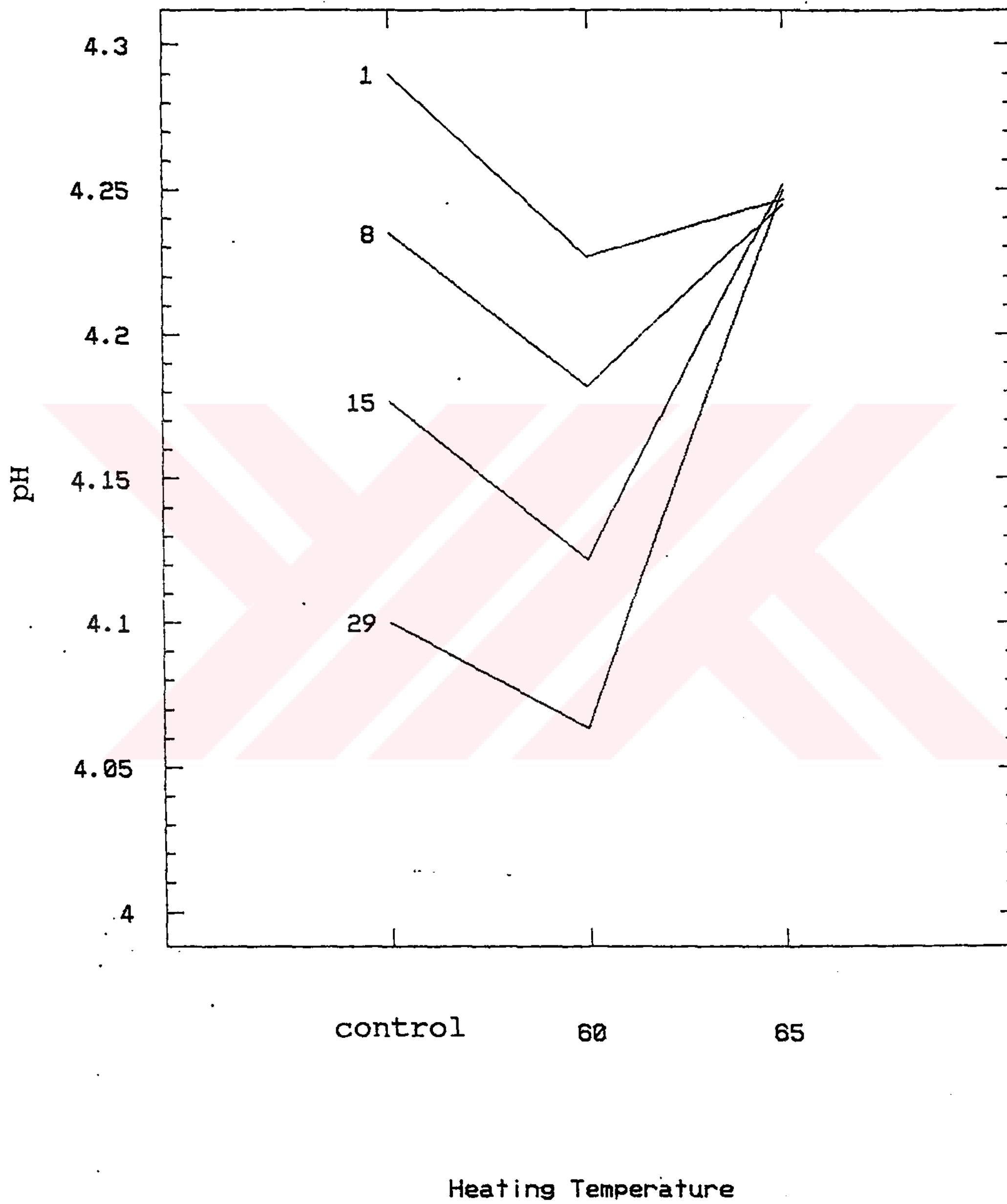

Figure 16. Plot of interactions for heating temperature by storage time (pH).

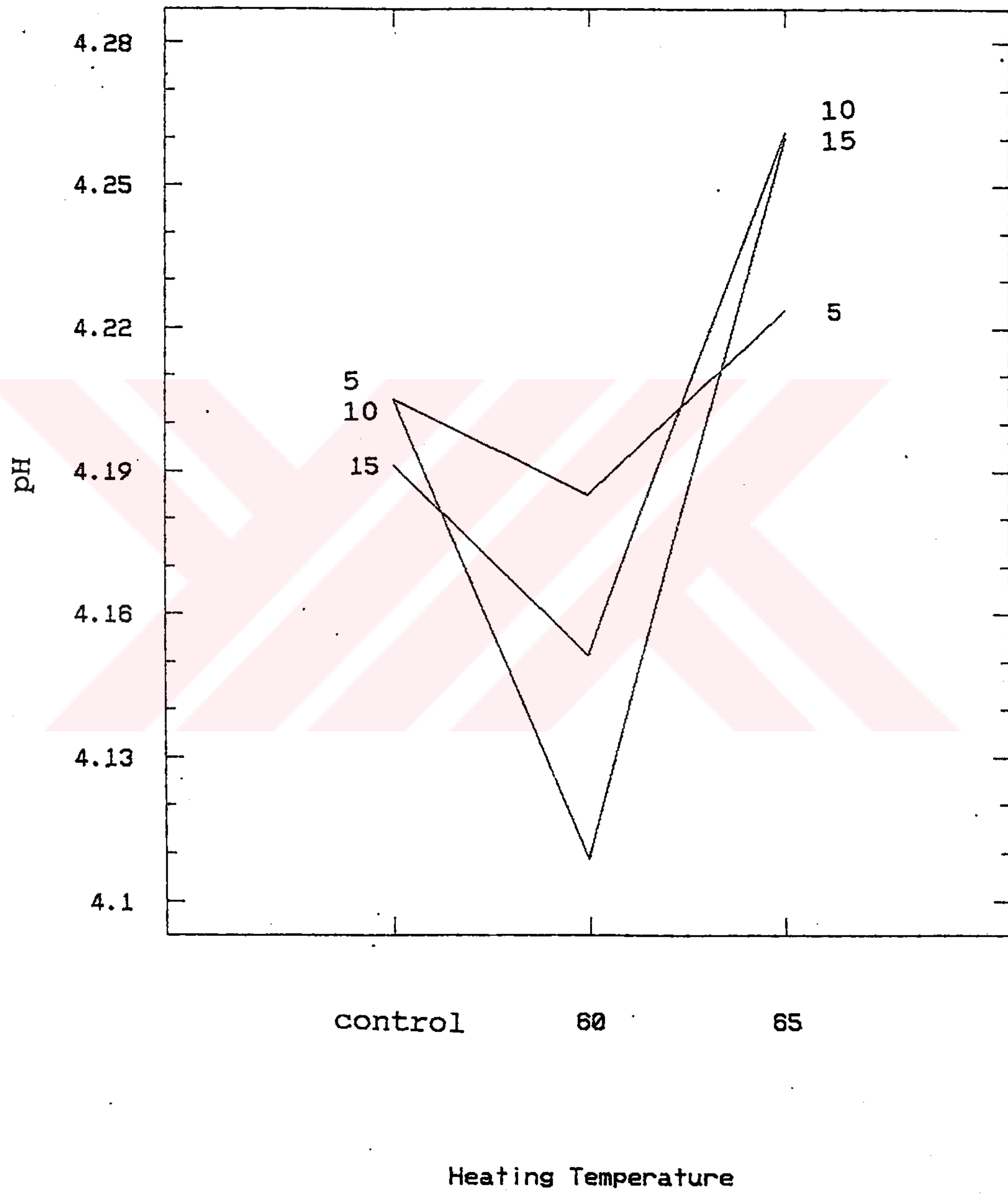

Figure 17. Plot of interactions for heating temperature by heating time (pH).

and it decreased significantly as the storage time increased.

Table 25. Multiple Range Analysis of pH for heating Temperature.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
60 °C	12	4.1483333	X
control	12	4.2004167	X
65 °C	12	4.2483333	X

It was clear from Table 25 that pH of control, 60 °C and 65 °C heated yogurt were significantly different from each other. Although LS means of control was between 60 and 65 °C, this was due to the differences in initial pH of heated yogurts and control.

3. 2. 2. Effects of Post Heat Treatment on Titratable Acidity

Lactic acid content of 5 and 10 minute heated yogurts (1. 04 %) were the same and it was nearly constant during four weeks storage. Titratable acidity of 15 min. heated sample (0. 945 %) was lower than 5 and 10 min. heated yogurts after overnight storage. Higher lethal effect of heat treatment at 65 °C for 15 min. comparing to 5 and 15 min. heat treatments prevented the excessive acid development due to higher cell injury. Later, acidity of 15 min. heated yogurt increased and during the last week it reached the lactic acid content of 5 and 10 min. heated yogurts (1.12 %). In general, lower the initial acidity, the more increase during storage [144, 145]. Data were plotted in Figure 18.

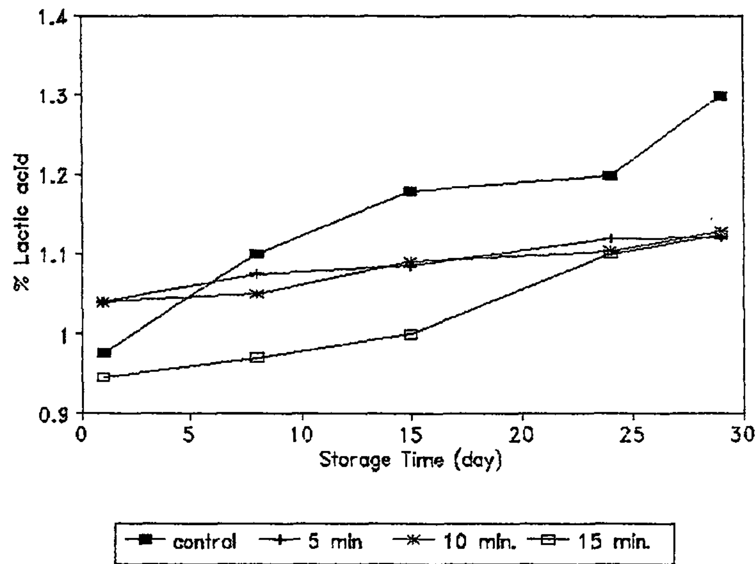

Figure 18. Changes in titratable acidity of unheated and heat treated yogurts during storage (65 °C).

Result of ANOVA Test for titratable acidity indicated that storage time and heating temperature had significant effect on titratable acidity of yogurt (Table 26). Similar to result of ANOVA test for pH, interaction between storage time and heating temperature; heating time and heating temperature were significant at α= 0.05 level (Figure 19, 20).

Table 26. Result of ANOVA for Titratable Acidity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.1593282	3	.0531094	58.745	.0000
Heating time	.0041494	2	.0020747	2.295	.1432
Heating temp.	.1354477	2	.06772394	74.911	.0000
Interactions					
S.time-H.temp.	.0047406	6	.0007901	.874	.5414
S.time-H.time.	.0428689	6	.0071448	7.903	.0013
H.time-H.temp.	.0109913	4	.0027478	3.039	.0604

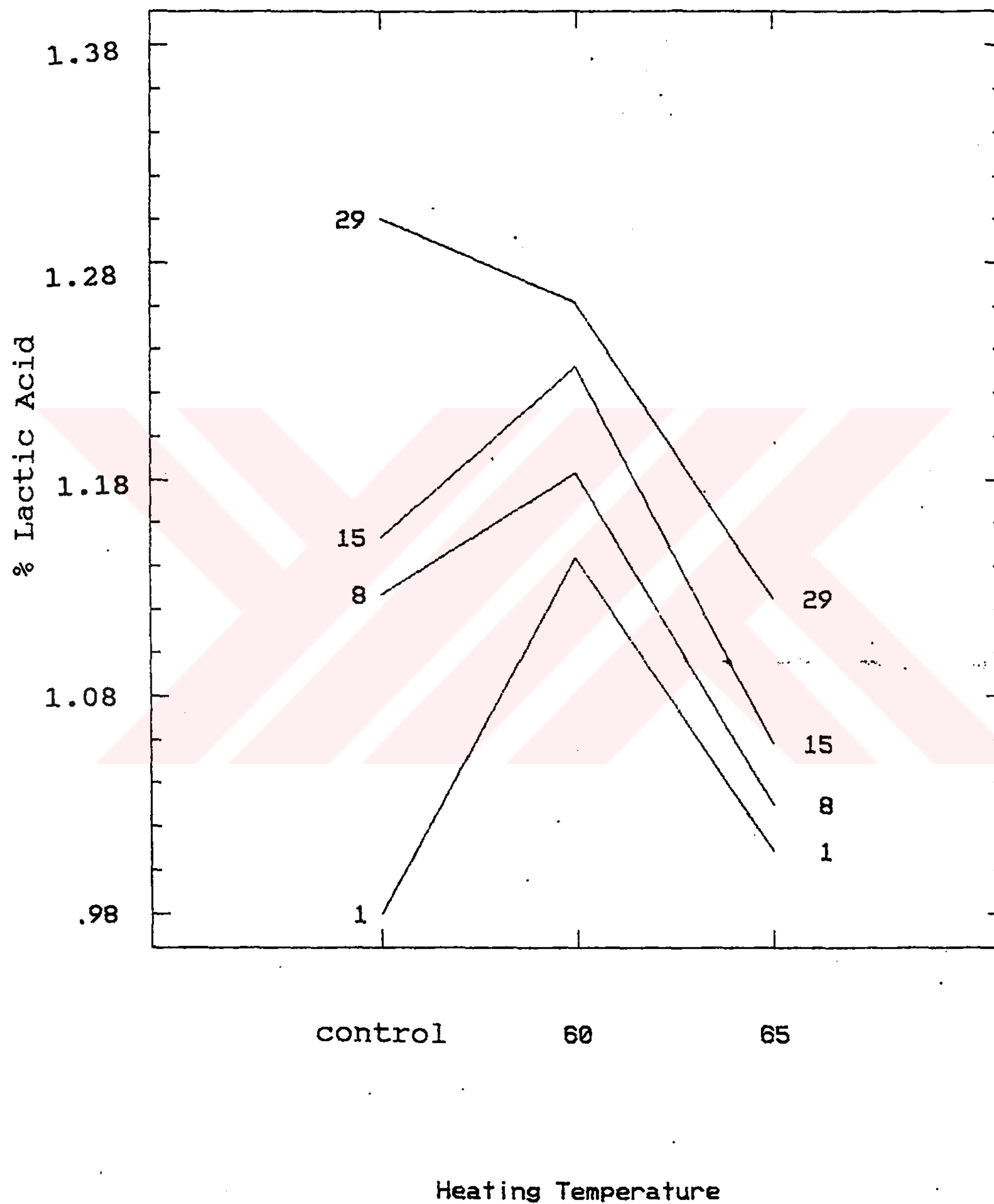

Figure 19. Plot of interactions for heating temperature by storage time (lactic acid).

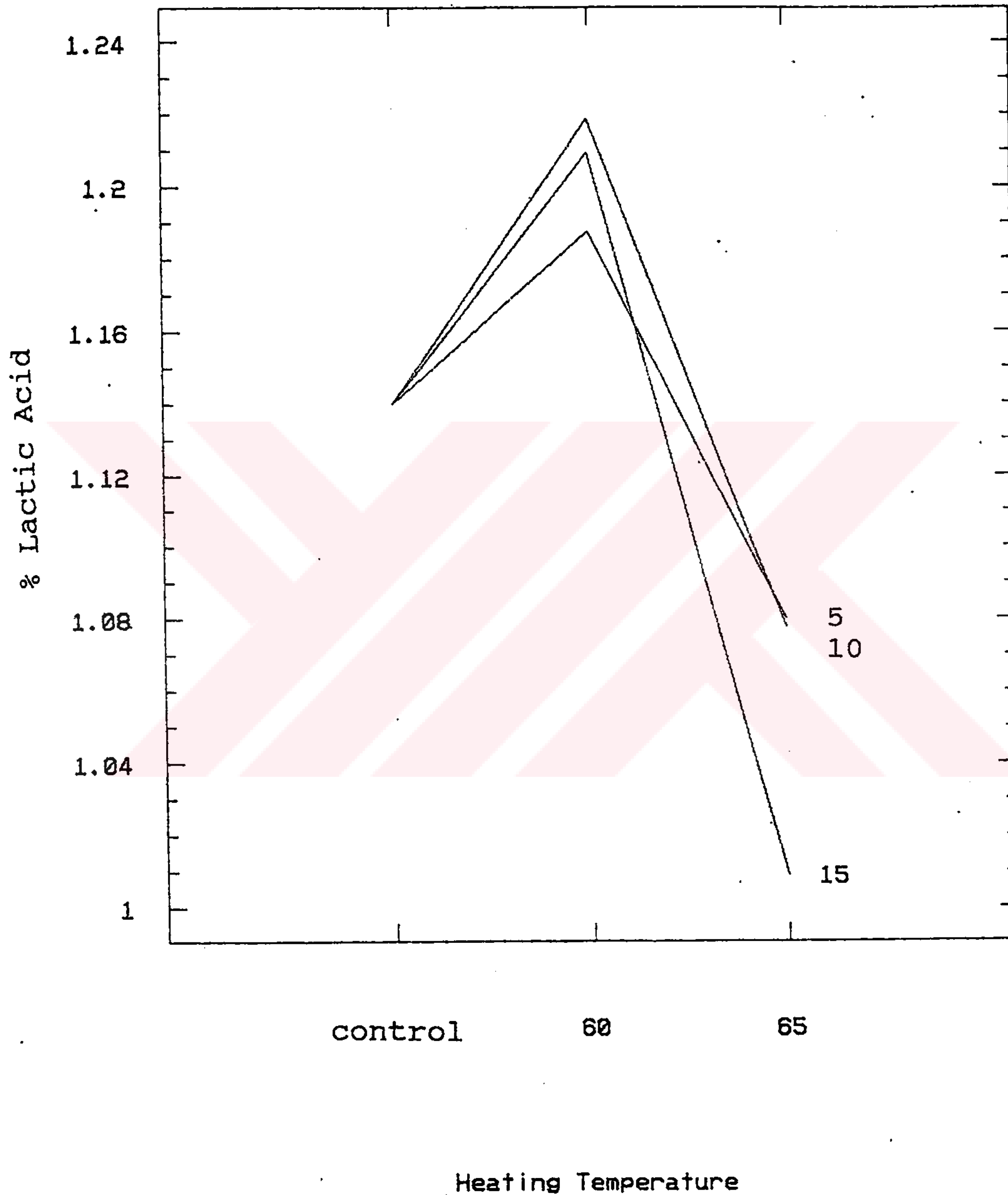

Figure 20. Plot of interactions for heating temperature by heating time (lactic acid).

Titratable acidity of yogurt stored for 1, 8, 15, 29 days were significantly different from each other Table 27).

Table 27. Multiple Range Analysis of Titratable Acidity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	9	1.0441111	X
15	9	1.1133333	X
8	9	1.2288889	X
1	9	1.2366667	X

Multiple Range Analysis test showed that titratable acidity of heat treated yogurt at 60 and 65 °C and control were significantly different from each other (Table 28).

Table 28. Multiple Range Analysis of Titratable Acidity for Heating Temperature.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
60 °C	12	1.0554167	X
control	12	1.1400000	X
65 °C	12	1.2052500	X

3. 2. 3. Effects of Post Heat Treatment on Protein Content

Similar to heat treatment at 60 °C, heating yogurt at 65 °C decreased protein content. Reduction in protein content was 28.80 % for 5 min. heated sample. It was 24.86 for 5 min. heated sample at 60 °C. Slight difference was noted for 10 and 15 min. heated yogurts at 60°C and 65 °C after overnight storage. Control had higher protein content than heat treated yogurts. Result was presented in Table 29.

Table 29. Variation in protein content during fermentation and post heat treatment at 65 °C for 5-10-15 min.

Heating Time (min.)	Protein Content (%)	% Reduction
Milk	3.48	-
Control yogurt	2.90	18.71
5	2.55	28.80
10	2.18	39.00
15	1.84	49.00

Data were tested by ANOVA (Table 30). Heating temperature significantly affected protein content of yogurt at $\alpha=0.05$. No significant difference was noted for heating time.

Table 30. Result of ANOVA for Protein Content.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Heating time	.4438222	2	.2219111	3.745	.1221
Heating temp.	.1354477	2	.5232444	8.778	.0344

Details of the effect of heating time on protein content was given in Table 31.

Table 31. Multiple Range Analysis of Protein content on Heating Temperature.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
60 °C	3	2.1833333	X
65 °C	3	2.1900000	X
control	3	2.9100000	X

Protein content of 60 and 65 °C heated yogurt significantly different from protein content of control. Difference in protein content was not significant between control and 60, 65 °C heated yogurt.

3. 2. 4. Influence of Post Heat Treatment on Whey Syneresis

Heat treatment at 65 °C slightly increased the whey syneresis of 5 and 10 min. heated yogurts comparing to

control. Increase was 0.5 %. Whey syneresis of 15 min. heated yogurt was decreased by 2 % after overnight storage. Heating at 65 °C for 10 and 15 min. increased the whey syneresis of yogurt during four weeks storage. Results were shown in Table 32. This effect was more clear at 65 °C than 60 °C. Possible explanation for this is that high heat treatment weaken the strength of linkages more and affect the interaction of casein particles negatively. Similar to heat treatment data at 60 °C, whey syneresis of all samples increased at the end of one week storage. Whey syneresis of 15 min. heated yogurts increased during storage and reach to 46 %. Increase was 3.5 %. It was 3 % at 60 °C. Whey syneresis of 10 min. heated yogurt increased by 0.5 % after four weeks. No difference in whey syneresis was detected for 5 min. heated yogurt at 60 and 65 °C.

Table 32. Effect of post heat treatment at 65 °C on whey syneresis during storage.

Storage time (day)	Whey Syneresis (%) control	5 min.	10 min.	15 min.
1	44.0	44.5	44.5	42.5
8	48.0	45.0	47.0	43.0
15	46.0	42.0	46.0	43.0
24	39.0	42.0	45.0	46.0
29	42.0	42.0	45.0	46.0

To estimate the effect of storage time, heating temperature and heating time on syneresis of yogurt and interaction between each other ANOVA was carried out (Table 33).

Table 33. Result of ANOVA for Whey Syneresis

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	22.888889	3	7.6296296	6.838	.0061
Heating time	2.055556	2	1.0277778	.921	.4245
Heating temp.	18.013889	2	9.0069444	8.073	.0060
Interactions					
S.time-H.time.	9.944444	6	1.657407	.874	.5414
S.time-H.temp.	71.152778	6	11.858796	10.629	.0003
H.time-H.temp.	13.277778	4	3.319444	2.975	.0639

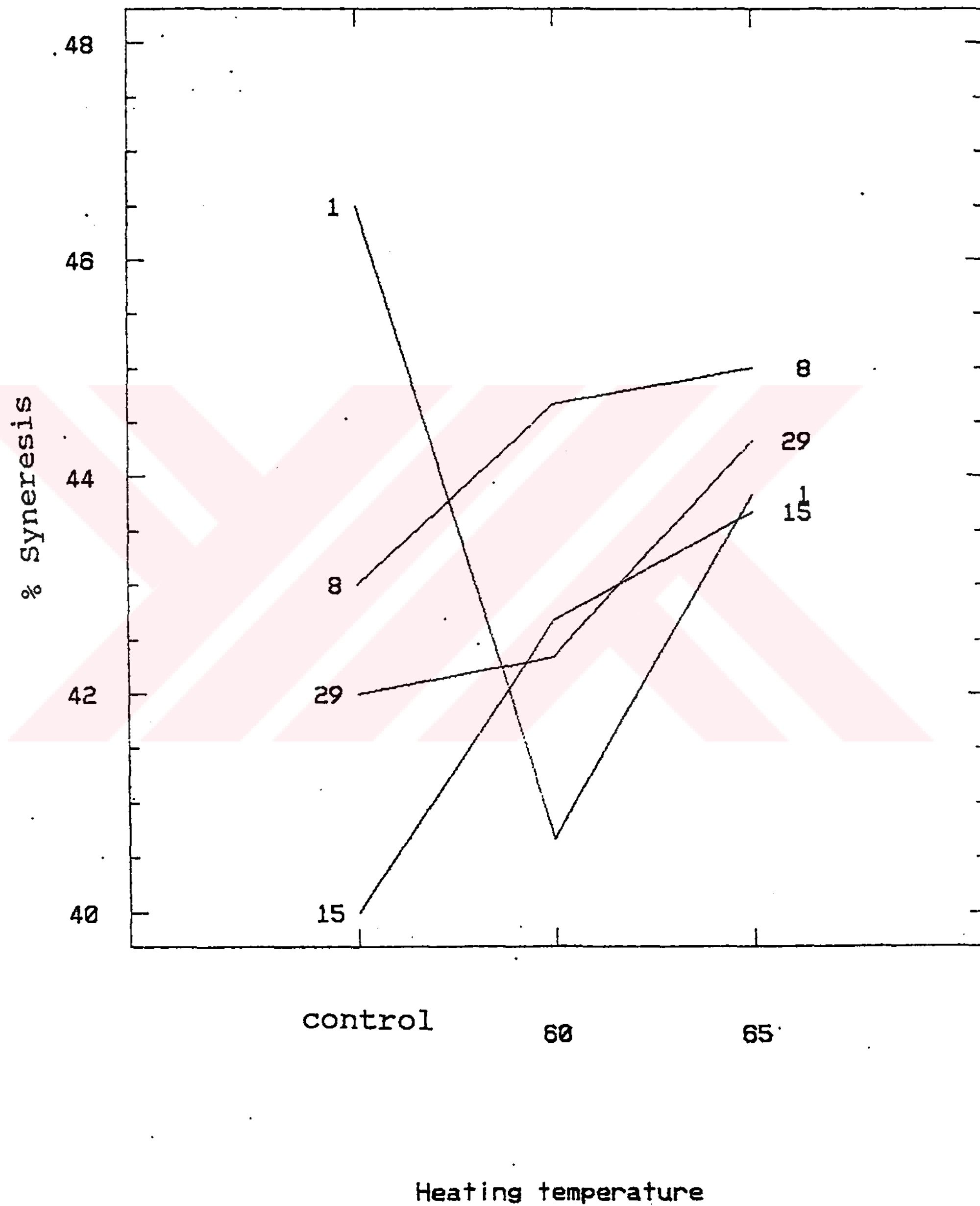

Figure 21. Plot of interactions for heating temperature by storage time (syneresis).

Storage time and heating temperature significantly affected the syneresis of yogurt at α=0.05 level (Table. 34). Interaction between storage time and heating temperature was also significant (Figure 21).

Table 34. Multiple Range Analysis of Syneresis for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
15	9	42.111111	X
29	9	42.888889	XX
1	9	43.666667	XX
8	9	44.222222	X

After 8 days of storage significant differences in syneresis of yogurt were observed. Effect of heating temperature on syneresis of yogurt shown in Table 35.

Table 35. Multiple Range Analysis of Syneresis for Heating Temperature

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
60 °C	12	42.583333	X
control	12	42.875000	X
65 °C	12	44.208333	X

There was no significant difference in syneresis of control and 60 °C heated yogurt. As the heating temperature increased from 60 °C to 65 °C, syneresis of yogurt significantly increased.

3. 2. 5. Effect of Post Heat Treatment on Viscosity

After overnight storage, viscosities of heated yogurts (3900 cp, 3460 cp, 3250 cp, respectively) were found to be higher than the control (3620 cp). During storage parallel to increase in syneresis viscosities of heated yogurt reduced. Because heating disturbs the network formed by the casein particles and let aqueous phase leak from void spaces and pores. This effect was more pronounced for 15

min. heating at 65 °C than 60 °C. % reduction for 5-10-15 min. samples were 45.0, 38.4, 31.7 respectively. In spite of this control maintained its higher viscosity during storage (Table 36). As control had lower acidity and the lower the acidity of the product the more resistance to serum separation and low viscosity.

Table 36. Influence of post heat treatment at 65 °C on viscosity during storage for four weeks.

Storage Time (day)	Viscosity (cp) control	5 min.	10 min.	15 min.
1	3620	3900	3460	3250
8	3760	3380	3320	3250
15	3640	2700	2630	2680
24	4150	2930	2070	2660
29	4290	2140	2130	2220

Effects of storage time, heating temperature and heating time were significant on viscosity of yogurt (Table 37). Also there was a significant interaction between storage time and heating temperature (Figure 22).

Table 37. Result of ANOVA for Viscosity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	1463675.0	3	487891.7	45.175	.0000
Heating time	85422.2	2	42711.1	3.955	.0479
Heating temp.	5176438.9	2	2588219.4	239.650	.0000
Interactions					
S.time-H.time.	46266.7	6	7711.11	.714	.6457
S.time-H.temp.	3751983.3	6	625330.56	57.901	.0000
H.time-H.temp.	45511.1	4	11377.78	1.053	.4206

Table 38. Multiple Range Analysis of Viscosity for Heating Temperature

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
65 °C	12	2921.6667	X
60 °C	12	3196.6667	X
control	12	3827.5000	X

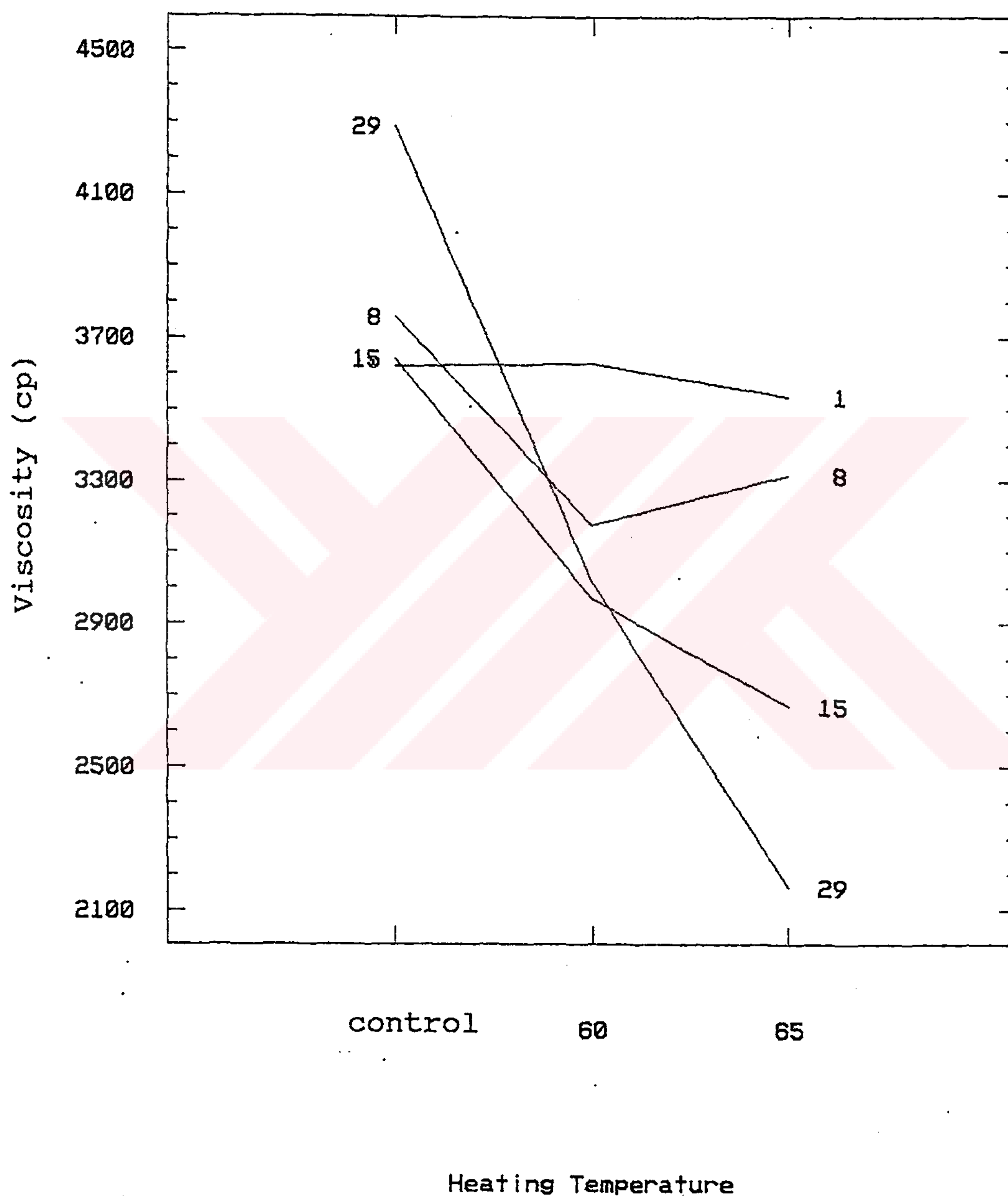

Figure 22. Plot of interactions for heating temperature by storage time (viscosity).

Viscosity of control, 60 and 65 °C heated yogurt significantly different from each other (Table 38).

The mean viscosity for 5 and 15 min heat treatment exhibited a statistical significant difference (Table 39).

Table 39. Multiple Range Analysis of Viscosity for Heating Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
15	12	3264.1667	X
10	12	3300.8333	XX
5	12	3380.8333	X

There was a significant effects of storage time on viscosity up to 15 days of storage but there after differences in viscosity were not significant (Table 40).

Table 40. Multiple Range Analysis of Viscosity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
15	9	3093.3333	X
29	9	3156.6667	X
8	9	3416.6667	X
1	9	3594.4444	X

3. 2. 6. Effects of Post Heat Treatment on Starter Culture Counts

If the post heat treatment at 65 °C was judged against the requirement $100*10^6$ cfu starter culture should be present per ml yogurt, 5-10-15 min. heated yogurt did not comply both after overnight storage and four weeks storage.

Same standard, tells that if the number of starter culture is less than $10*10^6$ cfu, result is unsatisfactory. When this standard is considered, heating at 65 °C for 15 min. is not acceptable. Number of starter culture decreased below $10*10^6$ cfu in one week storage. Increasing the heating temperature from 60 to 65 °C markedly lower the starter culture. Changes in number of starter culture

during storage were tabulated in Table 41.

Table 41. Influence of post heat treatment at 65 °C on the number of starter bacteria in yogurt during storage for four weeks.

Storage time (day)	Starter culture*10^6 (cfu) control	5 min.	10 min.	15 min.
1	1030	91	68	23
8	1180	56	80	12
15	1130	54	61	9
22	560	46	39	5
29	314	18	20	2.7

Control yogurt had high starter population and maintained this during storage. At the end of four weeks storage % reduction in the number of starter culture were 80.20, 70. 58, 88. 26 for 5-10-15 min. heated yogurts

Storage time, heating temperature and heating time significantly affected the total starter bacteria count (Table 42). Also there was a significant interaction between storage time and heating temperature; Heating time and heating temperature (Figure 23, 24).

Table 42. Result of ANOVA for Total Bacteria Count

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	1.2706521	3	.4235507	42.144	.0000
Heating time	.9474014	2	.4737007	47.134	.0000
Heating temp.	8.6079181	2	4.3039590	428.255	.0000
Interactions					
S.time-H.time.	.0263708	6	.0043951	.437	.5414
S.time-H.temp.	.8101208	6	.1350201	13.435	.0001
H.time-H.temp.	.8384444	4	.2096111	20.857	.0000

Details of these effects were given in Table 43. The mean log MRS count for each day of storage except one and 15 days exhibited statistical significant difference at α=0.05 level.

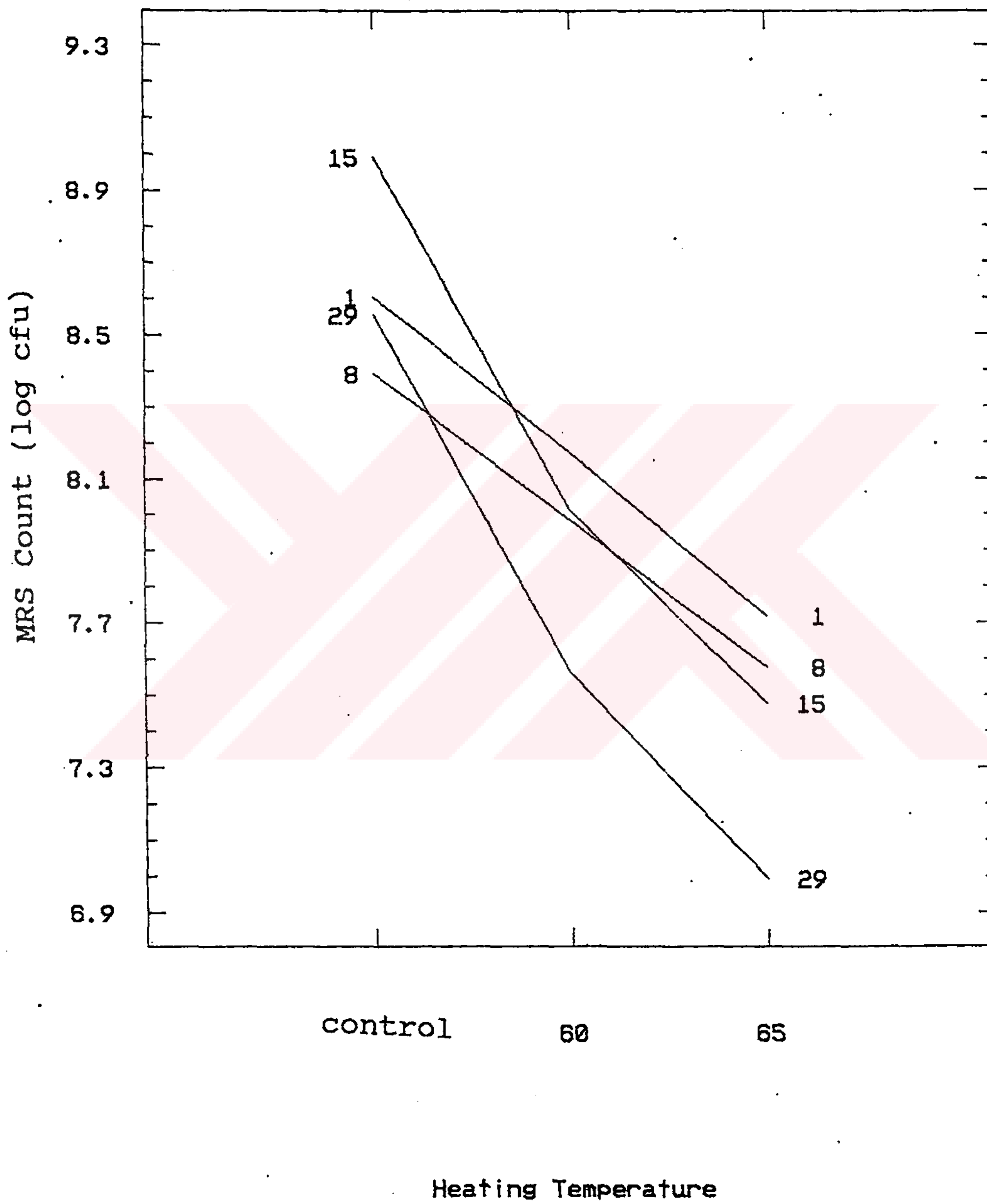

Figure 23. Plot of interactions for heating temperature by storage time (MRS count).

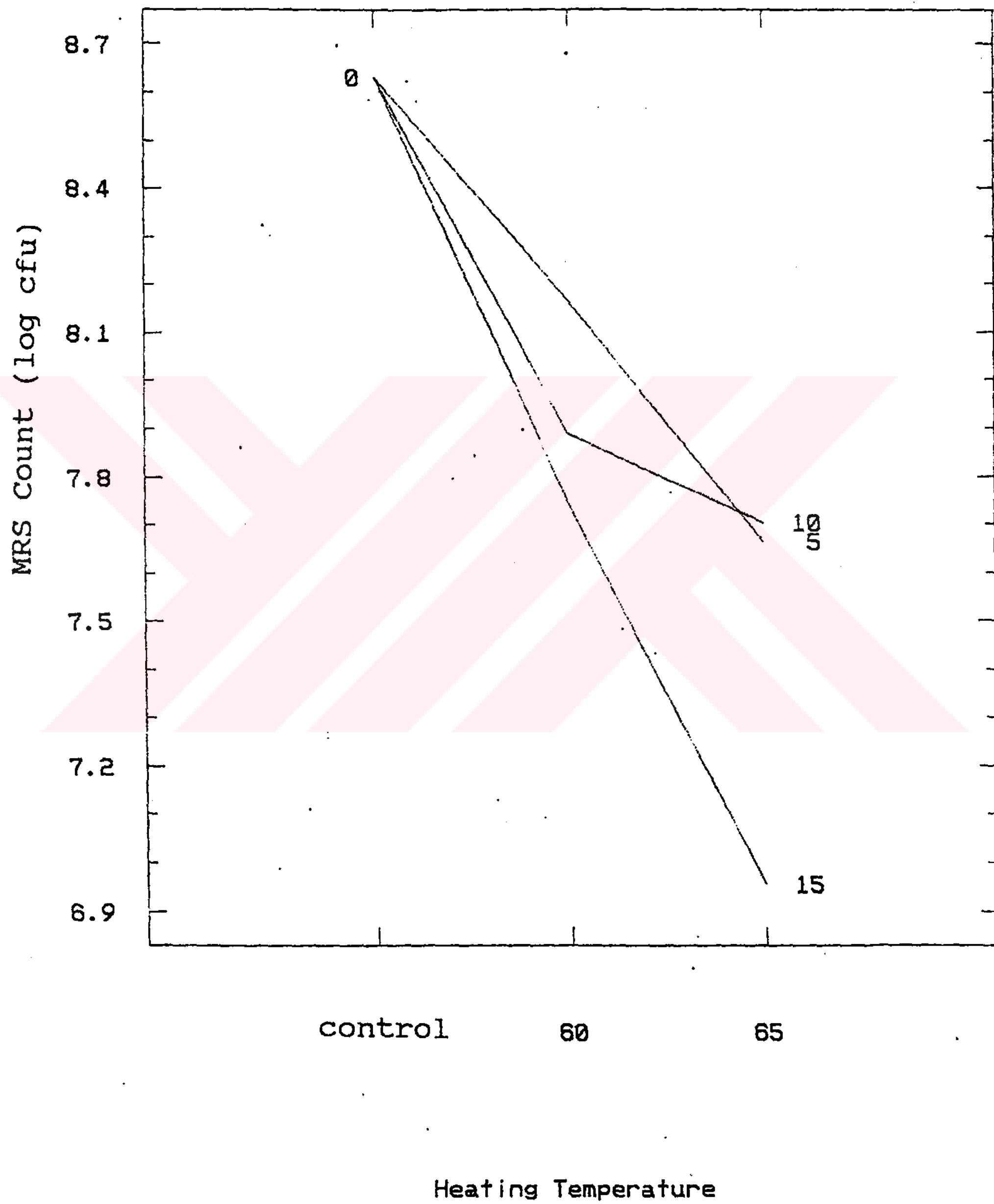

Figure 24. Plot of interactions for heating temperature by heating time (MRS) count.

Table 43. Multiple Range Analysis of Total Bacteria Count for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	9	7.7016667	X
8	9	7.9833333	X
15	9	8.1600000	X
1	9	8.1622222	X

Increase in the heating temperature significantly decreased the no. of starter bacteria. Highest count was observed for control, lowest one was for 65 °C heated yogurt (Table 44).

Table 44. Multiple Range Analysis of Total Starter Bacteria for Heating Temperature

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
65 °C	12	7.4408333	X
60	12	7.9320833	X
control	12	8.6325000	X

No significant difference was observed between 5 and 10 min. heating of yogurt. But LS Means of 15 min. heating significantly lower than the 10 and 5 min. heating yogurt (Table 45).

Table 45. Multiple Range Analysis of Total Starter Bacteria for Heating Time.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
15 °C	12	7.7770833	X
10	12	8.0741667	X
5	12	8.1541667	X

III. 2. 7. Total viable organisms

Difference between MRS and PCA gave the number of injured bacteria. Results were given in Table 46. Number of injured starter bacteria was higher at 65 C° than 60°C.

Table 46. Number of starter culture on MRS and PCA media.

Culture Media	Starter culture (cfu)*10^6		
	5 min.	10 min.	15 min.
MRS	91	68	45
PCA	104	120	110
Difference	13	52	65

III. 2. 8. Effect of Heat Treatment on Mold and Yeast

No yeast and mold were detected at any time during storage.

3. 3. Nisin Treatment of Yogurt

3. 3. 1. Diffusion of Nisin in Yogurt

Up to now, nisin has been used in stirred yogurt. Although set yogurt was produced in great extent in the world (there is no stirred yogurt production in Turkey), there is no study published concerning the use of nisin in set yogurt. This is possibly due to the lack of diffusion data of nisin in yogurt or in other products.

In this study, use of nisin in set yogurt was searched. In order to check whether nisin diffuse inside the yogurt and prevent the lactic acid production, nisin solution was spreaded on the surface of yogurt and number of starter culture, pH and titratable acidity of samples from top, middle and bottom of the glass were analyzed periodically.

Results were illustrated on Fig. 25, 27. As is evident from Figure 25, there is a progressive decline in pH of both control and nisin treated samples. Slight difference was detected between the layers in nisin treated samples. This implies that the nisin diffused inside the yogurt and can be used in set yogurt.

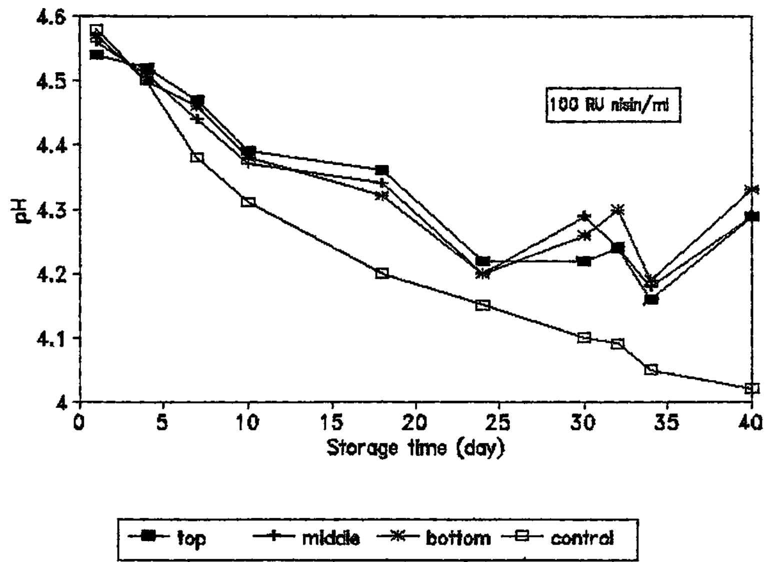

Figure 25. Effect of nisin addition on pH at different depth

Titratable acidities of each layer during storage were represented in Figure 26.

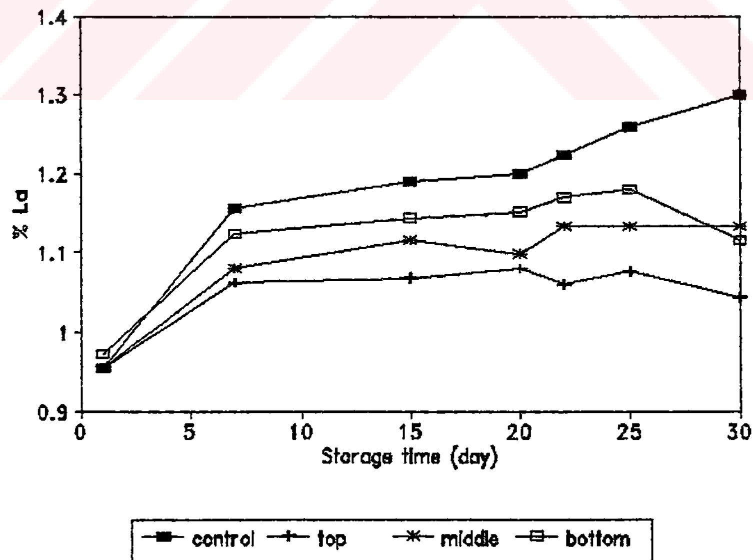

Figure 26. Effect of nisin addition on titratable acidity at different depth.

As seen from Figure 26 initial acidities were similar for control and samples from top-middle and bottom of the glass after one day of storage (0.954-9.72 %). Marked increase was observed during one week storage of all samples. % increase in sample from the top having the minimum acidity changes (11.32 %) with respect to control (20.5 %). It was expected, because surface of the yogurt directly contacts with the nisin compared to middle and bottom layer of the jars. % changes in titratable acidity for middle and bottom samples were found to be 13.21 and 16.98 % respectively.

At the end of refrigerated storage for four weeks minimum acidity was observed for sample from top (1.044 %) followed by middle (1.134 %) and bottom (16 %). Changes was the highest for control (1.3 %). % increase in lactic acid were 36.3 %, 9.43 %, 18.7 %, 14.8 % for control, top bottom and middle samples respectively.

Results of MRS count of yogurt from top, bottom and middle of the jar were illustrated in Figure 27. Maximum decrease was observed for the sample from top (61 %) and minimum decrease for bottom samples (6.7 %) as compared with the control after one day of storage. While log number of starter culture were 8.38 cfu and 7.97 cfu for control and top samples, bottom and middle samples had 8.35 cfu and 8.26 cfu. Later bacterial population of all samples reduced. At the end of storage, maximum decline in MRS counts was observed in case of sample from top (97.6 %) followed by middle (86.3 %) and bottom (90.7 %) of 100 RU/ml nisin containing yogurt and control.

Richard studied on the factors affecting nisin activity in cheese and concluded that the diffusion of nisin in acid clotted milk was not an important factor. There are two natural variations of nisin, nisin A and nisin Z which differ by only one amino acid. It is claimed that this minor change gives nisin Z better diffusion property in solid media than nisin A [99].

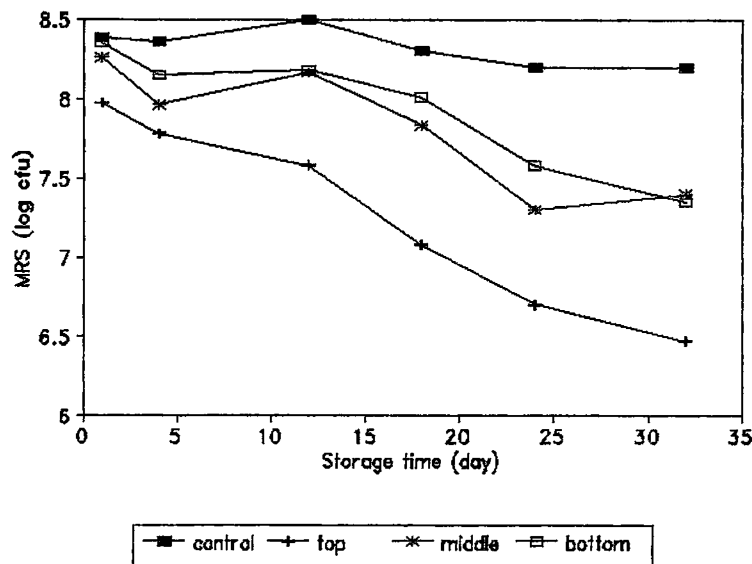

Figure 27. Effect of nisin addition on MRS count at different depth

3. 3. 2. Effect of Nisin Treatment on pH

It could be observed that the initial acidity of samples was around 4.4. Progressive decrease in pH was observed in all samples during one week storage. % reduction was minimum for (2.4 %) for 150 RU/ml nisin containing yogurt and maximum for 50 RU/ml nisin containing yogurt (3.99). % reductions were 2.7 and 3.2 for 100 RU/ml nisin containing and control yogurt respectively. A sharp decrease was noted in control during 4 weeks storage. Yogurt samples containing 50-100-150 RU nisin/ml showed slight increase in pH in the last three weeks and reached to initial values. At the end of storage, pH of 100 and 150 RU nisin containing yogurt had same pH (4.39). Slight difference was noted between 50 RU nisin containing yogurt and 100-150 RU nisin containing yogurt. The changes in pH with respect to nisin concentration were shown in Fig. 28.

% pH change at the end of 4 weeks storage was 6.3, 0.1, 0.1, 0.23 for control 50-100-150 RU nisin containing yogurt respectively.

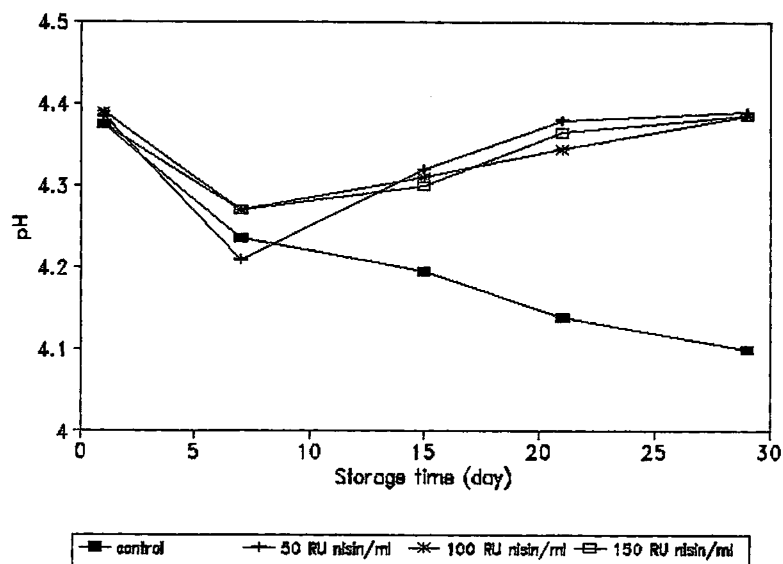

Figure 28. Effect of nisin treatment on pH

Data were analyzed by ANOVA and there found to be siginificant effect of nisin concentration and storage time on pH of yogurt (Table 47).

Table 47. Result of ANOVA for pH

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	.0341797	3	.0113932	2.543	.1216
Storage time	.0397047	3	.0132349	2.954	.0906

3. 3. 3. Effect of Nisin Addition to Titratable Acidity

It can be seen that initial acidities of yogurt samples were nearly same. Figure 29 represent gradual increase in acid production for control. It was important to indicate that addition of nisin reduced the lactic acid production comparing to control. % increase in titratable acidity was highest (12.26) for sample with 100 RU nisin after the first week. Slight increase was noted in acid

production for 50 (2.7 % to 5.4 %) and 150 RU nisin containing yogurt (6.6 % to 10. 8 %) during first and second week. Later lactic acid production remained nearly constant, addition of nisin gave stability to acid production. Total developed acidity on the 4th week was found to be maximum in control was 1.3 % (30 % increase) and minimum in the sample with 50 RU nisin was 1.053 (6.36 % increase). % lactic acid was 1.0755 (12. 7 %) and 1.0845 (11 %) for samples with 100 and 150 RU nisin.

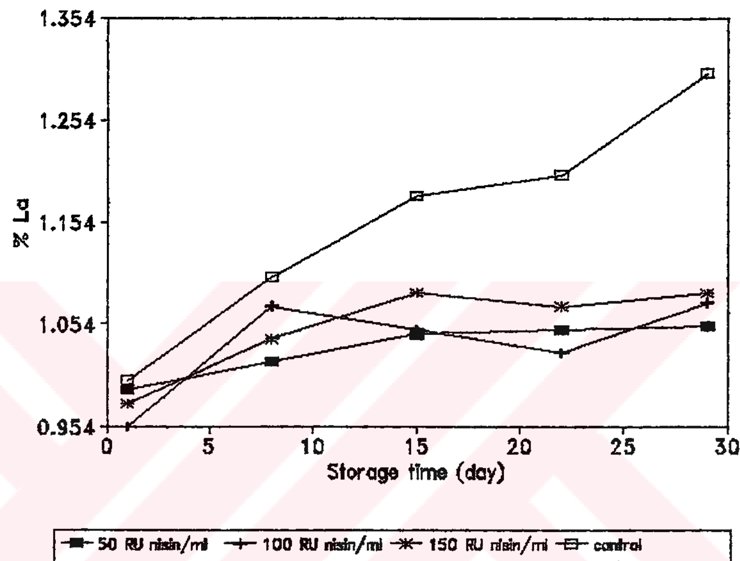

Figure 29. Lactic acid development in nisin treated yogurt

Table 48. Result of ANOVA for Titratable Acidity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	.0361003	3	.0120334	5.160	.0239
Storage time	0.474803	3	.0158268	6.787	.0109

ANOVA test showed that nisin concentration and storage time had siginificant effect on titratable acidity of yogurt at α=0.05 level (Table 48). Details of these effects were tabulated in Tables 49, 50.

Table 49. Multiple Range Analysis of Titratable Acidity for Nisin Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
50	4	1.0260000	X
100	4	1.0370000	X
150	4	1.0457500	X
control	4	1.1447500	X

Multiple Range Analysis showed that titratable acidity of control was significantly different from titratable acidities of 50-100-150 RU/ml nisin treated yogurt. LS mean was highest for the control. No siginificant differences were noted for titratable acidity among nisin treated yogurt.

Table 50. Multiple Range Analysis of Titratable Acidity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
1	4	.9797500	X
8	4	1.0567500	XX
15	4	1.0890000	X
29	4	1.1280000	X

Effect of storage time on titratable acidity was given in Table 50. Titratable acidities of 15 and 29 days stored yogurt significantly different from one day stored-yogurt.

Results were in agreement with the studies of incorporation of nisin in stirred yogurt. Prasad and Gupta observed sharp increase in control and 100 RU nisin containing yogurt on the 7th day of storage. In this study total developed acidity was found to be minimum with 25 RU nisin on the 10th day storage [146]. In conclusion, adding little amount of nisin to set yogurt increased its keeping quality by decreasing the acid production rate during storage and in turn increased the shelf-life without reducing its acceptability.

3. 3. 4. Effect of Nisin Treatment on Syneresis

It was seen that at day one, % syneresis was maximum (42.5 %) for control and minimum (40 %) for 100 and 150 RU nisin containing yogurt. It was 41 % for 50 RU containing yogurt. The results are illustrated in Fig. 30

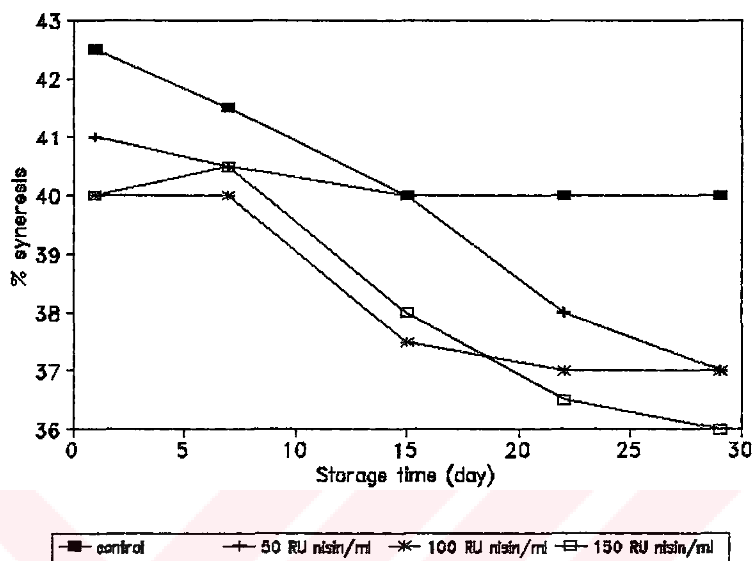

Figure 30. Effect of nisin treatment on syneresis

ANOVA test showed that nisin concentration and storage time had siginificant effect on syneresis of yogurt at α=0.05 level. Details of these effects were tabulated in Table 51.

Table 51. Result of ANOVA for Syneresis

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	15.171875	3	5.057292	10.367	.0028
Storage time	30.171875	3	10.057292	20.616	.0002

Syneresis of control was significantly different from syneresis of 50-100-150 RU/ml nisin treated yogurt. Difference in syneresis amoung nisin treated yogurt were not significant (Table 52).

Table 52. Multiple Range Analysis of Syneresis for Nisin Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
100	4	38.625000	X
150	4	38.625000	X
50	4	39.625000	X
control	4	41.000000	X

After two weeks storage, % syneresis of control reduced to 40 and remained constant. Syneresis decreased by the increasing the nisin concentration. Decrease was 36 %, 37 % and 39 % for 150-100 50 RU nisin containing yogurt respectively at the end of 4 weeks storage.

Table 53. Multiple Range Analysis of Syneresis for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	37.500000	X
15	4	38.875000	X
8	4	40.625000	X
1	4	40.875000	X

Storage time had significant effects on syneresis of yogurt. There was no significant effect for the first 8 days but thereafter it decreased significantly (Table 53).

3. 3. 5. Effect of Nisin Treatment on Yogurt Starter Culture

The observations on the number of yogurt starter bacteria were shown in Figure 31.

In general, the MRS count decreased during storage with an increase in the nisin concentration. Maximum inhibition (61 %) was noted in 150 RU nisin containing yogurts in day one. It was 35 % and 59 % for yogurts containing 50 and 100 RU nisin containing yogurt.

Further, there was a slight increase in yogurt starter count up to 7th day in control and nisin-containing samples. The decrease in counts after one week storage can be

attributed to cumulative action of nisin and the antimicrobial substances released by L. bulgaricus and S. thermophilus. Addition of nisin cause formation of large pores in the cytoplasmic membrane which allow the efflux of ions and small intracellular molecules (potassium, a.a's, ATP) finally resulting in cell death and possibly lysis [104]. In addition to pore forming activity, nisin is known to inhibit the outgrowth of bacterial spores and it might interfere with some enzymatic steps in the biosynthesis of the cell wall. Nisin also affects the activity of autolytic enzymes. Gupta and Parasad reported similar results with total plate count. They found that inhibition was 67 % for 100 RU nisin containing yogurt. They also observed continuous increase in control up to 5 th day in nisin containing samples [147].

After 4 weeks storage, max. destruction (81 %) was noticed for 150 RU nisin containing sample and min. for control (35 %). Inhibition was 74 % and 50 % for 50 and 100 RU nisin containing yogurt. As a result nisin retarded multiplication of starter culture.

It may be concluded that in the light of above facts controlled inhibition of growth of lactic acid organisms by nisin will result in minimal biochemical deterioration of set yogurt during storage. Nisin can therefore effectively be added for prolonging the shelf-life of set yogurt. This information could be of great interest in preservation of fermented milk products in which lactic contaminants pose major problem.

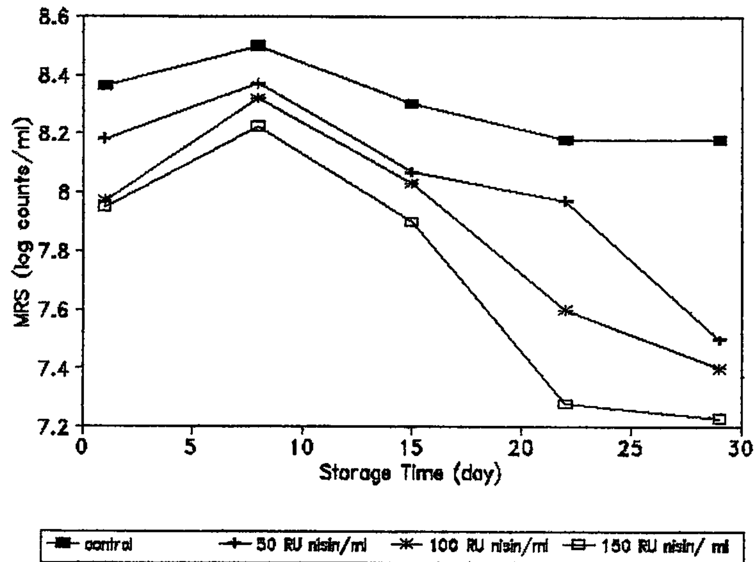

Figure 31. Effect of nisin level on MRS count

Table 54. Result of ANOVA for Total Starter Culture

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	.8270783	3	.2756928	8.608	.0052
Storage time	1.2799982	3	.4266661	13.321	.0012

Table 54 shows that the nisin concentration and storage time had significant efffect on number of starter bacteria.

Table 55. Multiple Range Analysis of Total Starter Bacteria Count for Nisin Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
150	4	7.7012500	X
100	4	7.9300000	XX
50	4	8.3342500	X
control	4	8.3342500	X

Number of starter culture of control was significantly different from 50-100-150 RU/ml nisin treated yogurt. Differences in syneresis amoung nisin treated yogurt were

not significant (Table 55).

Table 56. Multiple Range Analysis of Total Starter Bacteria for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	7.5767500	X
15	4	7.9500000	X
1	4	8.1150000	XX
8	4	8.3537500	X

No significant difference were noted in number of total starter bacteria up to 8 days of storage. As the storage time increased number of starter bacteria reduced significantly.

3. 3. 6. Effect of Nisin Treatment on *S. thermophilus*

Effect of nisin treatment on number of *S. thermophilus* was presented in Figure 32. All three concentration of nisin influence the count of *S. thermophilus* in the order 150 RU>100 RU> 50 RU. During storage there was a progressive decline in the counts of S. thermophilus. After one day of storage, maximum decrease was noted in 150 RU nisin treated yogurt (85 %). Decrease was 10.7 % and 27.6 % in case of 50 and 100 RU nisin containing yogurts.

After 4 weeks storage inhibitions were, 99 %, 94 %, 95 %, 79 % for 150-100-50 RU nisin containing yogurt and control respectively. Gupta and Parasad worked in this area and found similar results. Decrease was 81 % with 100 RU, 69 % 50 RU and 40 % with 25 RU nisin containing yogurt on day zero in stirred yogurt. They also found that MIC of *S. thermophilus* strains were 75-125 RU/ml in broth and 100-150 RU/ml in skim milk [148, 149]. They concluded that starter culture showed wide variation in nisin sensitivity at strain level [109].

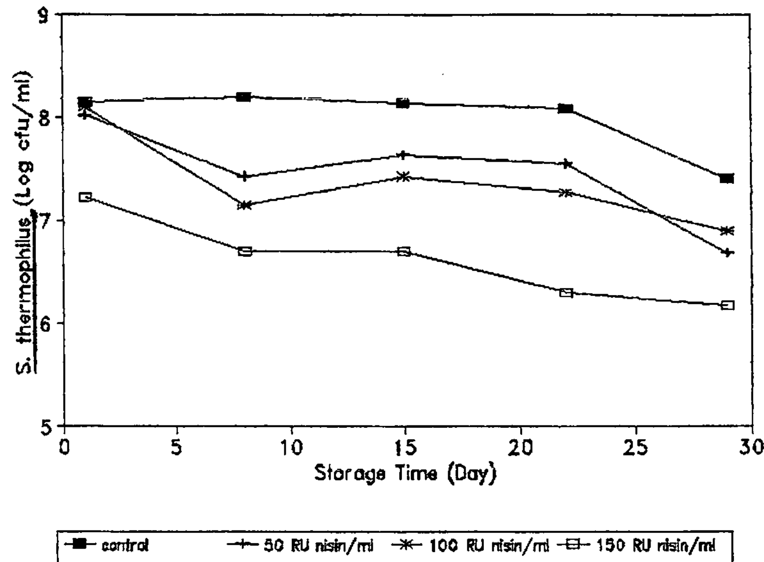

Figure 32. Effect of nisin treatment on the count of S. thermophilus.

Table 57. Result of ANOVA for S. thermophilus

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	3.4077188	3	1.1359063	26.498	.0001
Storage time	2.3293687	3	.7764562	18.113	.0004

Table 57 showed that the nisin concentration and storage time had significant effect on number of S. thermophilus.

Table 58. Multiple Range Analysis of S. thermophilus Count for Nisin Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
150	4	6.7000000	X
100	4	7.3950000	X
50	4	7.5175000	X
control	4	7.9900000	X

Number of S. thermophilus of control was significantly different from 50-100-150 RU/ml nisin treated yogurt.

Difference in number S. thermophilus between 50-100 Ru/ml nisin treated yogurt were not significant.

Table 59. Multiple Range Analysis of S. thermophilus for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	6.8075000	X
8	4	7.4450000	X
15	4	7.4775000	X
1	4	7.8725000	X

Differences in number of S. thermophilus at 8 days of storage were not significant from 15 days of stored yogurt.

3. 3. 6. Effect of Nisin Treatment on L. bulgaricus

Effect of nisin adddition on the number of L. bulgaricus was shown in Figure 33. While control maintained higher number of L. bulgaricus during storage, it decreased for nisin treated yogurt after one week of storage. Reduction in cell population was maximum for 150 RU/ml nisin treated yogurt. Although reduction was lower for 100 RU/ml nisin treated yogurt than 50 RU/ml nisin treated yogurt, at the end of storage count was in the order of 50>100>150 RU/ml.

Data were analyzed by ANOVA. There was a significant effect of storage time on number of L. bularicus. No significant effect was noticed for nisin concentration (Table 60).

Table 60. Result of ANOVA for L. bulgaricuss

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	.2681500	3	.0893833	2.588	.1176
Storage time	1.6994000	3	.5664667	16.401	.0005

Number of L. bulgaricus was significantly different for each storage time except one and 15 days stored yogurt

(Table 61).

Table 61. Multiple Range Analysis of L. bulgaricus for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	7.3300000	X
8	4	7.7850000	X
15	4	7.8355000	X
1	4	8.2500000	X

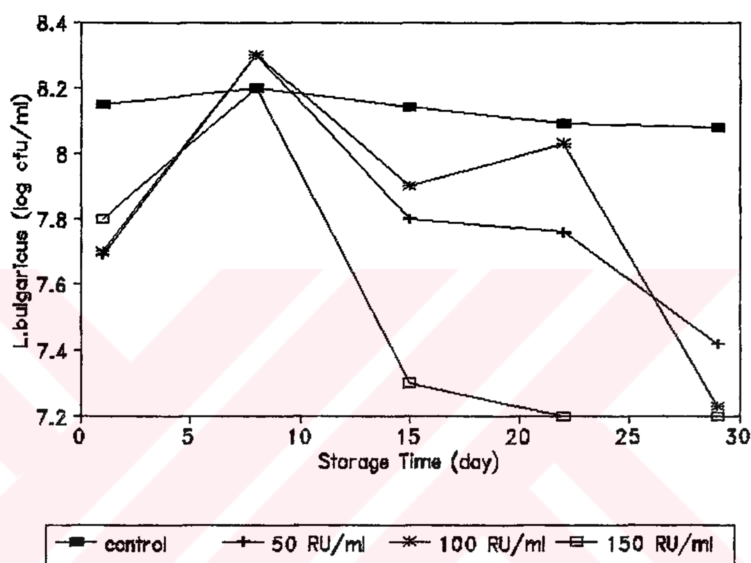

Figure 33. Effect of nisin treatment on the number of L. bulgaricus

3. 3. 7. Effect of Nisin Treatment on Viscosity of Yogurt

As seen in Figure 34, viscosities of control, 50-100-150 RU containing yogurt were 5800, 5900, 5640, 5410 cp respectively. After one week storage viscosities of all yogurt samples decreased, this may be due to the increase in starter culture count and increase in proteolytic action of bacteria during first week. At the end of 4 weeks storage, viscosities were 5100, 5700, 5570, 4960 for control, 50, 100, 150 RU nisin containing yogurt. %

reductions were 12.8, 12.5, 10.46, 5.36 for control, 50, 100 and 150 RU/ml nisin containing yogurts.

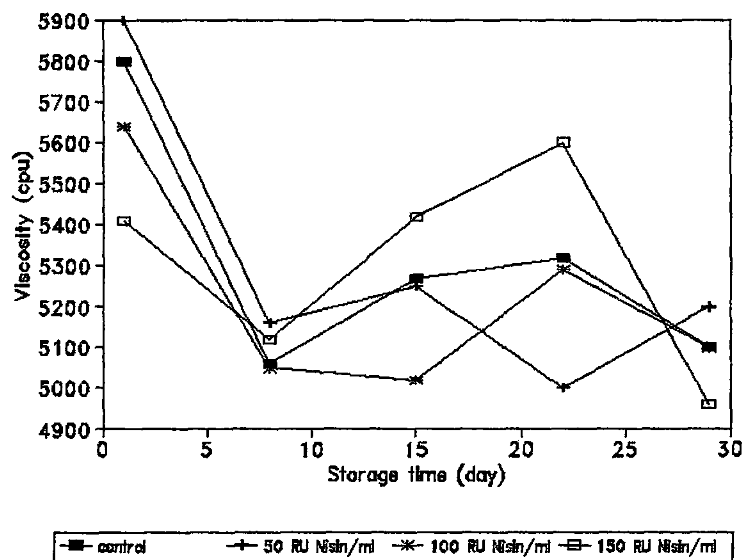

Figure 34. Effect of nisin treatment on viscosity

Table 62. Result of ANOVA for Viscosity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Nisin conc.	80100.00	3	26700.00	1.329	.3245
Storage time	940750.00	3	313583.3	15.614	.0007

Storage time had significant effect on viscosity of yogurt. No significant differences were noted for nisin concentration (Table 62).

Table 63. Multiple Range Analysis of Viscosity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	5095.0000	X
8	4	5097.5000	X
15	4	5240.0000	X
1	4	5687.5000	X

Details of the effects of storage time on viscosity of yogurt was evaluated by Multiple Range Analysis (Table 63). Viscosity of one day stored yogurt significantly different from viscosities of 8, 15, and 29 days stored yogurt.

3. 8. Effect of Nisin Treatment on Protein

As can be seen from Table 64 nisin addition did not affect the protein content of yogurt significantly. Nisin directly affect the cell wall of microorganism and protein content of yogurt mainly affected during fermentation stage rather than storage period.

Table 64. Effect of nisin treatment on protein content.

Sample	Protein Content (%)		
	1 day	1 week	4 week
Control	2.3	2.3	3.0
Yogurt (50 RU)	2.5	2.5	2.3
Yogurt (100 RU)	1.9	2.3	2.2
Yogurt (150 RU)	2.4	2.1	2.9
Milk 3.58			

Nisin concentration and storage time did not significantly affected protein content of yogurt.

3. 9. Effect of Nisin treatment on Yeast and Mold

Yeast and molds were absent in all samples with or without nisin. Gupta and Parasad concluded that no effect of nisin addition on yeast and mold was observed in controlling their growth [149].

IV. LYSOZYME TREATMENT OF YOGURT

4. 1. Preliminary Test

At present the experimental data on the effect of lysozyme treatment in yogurt making are still insufficient to assess its real value on the prevention of excessive acid development either alone or incombination with other preventing methods. In this study the possibility of using lysozyme to control lactic acid bacteria and consequently lactic acid fermentation was studied. 10 and 20 mg/L lysozyme solution was added on the surface of yogurt, after fermentation was completed. Yogurt samples from top, middle and bottom of the jars were analyzed for 10 days of storage. Results were tabulated in Table 65, 66. Slight differences were detected between the pH and acidities of control and lysozyme treated yogurts.

Table 65. Effect of lysozyme treatment on pH of yogurt.

Storage Time (day)	pH						
		10 mg/L			20 mg/L		
	c	L1	L2	L3	L1	L2	L3
1	4.57	4.58	4.51	4.54	4.57	4.51	4.54
7	4.43	4.42	4.38	4.38	4.44	4.44	4.44
10	4.51	4.53	4.51	4.50	4.55	4.54	4.53

L1: Top L2: Middle L3: Bottom

Similar to pH data, lysozyme treatment at 10 and 20 mg/L concentrations did not affect the lactic acid production possibly due to low concentration of lysozyme.

Table 66. Effect of lysozyme treatment on titratable acidities of control and lysozyme treated yogurt.

Storage Time (day)	Titratable Acidity (%)						
		10 mg/L			20 mg/L		
	c	L1	L2	L3	L1	L2	L3
1	0.999	0.936	1.044	-	0.99	1.170	1.008
7	1.080	1.008	1.134	1.152	1.16	1.098	0.882
10	1.098	1.152	1.170	1.008	1.188	1.188	1.242

Literature survey indicated that lysozyme is mainly used in cheese production to prevent butyric acid fermentation [115]. Effect of lysozyme treatment on L. bulgaricus, L. jugur, L helveticus, L. fermenti, S. thermophilus was studied by Lodi et all. It was concluded that lysozyme had no affect on the growth of acid production and consequently activity except L. helveticus [150]. Ottogalli et all. worked in similar area and found that except some L. helveticus strains all strains (L. bulgaricus, S. thermophilus, L. lactis, L.acidophilus) showed normal growth and acid production in the presence of lysozyme hydrochloride [151]. In Turkey 25 mg/L lysozyme addition to cheese was permitted. In general lysozyme concentrations used in cheese industry was in the range of 10 to 30 mg/L [115]. Pitotti et al. concluded that lysozyme can be used as a food additive in the quantity of higher than 100 mg/ml [152].

A number of studies showed that lysozyme addition at that concentrations stop the activity of Clostridium tributyricum and other food borne bacteria in cheese making without affecting the growth and development of starter bacteria and casein hydrolysis [153]. Also there is little information concerning the use of lysozyme treatment on yogurt making.

It may be concluded in the light of above facts that lysozyme concentration in preservation of yogurt should be higher than used in cheese making in order to slow down the lactic acid fermentation and 300-600-1200 mg/L lysozyme concentration was used in yogurt making.

4. 2. Diffusion of Lysozyme

600 mg/L lysozyme treated yogurt was used to follow the diffusion of lysozyme. Yogurt from top bottom and middle of the jars were analyzed for pH, titratable acidity and total number of starter bacteria during one month period.

After overnight storage pH of the control and bottom yogurt were the same (4.51) because lysozyme did not reach

the bottom of the jars. Yogurt from surface of the jars had higher pH (4.56) than middle (4.52). Since surface directly interact with the lysozyme and reduce the activity of starter culture. Yogurt from surface of the jars maintained its higher pH during storage and stayed nearly constant.

Differences in pH's of yogurt from middle and bottom were small. pH reduced during 12 days of storage due to low diffusion rate of lysozyme. Yogurt from bottom of the glass had lower pH than middle at the end of storage (Figure 35).

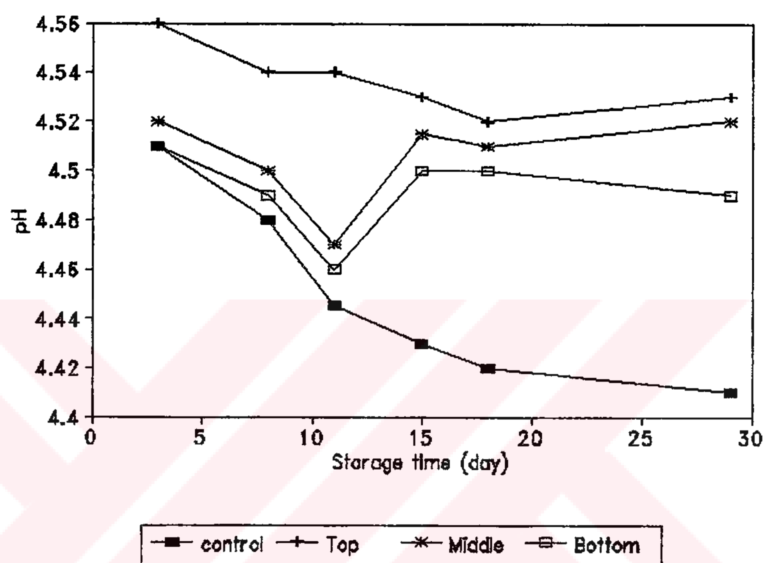

Figure 35. Effect of lysozyme treatment on pH of the top, bottom and middle yogurt

Titratable acidities of lysozyme treated yogurt at different levels were shown in Figure 36. Titratable acidity of surface yogurt was minimum (0.85 %) and bottom yogurt was maximum (0.99 %). It means that lysozyme did not reach the bottom of the jars after overnight storage. Titratable acidities of control and middle yogurt was 0.918 %.

During storage acidity of control increased continuously. Increase in titratable acidity was minimum for surface yogurt (9.8 %) due to higher reduction in

starter bacteria count. Small differences were noticed in acidities of yogurt from middle and top up to 18 days, later acidity of middle yogurt increased. Yogurt from bottom of the glass had highest acidity during storage.

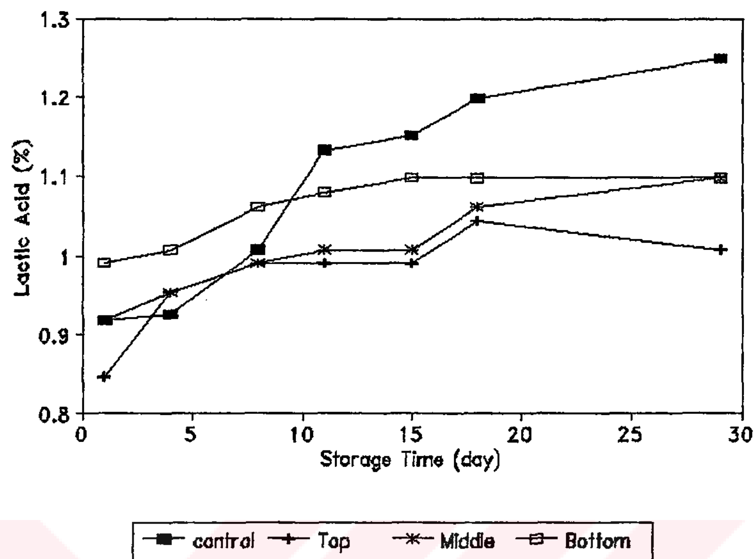

Figure 36. Effect of lysozyme treated yogurt on titratable acidity of top, middle and bottom yogurt

As seen from Figure 37 that number of starter culture for the control was log $147*10^6$ cfu/ml. It was found to be minimum for yogurt from top of the glass (log 7.19 cfu/ml). Yogurt from middle of the glass had higher starter bacteria load (log 7.24 cfu/ml) than top (log 7.19 cfu/ml). Starter culture count for the bottom yogurt (log 8.14 cfu/ml) was close to control (log 8.17 cfu/ml) because lysozyme did not reach to the bottom after overnight storage.

During storage period lysozyme diffused through the yogurt and decreased the cell population in all layer due to cell lysis. At the end of storage number of starter bacteria reduced to log 4.69 cfu/ml, log 5.2 cfu/ml and log 5.36 cfu/ml for top, middle and yogurt respectively.

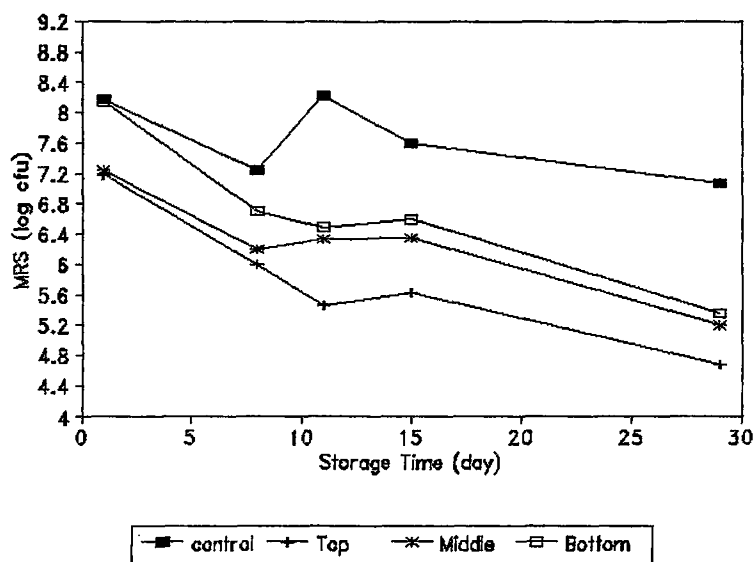

Figure 37. Effect of lysozyme treatment on the number of starter bacteria at top, middle and bottom of the glass

4. 3. Effect of Lysozyme Treatment on pH

Initial pH of control was 4.55. pH of lysozyme treated yogurts was higher than control. Lysozyme reduced the metabolic activity of starter culture so slowed down the lactic acid production. Lower the titratable acidity higher the pH. Small differences were noted between the initial pH's of 300 mg/L (4.46), 600 mg/L (4.485) and 1200 mg/L (4.465) lysozyme treated yogurt. Effect of lysozyme treatment on pH was shown in Figure 38.

During storage period, pH of 300 mg/L lysozyme treated yogurt had lower pH than 600 mg/L and 1200 mg/L lysozyme added yogurt. Final pH of 300 mg/L lysozyme treated yogurt was 4.51. followed by 4.56 and 4.55 for 600 mg/L and 1200 mg/L lysozyme treated yogurt respectively.

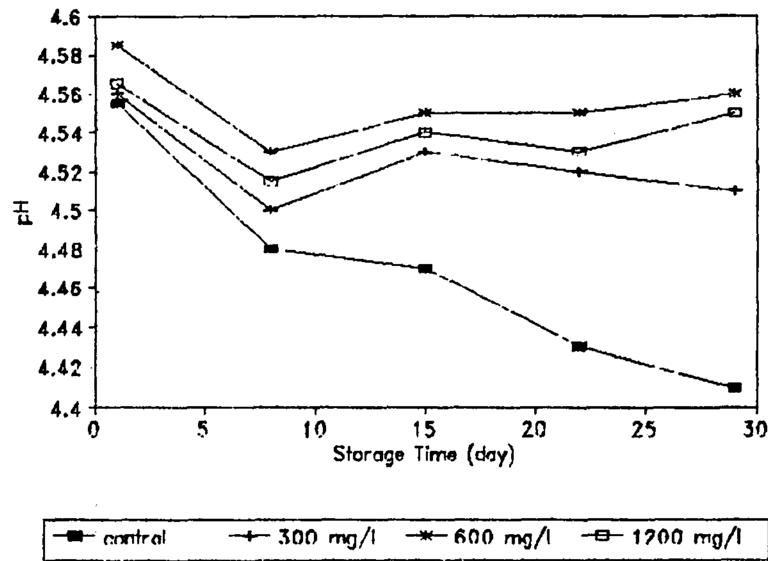

Figure 38. Influence of lysozyme treatment at different concentration on pH of the yogurt

Table 67. Result of ANOVA for pH

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	.0136812	3	.0045604	6.667	.0115
Storage time	.0094563	3	.0031521	4.608	.0323

ANOVA test showed that lysozyme concentration and storage time had significant effect on pH of yogurt at α=0.05 level (Table 67). Details of these effects were tabulated in Tables 68, 69.

Table 68. Multiple Range Analysis of pH for Lysozyme Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
control	4	4.4787500	X
300	4	4.5250000	X
600	4	4.5562500	X
1200	4	4.5625000	X

Multiple Range Analysis showed that pH of control was significantly different from pH of 300-600-1200 mg/ml lysozyme treated yogurt. LS mean was lower for the control. No significant differences were noted for pH among lysozyme treated yogurts.

Table 69. Multiple Range Analysis of pH for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups	
8	4	4.5062500	X	
29	4	4.5075000	X	
15	4	4.5225000	X	
1	4	4.5662500		X

pH of one day stored yogurt was significantly different from pH of 8-15-29 days stored yogurts.

4. 4. Effect of Lysozyme Treatment on Titratable Acidity

It was seen from Figure 39 that initial acidity increased by decreasing the concentration of lysozyme. Titratable acidity of control was 0.89 %. 1200 mg/L lysozyme treatment gave low acidity (0.87 %) followed by 600 mg/L (0.92) and 300 mg/L (1.017) after overnight storage.

300 mg/L lysozyme treated yogurt maintained higher acidity during storage with respect to 600 and 1200 mg/L lysozyme treated yogurt. Small differences were noted in acidities of 600 and 1200 mg/L lysozyme treated yogurt. In one study, titratable acidity of lysozyme treated cheese whey and control were compared and it was concluded that lysozyme treated cheese whey had lower titratable acidity than control up to 300 ppm. On the other hand 26 strains of <u>L. helveticus</u> isolated from the lysozyme cheese whey grew better in the presence of lysozyme than control whey [154].

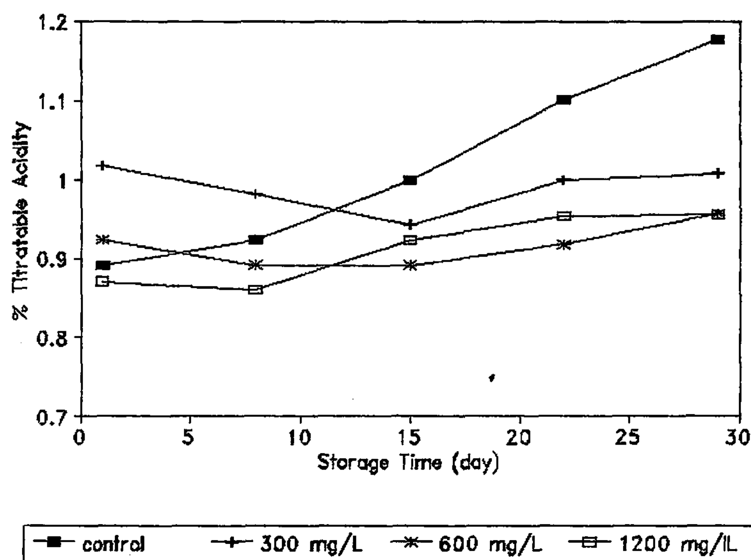

Figure 39. Effect of lysozyme treatment at different concentration on titratable acidity of yogurt

ANOVA test showed that lysozyme concentration and storage time had no significant effects on titratable acidity of yogurt at α=0.05 level (Table 70).

Table 70. Result of ANOVA for Titratable Acidity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	.0283602	3	.0094534	2.731	.1061
Storage time	.0301412	3	.0100471	2.902	.0939

4. 5. Effect of Lysozyme treatment on Syneresis and Viscosity of Yogurt

Syneresis of control and 300 mg/L lysozyme treated yogurt were 40 %. Lysozyme treatment at 600 mg/L and 1200 mg/L increased the syneresis of yogurt to 42 % and 43 % respectively after one day of storage. During storage syneresis reduced for control and reached to 36 % at the end of storage due to increase in acidity and water binding capacity of protein. But syneresis of lysozyme treated

yogurt decreased during storage. Final syneresis were 42 %, 43 %, 46 % for 300 mg/L, 600 mg/L and 1200 mg/L lysozyme treated yogurt respectively after 29 days of storage.

Possible reason for the increase in syneresis was the reduction in number of starter bacteria as a result of lysozyme treatment. Electron microscopy of yogurt showed that L. bulgaricus grew in a bundle of cells while S. thermophilus resulted in the appearance of globular bunches that were often very large. Around the S. thermophilus very large void space were formed while the former were found narrow channels. Moreover starter bacteria produce threads of polysaccharide which linked both the cells to each other and at the same the starter culture to the yogurt matrix of coagulated casein. Decrease in starter culture count prevent the formation of polysaccharide filaments that cause leakage of water from void and produce soft texture [139].

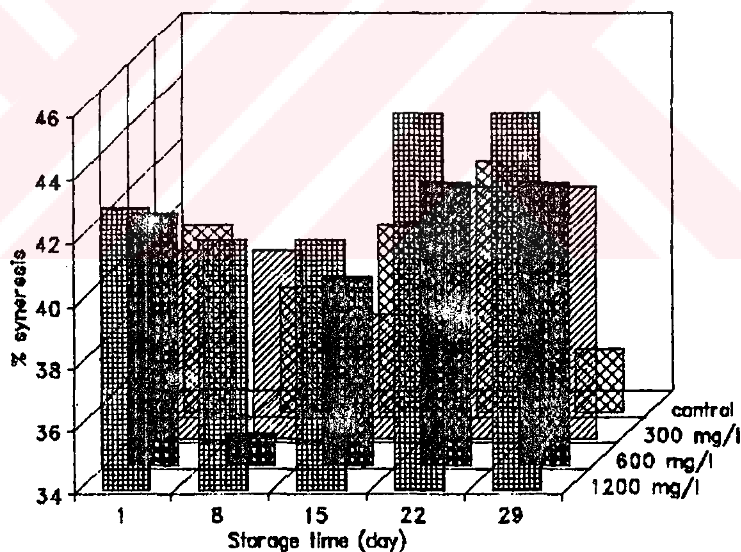

Figure 40. Effect of lysozyme treatment on syneresis of yogurt

Similar result was observed for the viscosity of yogurt. Viscosity of control yogurt was 5940 cp after overnight

storage. They reduced to 5430 cp, 5390 cp, and 5250 cp in the case of 300 mg/L, 600 mg/L and 1200 mg/L lysozyme treatment. Control maintained higher viscosity during storage. Final viscosity was 5640 at the end of storage. Viscosities of lysozyme treated yogurt tend to decrease and were 4600 cp, 4500 cp and 4420 cp after four weeks storage is mainly due to decrease in cell population.

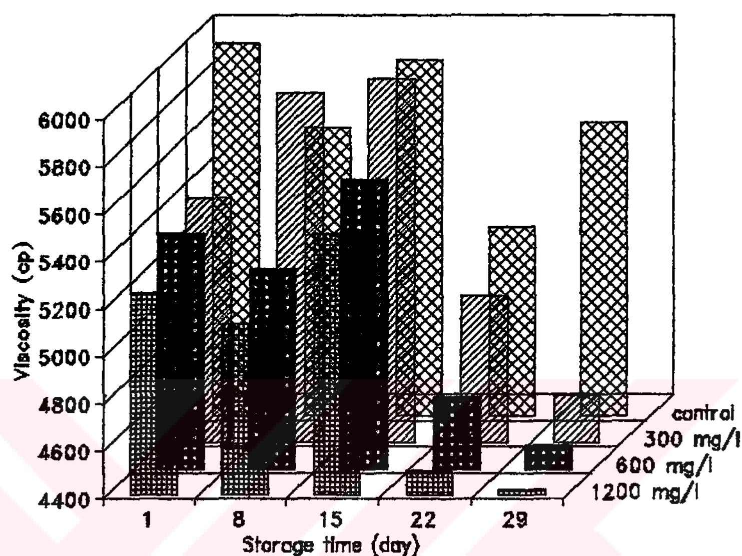

Figure 41. Effect of lysozyme treatment on viscosity of yogurt

Table 71. Result of ANOVA for Syneresis

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	52.250000	3	17.416667	2.889	.0947
Storage time	19.250000	3	6.416667	1.065	.4114

No significant difference in syneresis was noted for lysozyme concentration and storage time (Table 71).

Table 72. Result of ANOVA for Viscosity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	1198100	3	399366.67	6.274	.0138
Storage time	1998050	3	666016.67	10.464	.0027

There was significant effect of lysozyme concentration and storage time on viscosity of yogurt (Table 72).

Table 73. Multiple Range Analysis of Viscosity for Lysozyme Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
1200	4	5072.5000	X
600	4	5187.5000	X
300	4	5457.5000	XX
control	4	5782.5000	X

Multiple Range Analysis showed that 600-1200 mg/ml lysozyme treatment significantly affected viscosity of yogurt. No significant differences in viscosity were noted for 300 mg/ml lysozyme treated yogurt.

Table 74. Multiple Range Analysis of Viscosity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	4790.0000	X
8	4	5462.5000	X
1	4	5510.0000	X
15	4	5737.5000	X

Differences in viscosity were not significant during three weeks of storage. Viscosities of yogurt significantly reduced after 29 days of storage.

4. 7. Effect of Lysozyme Treatment on Number of Starter Culture

Lysozyme treatment reduced the viable cell number of starter bacteria comparing to control after overnight storage. While control had initial inoculum of log 8.1

cfu/ml number of starter bacteria reduced to log 7.4 cfu/ml and log 7.5 cfu/ml for 300 600 mg/L lysozyme treated yogurt. 1200 mg/L lysozyme treatment reduced the starter bacteria count to log 6.95 cfu/ml. As the concentration increased, lethality of starter culture increased.

During refrigerated storage, number of starter bacteria in control was higher and at the end of storage it reached to log 7.97 cfu/ml. Number of starter bacteria continuously reduced during storage in the presence of 1200 mg/L lysozyme and reached to log 3.25 cfu/ml. Starter bacteria count were nearly same for 300 and 600 mg/L lysozyme treated yogurt (log 4.8 cfu/ml) at the end of storage.

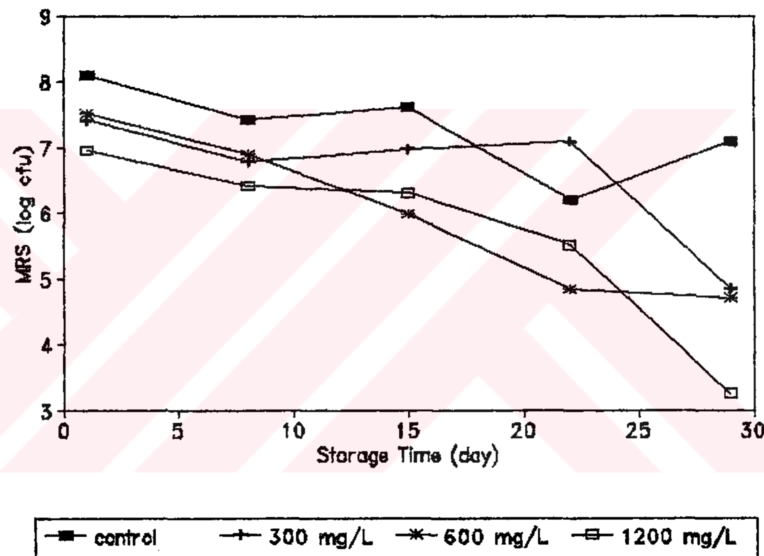

Figure 42. Influence of lysozyme treatment on number of starter bacteria

Decrease in number of starter bacteria with the addition of lysozyme can be explained on the basis that lysozyme cause lysis of bacterial cells in the culture, resulting in inhibition of growth and death of the cell. The injury is manifested by alterations in different structural and functional components of cell wall. Then bacterial cells loose their barrier functions allowing

different types of molecules to get inside and outside the cell, hence cells that are normally resistant antimicrobial agents loose their viability.

Table 75. Result of ANOVA for Starter Bacteria

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	11.330069	3	3.7766896	5.685	.0183
Storage time	10.481569	3	3.4938563	5.259	.0227

Lysozyme concentration and storage time had significant effect on the number of starter bacteria (Table 75).

Table 76. Multiple Range Analysis of Starter Bacteria Count for Lysozyme Concentration.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
1200	4	5.5250000	X
600	4	6.2675000	X
300	4	6.6375000	XX
control	4	7.7675000	X

Multiple Range Analysis showed that 600-1200 mg/ml lysozyme treatment significantly decreased the number of starter bacteria. 300 mg/ml lysozyme addition did not affected the starter bacteria count.

Table 77. Multiple Range Analysis of Number of Starter Bacteria for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	5.1925000	X
15	4	6.6500000	X
8	4	6.8675000	X
1	4	7.4875000	X

There were no significant differences in number of starter bacteria up to 29 days of storage. At the end of 29 days number of starter bacteria significantly reduced.

4. 8. Effect of Lysozyme Treatment on Protein Content of Yogurt

Lysozyme treatment did not affect the protein content of yogurt. Since main function of the lysozyme was the bacterial cell wall and as a result lysis of cell. There was no direct effect on the network of the yogurt, but proteolysis could be slowed down by the inhibition of starter culture. As seen from Table 78 very small differences were detected during storage.

Table 78. Effect of lysozyme treatment at different concentrations on protein content

Storage time (day)	Protein content (%)			
	control	300 mg/L	600 mg/L	1200 mg/L
1	2.89	2.98	2.76	2.68
29	2.53	2.83	2.74	2.65

Table 79. Result of ANOVA for Protein Content

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Lysozyme conc.	.0392000	1	.0392000	3.136	.1747
Storage time	.0652500	3	.0217500	1.740	.3302

Lysozyme concentration and storage time did not significantly affected protein content of yogurt.

V. MICROWAVE TREATMENT OF YOGURT

5. 1. Time-Temperature Profile of Yogurt

In order to obtain time-temperature profile of yogurt samples were microwaved at 5 different power setting for 60 sec and at each 10 sec temperature was measured. Two data set were obtained by measuring the temperature of yogurt having different initial temperature. Results were given in Figure 43 and 44. It was observed that increasing the microwave treatment time and power level increased the temperature of yogurt. At low power setting little increase in temperature of yogurt was observed. At high power setting linear relationships observed between the time of heating and temperature. Heddleson et al. found similar time-temperature profile for milk [91]. When microwaves enter the yogurt, they interact with the positive and negative charges region on water molecules and generates heat by molecular frictions. As interaction time and power level increases more heat generates and temperature of yogurt increases. Experimental study showed that microwave penetration level vary within yogurt depending upon the depth. So temperature of yogurt was different at the edge of the jars and at the center. Edge overheats which is due to energy transfer at foods surfaces from two to three directions. Also possible reason for this variation could be explained with the high moisture content and low heat capacity of yogurt. The greater the moisture content and lower heat capacity the insubstantial the microwave penetration depth and consequently the less uniform heating throughout the product [88]. Preliminary tests indicated that high power setting and long time treatment first caused whey separation at the surface of yogurt and acidic flavour was felt. Since more energy is absorbed near product surface and temperatures became higher nearer the surface than the center at 2450 MHz frequency. As a result evaporation of flavour giving compound gave acidic flavour. Later texture of yogurt completely disturbed due to increase in temperature (around 80 °C) and curd was completely separated from whey. Recent studies showed that temperature at the surface of the product was lowest than bottom and edge. Because much heat dissipated from the surface of microwaved product. Cool oven

air acts as a heat sink. But in this research jars were wrapped with microwavable foil to prevent contamination and consequently heat loss from surface of yogurt was prevented and surface temperature became higher.

Tests showed that texture of yogurt can resist following time-power combination without any deterioration. Longer treatment time, than given below caused complete upset of yogurt texture.

Power 5 (100 %) 15 sec
Power 4 (70 %) 20 sec
Power 3 (35 %) 35 sec
Power 2 (30 %) 100 sec

Although at power setting 1 (10 %) yogurt seemed to stand 180 sec treatment without appreciable effect observation showed that yogurt was liquefied and lost its resistance to flow during storage. In addition to this when the yogurt surface was broken by using a glass rod immediately after microwave treatment applied, it was observed that inner texture was not smooth and sudden whey separation was seen.

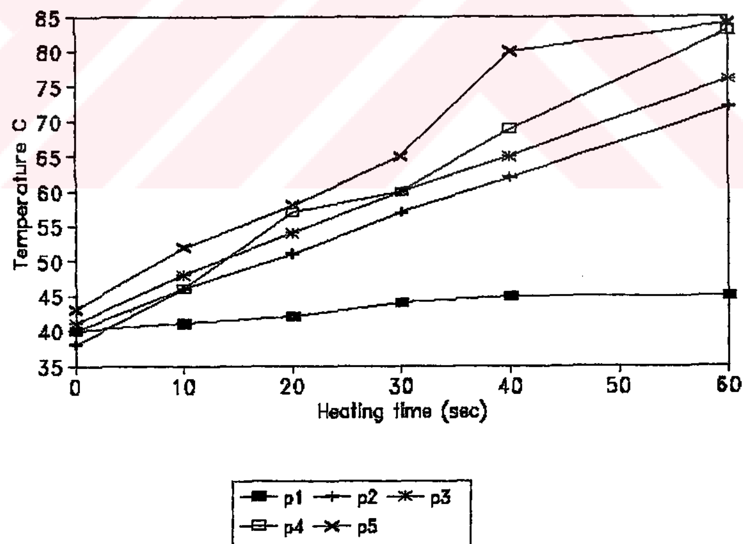

Figure 43. Time temperature profile of yogurt (initial temperature of around 43 °C) at the center of the plastic glass.

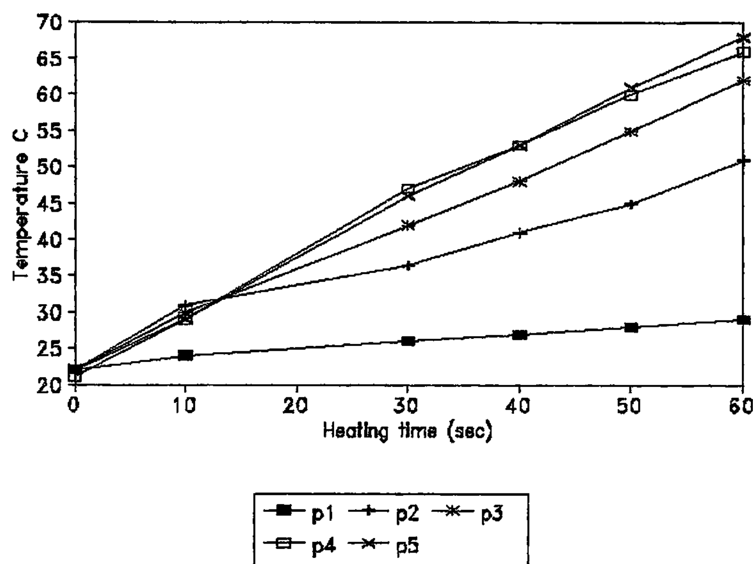

Figure 44. Time temperature profile of yogurt (initial temperature of 23 °C) at the center of the jar

In the light of above facts that following time-power setting combinations were selected for microwave treatment of yogurt;

Power setting 1 (P1)- 10 and 40 sec

Power setting 3 (P3)- 10 and 20 sec

Power setting 5 (P5)- 5 and 10 sec

5. 2. Effects of Microwave Treatment on pH

It was observed that application of microwave treatment reduced the pH of yogurt with respect to control after overnight storage. Increasing treatment time from 10 to 40 sec at power setting 1 reduced pH from 4.165 to 4.12. While pH of microwave treated yogurts, P3- 10 and 20 sec, P5-5, P5- 10 were 4.08, 4.095, 4.12 respectively, control had higher pH value (4.20).

It is expected that differences in initial pH between microwave treated yogurt and control will be lower than differences in conventional heat treatment due to faster transfer of thermal energy in microwave treatment than

conventional (10-20 times faster) [88]. Transfer of thermal energy depends on the electrical and physical properties of product (dielectric constant which is affected by moisture and salt content,loss factor, heat capacity, density) chemical composition, temperature of product [82]. In other words microwave treatment is more food dependent than conventional heat treatment. But it was seen that pH decreased as the power level and treatment time increase compare to control. Result were represented in Figure 45.

During storage longest treatment time and lowest power level (P1-40 sec) gave stability to pH (4.12-4.135). pH of P1- 10 sec microwave treated yogurt tended to decrease during one week storage (4. 165 to 4.11) but after a while their pH's stayed approximately constant. P3- 20 sec microwave treated yogurt had always higher pH than P3- 10 sec.

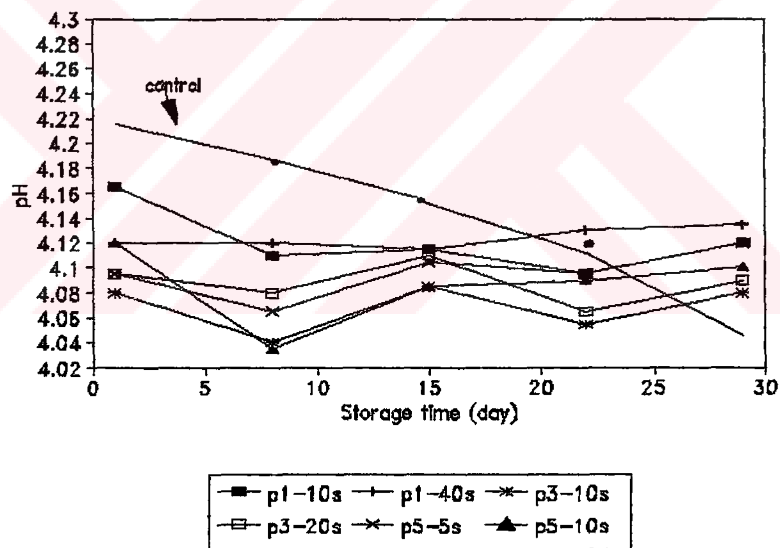

Figure 45. Influence of microwave treatment on the pH of yogurt.

At the end of four weeks of storage % increase in pH was minimum (0.364 %) for treatment of P1 for 40 sec. and maximum (1.08 %) for P1 for 10 sec. Increase in pH was

higher for P3 20 sec (0.855 %) than P5 5 sec. (0.61 %). A small difference was noted in pH between P1 40 sec (0.364 %) and P5 10 sec (0.485 %). P3 10 sec microwave treated yogurt reached to its initial pH (4.08 %).

In order to estimate the effects storage time and power level at treatment time 10 sec ANOVA at α=0.05 level was done. Storage time and power level didn't affect the titratable acidity, syneresis, viscosity of yogurt significantly due to the lower treatment time, 10 sec.

Power level significantly affected pH of yogurt (Table 80).

Table 80. Result of ANOVA for pH

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.0088687	3	.0029562	1.757	.2251
power level	.0334813	3	.0111604	6.633	.0117

Table 81. Multiple Range Analysis of pH for Power Level

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
Power1	4	4.0712500	X
Power3	4	4.0855000	X
Power5	4	4.1275000	XX
control	4	4.1887500	X

Differences in pH was significant for control and power setting 1-3. No significant difference was detected among power setting.

5. 3. Effect of Microwave Treatment on Titratable Acidity

Parallel to reduction in initial pH value of microwave treated sample, microwave treatment increased the initial lactic acid content of microwave treated yogurt with respect to control. As power levels increased differences in lactic acid content of yogurt increased in the order of P5>P3>P1. Power absorption of foods varies with volume and

moisture content of product. Power is less efficiently absorbed by small load volumes of low and intermediated moisture content [87]. P1 10 and 40 sec treated yogurt had lowest lactic acid content (0.918 % and 0.909 %). In the case of P5 5 and 10 sec treatment lactic acid were maximum (1. 02 % and 1.035 %). Treatment at P3 for 10 and 20 sec gave lactic acid content of 0.95 % and 0.963 % after overnight storage.

It was seen from Figure 46 that titratable acidity of P3-20 sec microwave treated yogurt stayed lower than 10 sec treatment due to the longer treatment time at the same power setting during storage period. Titratable acidity of microwave treated yogurt at P5 5 sec showed increase from 0.864 to 1.026 % during the last week storage due to shortest treatment time. P5 for 10 sec microwave treated yogurt stayed lower than P5 for 5 sec during storage. It was important to note that longer the microwave treatment time, lower the lactic acid development rate.

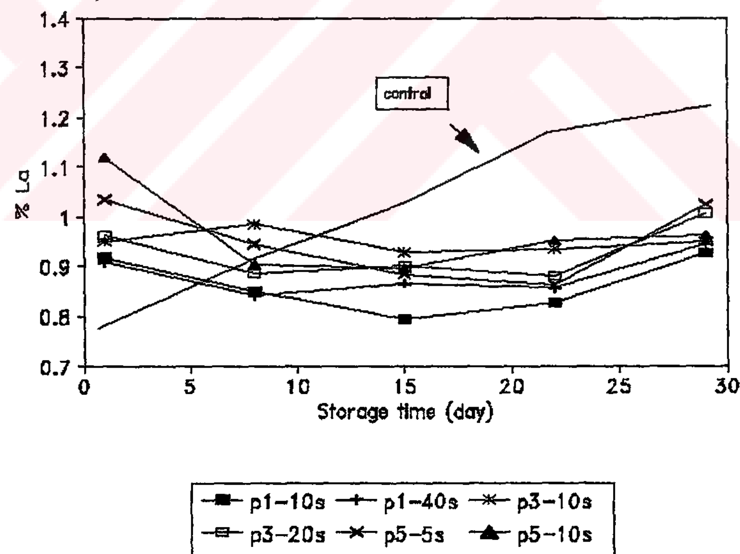

Figure 46. Effect of Microwave treatment on lactic acid content of yogurt

5. 4. Effect of Microwave Treatment on Syneresis of Yogurt

It was seen from the Figure 47 that initial % syneresis of P1 10 sec microwave treated yogurt was minimum and same with P3 10 sec (36 %). It was 38 % for P1 -40 sec treatment followed by P5 5 sec (39 %), P3 20 (40 %). Maximum syneresis was obtained for P5 10 sec treatment (41 %). During 10 days of storage % syneresis of microwave treated yogurt increased parallel to decrease in viscosity.

At the end of storage period % syneresis of P5 5 sec treated yogurt were found to be highest (43 %) and lowest for control (37.5 %). While % syneresis was 42 % for P3 20 ,P1 40 had syneresis of 40 %.

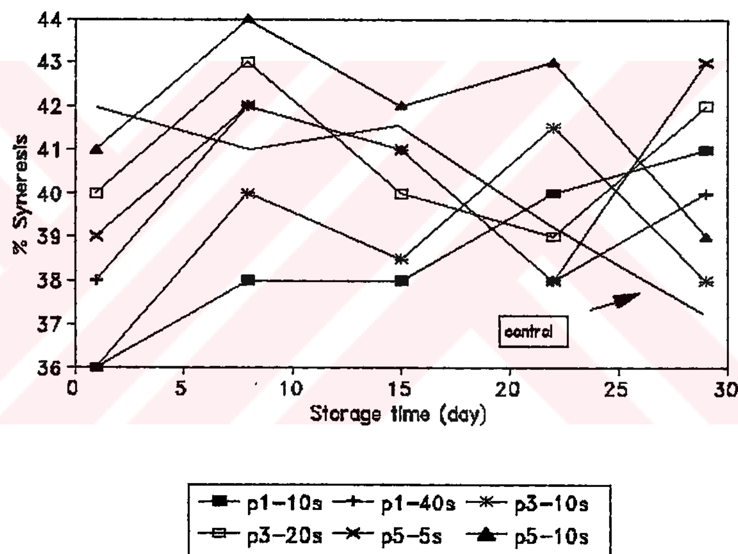

Figure 47. Effect of microwave treatment on whey syneresis of yogurt

5. 5. Effect of Microwave Treatment on Viscosity of Yogurt

As it can be seen from Figure 48 viscosity data gave parallel results with syneresis. Viscosity was minimum for P5-5 sec treated yogurt (5030 cp), and maximum for P1-10 sec treated yogurt (6220 cp) after overnight storage.

Similar to syneresis data viscosities of yogurt at

following conditions, P1 40, P1 10, P3 10, P3 20, reduced after 10 days storage. Maximum reduction was observed for P1 40 sec treated yogurt (from 6050 cp to 4020 cp). At the end of storage P3 10 sec (4650 cp) and P5 5 sec (5040 cp) microwave treated yogurt had minimum viscosity while P1 10 sec (5800 cp) treated yogurt had highest viscosity.

In general longer the treatment time lower the viscosity of yogurt. Possible reason for this behaviour could be the more energy absorption of protein and reduction in protein content of yogurt. Since protein (casein) particles are linked together and form network containing void and pores, electrophoretic effect upset this network and consequently protein may loss its water binding capacity. As a result, leakage of water from these voids and pores yield low viscous yogurt [130].

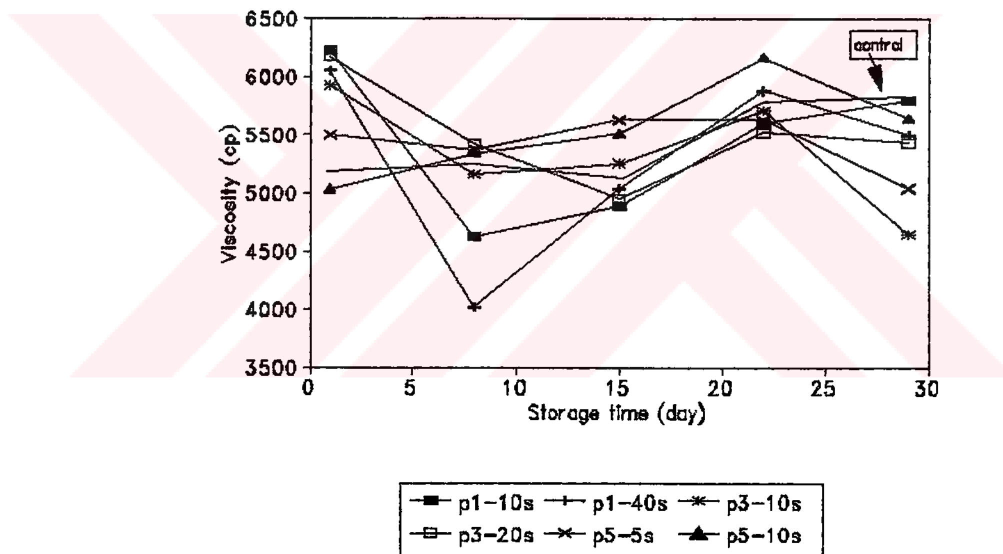

Figure 48. Effect of microwave treatment on the viscosity of yogurt

5. 6. Effect of Microwave Treatment on Protein Content of Yogurt

Protein content of control was 3.05 %. Protein content of microwaved yogurt decreased after overnight storage. Minimum reduction was observed for P5 5 sec microwave

treated yogurt (2.94 %) and maximum reduction was for P1 40 sec treatment (2.0 %). Differences were smaller in protein content between P5 10 sec and P3 10 sec microwave treatment and was around 2.7 %. P3 20 sec microwave treatment gave 2. 17 % protein content. It could be concluded that as the interaction time increased undenatured protein content decreased. Results were listed in Table 82.

Kudra et al. studied heating characteristics of milk constituents (fat, lactose and protein) in microwave pasteurization system. They concluded that milk heated more rapidly than water and protein was a major contributor to heating [156]. In other words protein absorbed significantly more energy as a function of concentration consequently increase in temperature was higher at high protein content. This may be major reason for the reduction of protein content of yogurt after microwave treatment. This is reasonable since proteins in milk or in yogurt are charged molecules and are associated with calcium ions and other mineral components. These undergo strong heating due to electrophoretic effect produced in a microwave field.

Table 82. Effect of Microwave treatment at different power setting and time on protein content of yogurt.

Storage time (day)	Protein Content (%)						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	3.05	2.80	2.00	2.70	2.17	2.94	2.73

5. 7. Effect of microwave Treatment on the Number of Starter Bacteria.

Microwave treated yogurt had lower number of starter bacteria than control after overnight storage. While microwave treatment at P3 20 sec had minimum cell viability (7.54) P5 5 sec treatment gave highest number of starter bacteria (8.06). P1 40 and P5 10 had same cell population (7.83). Small difference in MRS count was noted between P3 10 and control (7.69). Result was 8.24 for control yogurt.

During storage period control maintained its higher

cell population while number of starter bacteria in microwave treated yogurt decreased.Microwave energy inactivate microorganisms by thermal denaturation of proteins and nucleic acid. Microwave and conventional heat treatment of culture showed differences in cell viability and specific enzyme activity. But during a week storage cell population of yogurt increased. This increase showed the increase in lactic acid content at the end of one week storage.

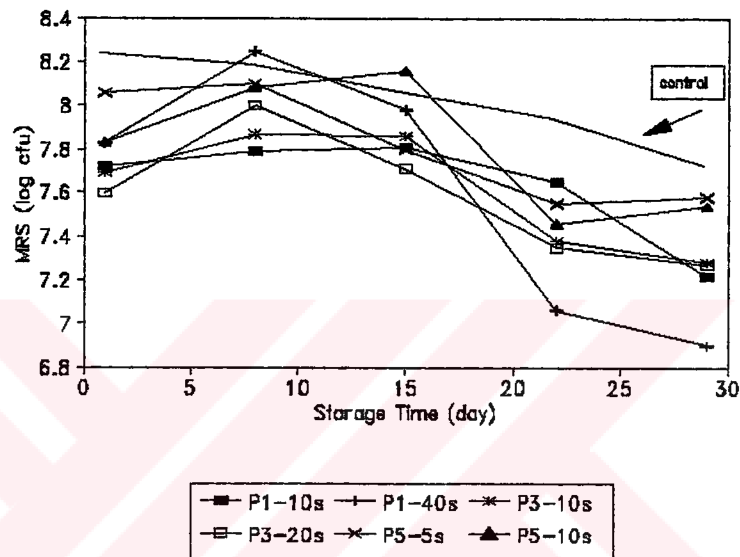

Figure 49. Effect of microwave treatment on the number of starter bacteria

Maximum reduction was noted for P1 40 sec treatment at the end of storage (95.5 %) although temperature did not exceeded 45 °C. If microbial inactivation occurred mainly by thermal effect, final temperature need to be higher than 45 °C, since litarature survey showed that starter bacteria resist to conventional heating at 60 °C for 30 min. (medium was not indicated), same conclusion was true at other power settings and time combinations. Final mean temperatures were around 47. 5 °C, 52 °C, 48 °C, 57°C, 41 °C for P5 5 and 10 sec, P3 10 and 20 sec, P 10 and 40 sec respectively.

There are conflicting reports in literature regarding

the lethal effects of microwaves on microorganisms. Some researchers believed that non-thermal electromagnetic effects of microwave responsible for microbial inactivation while others indicate that microwave causes only thermal effects. But there is still unagreement concerning the superiority of conventional or microwave treatment due to difficulty comparing results under different experimental conditions [81, 87, 89, 157].

Higher temperature is needed in conventional heating to kill microorganisms but yogurts after microwave treatment did not reach such a high temperature. At the same time number of starter bacteria decreased significantly. Hence heat may not be only agent for inactivation.

% destruction was found to be maximum for P1 40 sec microwave treated yogurt (95.5%) followed by P3 20 sec treatment (81.4 %). Small difference in MRS count was noticed for P1 10 sec (73.1 %) and P3 10 sec (74. 3 %) treatment. At the end of storage number of starter culture reduced by 66.9 % and 69.8 % for P5 10 and p5 5 sec microwave treated yogurt.

ANOVA Table showed that Power level had significant effects on the number of starter bacteria (Table 83).

Table 83. Result of ANOVA for Starter Bacteria Count

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.1763000	3	.0587667	1.434	.2960
power level	.9569000	3	.3189667	7.786	.0072

Table 84. Multiple Range Analysis of Starter Bacteria Count for Power Level

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
Power1	4	7.63500500	X
Power3	4	7.67550000	X
Power5	4	8.05000000	X
control	4	8.21000000	X

5. 8. Effect of Microwave Treatment on S. thermophilus

Microwave treatment reduced the initial number of S. thermophilus in the order of P3 20 and 10 sec, P5 10 and 5 sec, P1 40 and P1 10 sec. The longer the microwave treatment time the more inhibition of S. thermophilus. Number of starter culture of P3 20 and 10 sec, P5 10 sec microwave treated yogurt increased slightly in one week storage, later reduced for all sample due to denaturation of protein and nonthermal effect of microwave. Maximum reduction was observed for P3 10-20 sec and P1 40 sec treatment (log 5 cfu) followed by P5 10 sec (log 5.5 cfu). Slight difference in count were noted for P5 5 sec (log 6.43 cfu) and P1 10 sec treatment (log 6.20 cfu) at the end of storage.

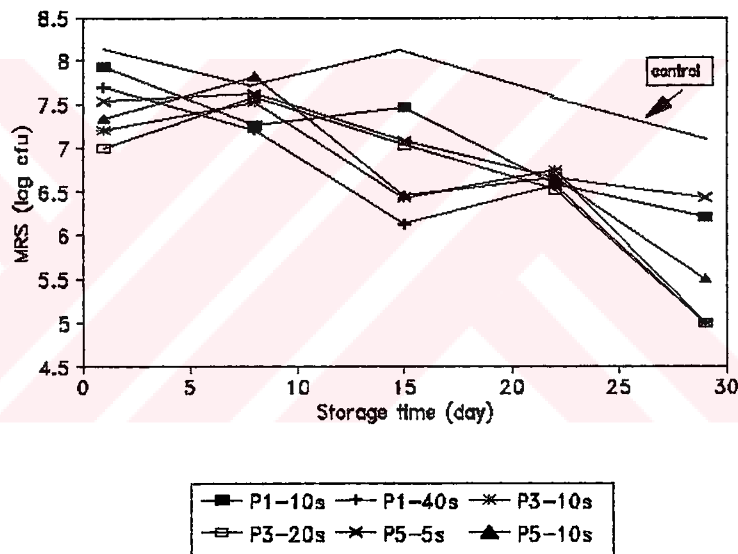

Figure 50. Effect of microwave treatment on the number of S. thermophilus

Storage time significantly affected the number of S. thermophilus.

Table 85. Result of ANOVA for S. thermophilus

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	4.8424688	3	1.6141563	5.293	.0223
power level	3.2035687	3	1.0678562	3.501	.0628

Table 86. Multiple Range Analysis of S. thermophilus for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	6.2400000	X
15	4	6.9975000	XX
8	4	7.5300000	X
1	4	7.6250000	X

There was no significant differences in number of S. thermophilus. up to 8 days of storage. Count of bacteria significantly decreased after 8 days of storage.

5. 9. Effect of Microwave Treatment on L. bulgaricus

Effect of microwave treatment on L. bulgaricus was given in Figure 51.

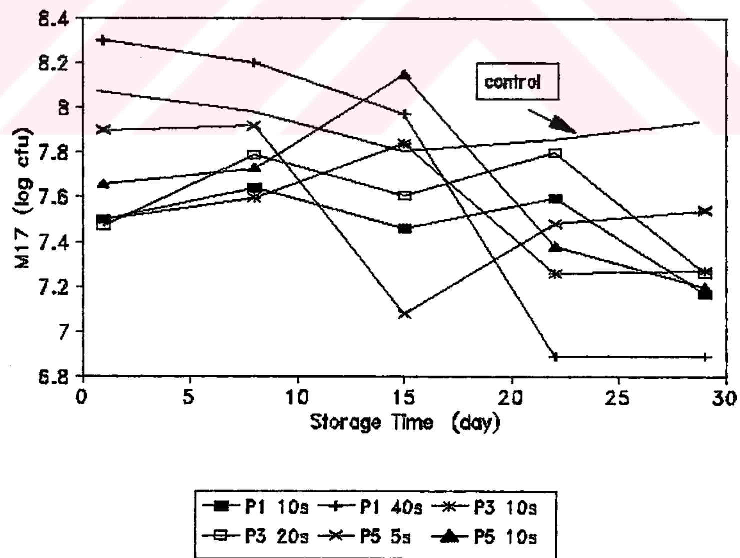

Figure 51. Effect of microwave treatment on the number of L. bulgaricus

No great change in number of L. bulgaricus was observed during one week of storage. After one week of storage, sudden reduction in L. bulgaricus was seen for P5 5 sec microwave treatment. Same reduction was observed for P1 40 sec and P5 10 sec microwave treated yogurt after two weeks of storage. There was no significant effect of power level on L. bulgaricus.

5. 10. Effect of Microwave Treatment on Total Viable Bacteria

Difference between MRS count and PCA count were listed in Table . As the power level and treatment time increased difference between PCA and MRS count increased due to higher injury. They were $134*10^6$ and $164 *10^6$, $104*10^6$ and $93*10^6$ for P1 40, P3 20, P3 10, P5 10 sec microwave treatments. Same trends was not true for P5 5 sec treatment possibly due to shorter treatment time. Minimum differences $8*10^6$ were detected for P1 10 sec microwave treatment. Lower power setting minimized number of injured bacteria.

Table 87. Number of starter bacteria in PCA and MRS agar.

Medium	cfu*10^6						
	control	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
MRS	173	52	68	49	41	115	67
PCA	170	60	202	153	205	110	160

6. Pekmez Yogurt Production

In order to determine optimum pekmez concentration for the pekmez yogurt production, preliminary tests were done. 5-10-15 % pekmez was mixed with skim milk and it was inoculated with starter culture. pH was measured during fermentation period. pH profile of pekmez yogurt was shown in Figure 51. Increase in pekmez concentration increased the fermentation time. In other words pekmez slowed down the fermentation. Initial pH of the milk was 6. 35. Addition of pekmez at 5-10-15 % (v/v) reduced the initial pH of the milk to 6.32, 6.28, 6.24 respectively due to the lower pH value of pekmez (4.96). After 4 hours of incubation pH of the control reduced to 4.26 while pH of 5-10-15 % pekmez yogurt were 4.44, 4.98, 5.9 respectively. It was evident from Figure 52 that pekmez addition inhibited the growth of starter culture as a result of this acid development reduced and pH reduction rate became slower. 15 % pekmez addition increased the fermentation time much more than 5 % and 10 % pekmez and slowed fermentation is not preferred for yogurt making due to the increase in syneresis and breakdown of symbiotic relationship between starter culture [27]. On the other side 5 % pekmez didn't supply sufficient sweetness to yogurt. Because of these reasons further experiments were carried out with 10 % pekmez addition. After the determination of optimum pekmez concentration, nisin addition (150 RU/ml yogurt) and microwave treatment, power setting 5 for 10 sec (preliminary determined) were applied to pekmez yogurt. Properties of control, pekmez yogurt, nisin treated pekmez yogurt and microwaved pekmez yogurt were followed during refrigerated storage.

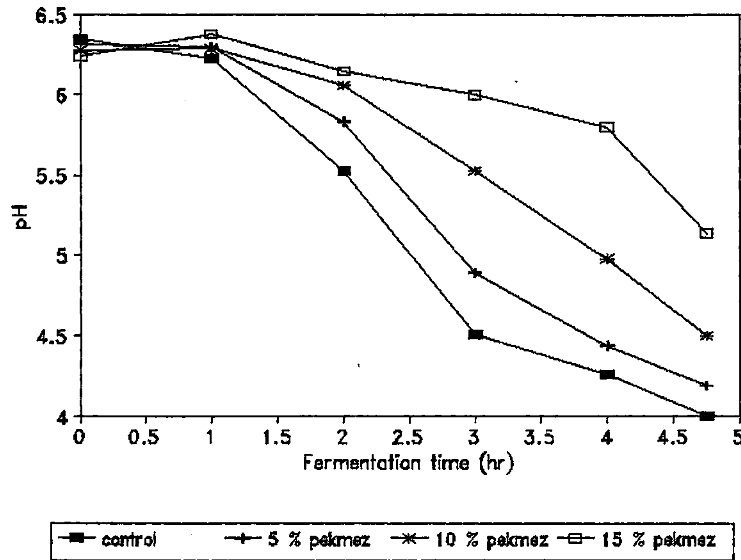

Figure 52. pH profile of pekmez yogurts.

6. 1. Titratable Acidity of Pekmez Yogurts

Titratable acidity of nisin treated yogurt was nearly constant during storage period. Increase in titratable acidity of pekmez yogurt, microwave treated pekmez yogurt and control was observed within one weeks of storage. At the end of the storage microwave treated pekmez yogurt and nisin added pekmez yogurt had lower titratable acidity than untreated pekmez yogurt. Titratable acidity was maximum for the control (Figure 53).

To estimate the effects of storage time and treatments; pekmez addition, microwave treatment of pekmez yogurt and nisin added pekmez yogurt, on titratable acidity ANOVA was done. Result indicated that storage time had significant effect on titratable acidity of yogurt (Table 88).

Table 88. Result of ANOVA for Titratable Acidity.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.0491252	3	.0163751	5.604	.0191
Treatments	.0097807	3	.0032602	1.116	.3927

Table 89. Multiple Range Analysis of Titratable Acidity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
1	4	.8907500	X
8	4	.9635000	XX
15	4	.9782500	XX
29	4	1.0467500	X

There was no significant difference between titratable acidity of 1, 8, 15 days stored yogurt and 8, 15, 29 days stored yogurt. Difference between 1 and 29 days yogurt was significant.

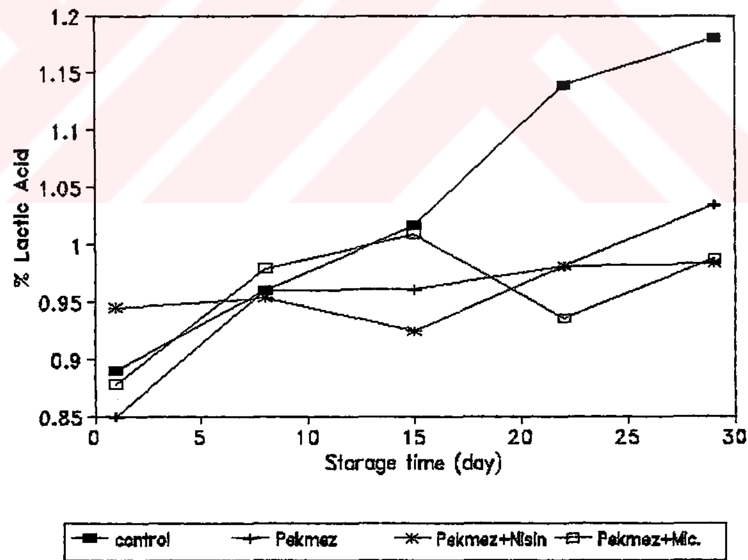

Figure 53. Titratable acidity of Pekmez yogurts.

6. 2. pH of Pekmez Yogurts

Parallel to the change in titratable acidity of nisin treated yogurt, pH of it was nearly constant during storage period. Similar fluctuation in titratable acidity was observed for pH of microwaved treated pekmez yogurt. pH of control and pekmez yogurt decreased during two weeks of storage. During the rest of storage pH of the pekmez yogurt stayed constant while control continued to decrease.

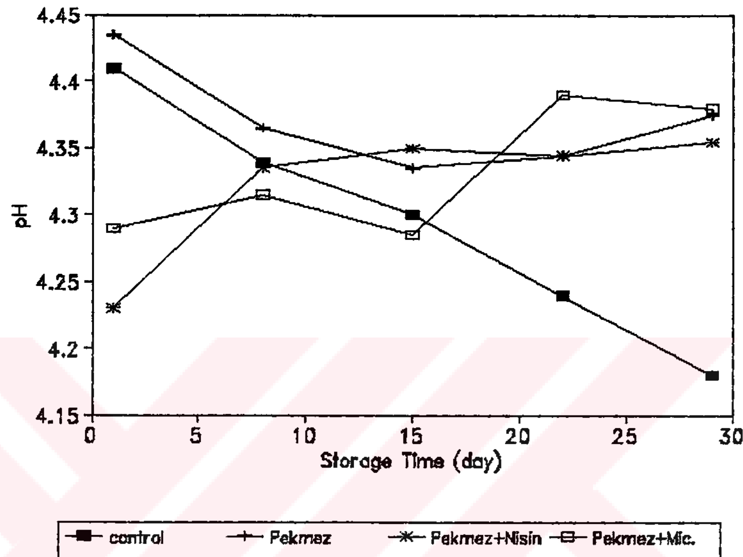

Figure 54. pH of pekmez yogurts.

Table 90. Result of ANOVA for pH.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	.0025873	3	.0008624	.147	.9291
Treatments	.0113898	3	.0037966	.647	.6044

Statistical analysis showed that there was no significant effect of storage time and treatments on pH of yogurts (Table 89).

6. 3. Viscosities of Pekmez Yogurts

Initial viscosity of control, pekmez yogurt and nisin

treated yogurt was around 3600 cp. Microwave treated pekmez yogurt had lower viscosity (3400 cp) than others due to the heating effect of microwave energy. During two weeks of storage viscosity of control increased. Viscosity profile of pekmez yogurt, nisin and microwave treated pekmez yogurt seemed parallel to the viscosity profile of control. But increase in viscosity for pekmez yogurts was lower than control. Figure 55 showed the viscosity profile of control, pekmez yogurt, nisin and microwave pekmez yogurt. Nisin treatment and microwave treatment slowed down the reduction in viscosity. Microwave treated pekmez yogurt had higher viscosity than pekmez and nisin treated pekmez yogurt. At the end of the storage, control had highest viscosity.

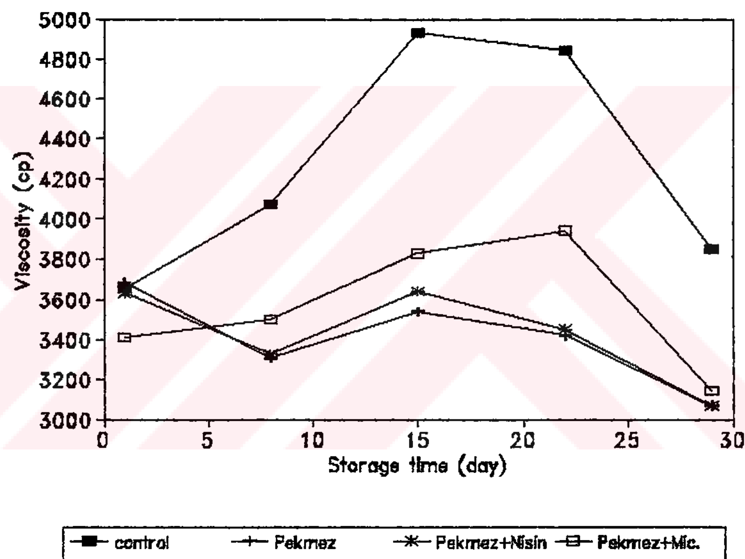

Figure 55. Viscosity of pekmez yogurts.

Table 91. Result of ANOVA for Viscosity

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Treatments	1005218.8	3	335072.92	4.801	.0290
Storage time	1463168.8	3	487722.92	6.988	.0100

ANOVA test showed that storage time and the treatments (pekmez addition, microwave and nisin treatment of pekmez yogurt) had significant effect on viscosity of pekmez yogurt at α=0.05 level (Table 91). Details of these effects were tabulated in Tables 92, 93.

Table 92. Multiple Range Analysis of Viscosity for Treatments.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
Pekmez	4	3400.0000	X
Pekmez+Nis	4	3417.5000	X
Pekmez+Mic.	4	3470.0000	X
Control	4	4125.0000	X

Multiple Range Analysis showed that viscosity of control was significantly different from viscosity of pekmez yogurt, microwave treated pekmez yogurt and nisin added pekmez yogurt. Pekmez added yogurts had significantly lower viscosity than control.

Table 93. Multiple Range Analysis of Viscosity for Storage Time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	3282.5000	X
8	4	3552.5000	XX
1	4	3592.5000	XX
15	4	3985.0000	X

15 days stored yogurt had significantly lower viscosity than 29 days stored yogurt. No significant difference was detected among others.

6. 4. Syneresis of pekmez Yogurts

After one day of storage syneresis of control (42 %) was lower than the syneresis of pekmez yogurt, microwave treated pekmez yogurt (43 %) and nisin treated pekmez yogurt (42 %). While control maintained its lower syneresis, syneresis of pekmez yogurts increased during 8 days of storage. At the end of 29 days of storage syneresis

of microwave treated pekmez yogurt and pekmez yogurt was 46 % and nisin treated pekmez yogurt was 45 %.

Storage time and pekmez treatment significantly affected syneresis of yogurt (Table 93). Details of this effect were given in Table 94.

Table 94. Result of ANOVA for syneresis

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Treatments	14.000000	3	4.6666667	6.462	.0127
Storage time	14.500000	3	5.8333333	8.077	.0064

Table 95. Multiple Range Analysis of syneresis for treatments.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
Control	4	42.750000	X
Pekmez+Mic.	4	44.750000	X
Pekmez+Nis.	4	45.000000	X
Pekmez	4	45.500000	X

Pekmez addition significantly decreased the syneresis of yogurt. But differences were not significant among the pekmez treated yogurt, microwave treated yogurt and pekmez yogurt. LS Means of control was lower than the pekmez yogurts.

Flavored yogurts are made by adding fruit concentrates or flavoured syrups to the cultured milk before and after the incubation process [158]. Added ingredients generally tend to decrease the consistency of the products due to the reduction in water binding capacity of protein and therefore such products are often stabilized with viscosity modifiers such as starches or pectin [39]. As a result, reduction in viscosity or increase in syneresis is a common problem for fruit yogurts [159]. But it is possible to improve the texture of pekmez yogurt by using stabilizer such as pectin, starch, gum, gelatin etc.

Table 96. Multiple Range Analysis of syneresis for storage time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
1	4	43.000000	X
8	4	44.500000	X
15	4	45.000000	X
29	4	45.500000	X

Syneresis of one day stored yogurt was significantly lower than the 8-15-29 days stored yogurt. After one week of storage syneresis of yogurt significantly increased.

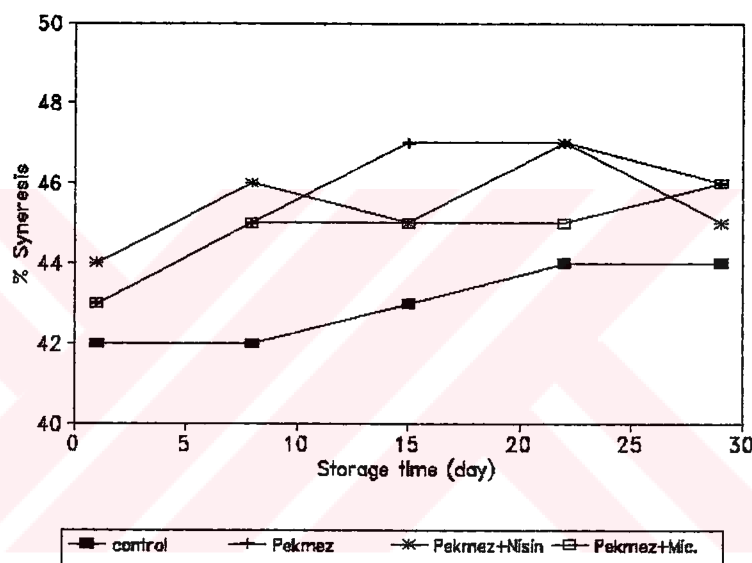

Figure 56. Syneresis of pekmez yogurts.

6. 5. Starter Bacteria of Pekmez Yogurts

Number of starter bacteria of control was higher than pekmez yogurt, microwave treated and nisin added pekmez yogurts after one day of storage. Influence of pekmez on total bacteria count was shown in Figure 57. Pekmez addition significantly reduced the number of starter bacteria. After two weeks of storage number of starter bacteria decreased. Pekmez can supply the required sweetness needed to produce an acceptable yogurt.

Nevertheless, due to its higher osmotic pressure [39] or hydoxymethyl furfural (HMF) content may be inhibited the growth of the yogurt starter culture. Jay reported that HMF has inhibitory effect on fermentable bacteria [27].Therefore amount used may be critical to culture growth. Growth of total starter bacteria were retarded, such that acid production slowed down. Reduction was maximum for nisin added pekmez yogurt. At the end of four weeks of storage number of starter bacteria of microwave treated pekmez yogurt and pekmez yogurt was nearly same and had higher cell population than nisin added pekmez yogurt.

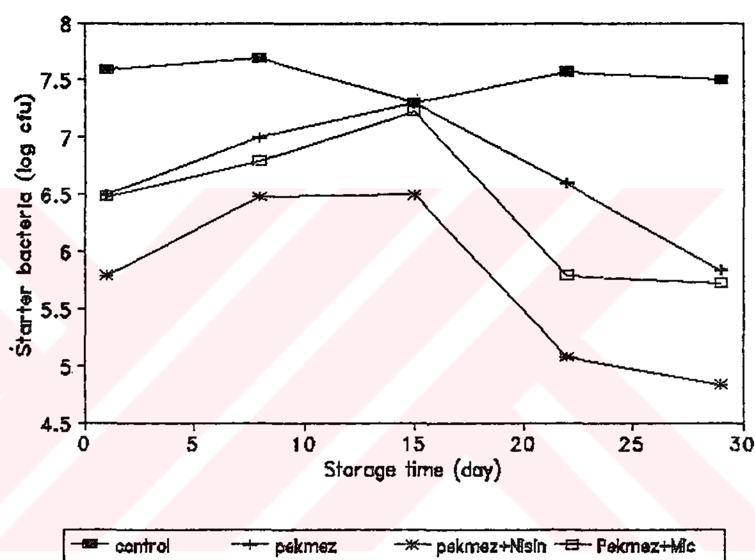

Figure 57. Total starter bacteria of pekmez yogurts.

Table 97. Result of ANOVA for starter bacteria

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	sig. level
Storage time	2.7361188	3	.9120369	6.768	.0110
Treatments	5.3433688	3	1.7811229	13.218	.0012

Storage time and pekmez treatment significantly affected number of starter bacteria count. Details of this effect was given in Table 97, 98.

Table 98. Multiple Range Analysis of starter bacteria (log cfu) for treatments.

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
Pekmez+Nis.	4	5.9050000	X
Pekmez	4	6.5600000	X
Pekmez+Mic.	4	6.5625000	X
Control	4	7.5250000	X

Pekmez addition significantly decreased the number of starter bacteria of yogurt. But differences were not significant between the pekmez and microwaved treated pekmez yogurt. LS Means of nisin treated pekmez yogurt was significantly lower than pekmez yogurt and microwave treated pekmez yogurts.

Table 99. Multiple Range Analysis of starter bacteria (log cfu) for storage time

Method: 95 Percent Duncan

Level	Count	LS Mean	Homogeneous Groups
29	4	5.9775000	X
1	4	6.5975000	X
15	4	6.9825000	X
8	4	6.9950000	X

Differences in number of starter bacteria was not significant up to 3 weeks of storage. But starter bacteria count significantly reduced within 4 weeks of storage.

6. 6. Protein Content of Pekmez Yogurts

Protein content of control (2.33 %) was lower than the protein content of the pekmez yogurts. Protein content of pekmez yogurts (2.64 %), nisin added pekmez yogurt (2.60 %) and microwave treated pekmez yogurt (2.52 %) were close to each other.

CHAPTER IV
CONCLUSION

Storage stability of yogurt was increased by using four different methods; pasteurization, lysozyme addition, nisin addition and microwave treatment.

Pasteurization process reduced the initial acidities of yogurt samples because of continuing fermentation during the come up period. Pasteurization process slowed down the acidity during storage in turn all titratable acidities and pH values of pasteurized yogurts stayed within the limits of TSE (minimum 3.8, maximum 1.6 %).

At the end of 4 weeks of storage none of the samples except heat treated at 65 °C for 15 min. showed bacterial counts less than 20 millions which is the lower limit of some international standards. Pasteurization at 60 °C did not negatively affect other parameters.

S. thermophilus was more heat stable than *L. bulgaricus* during pasteurization at 60 °C for 5-10-15 min. This prevented the excessive protein hydrolysis and bitter taste during storage.

Effect of heat treatment on extending storage stability of yogurt was significant.

Addition of nisin gave stability to acid production. Nisin can sufficiently diffuse through the yogurt and can be used for set yogurt production.

Nisin decreased the cell population in the order of 150>100>50 RU/ml nisin yogurt. Even at lowest concentration (50 RU/ml), nisin can be effectively be added for prolonging the storage stability of set yogurt. It may be concluded that controlled inhibition of growth of lactic acid bacteria by nisin will result in minimal deterioration

of set yogurt during storage

Low concentration of lysozyme (0.1-0.2 g/l lysozyme) did not affect the number of starter bacteria.

Number of starter bacteria continuously was reduced as the lysozyme concentration increased (from 0.3 to 1.2 g/l lysozyme) and 1.2-0.6 g/l lysozyme can be used in order to increase the storage stability of yogurt.

Diffusion of lysozyme was slower than the diffusion of nisin in yogurt. But detailed tests are necessary to measure the diffusivity of nisin and lysozyme in yogurt.

It was concluded that lysozyme which is mainly used in cheese production, can also be used in yogurt manufacturing to increase the storage stability of yogurt.

Higher power setting and longer treatment time, P3 20 sec, P5 10 sec, P1 40 sec, of 100 ml yogurt at 43 °C (after fermentation process) was effective in the preservation of yogurt with microwave treatment. Microwave at high power setting and longer time than P5 15 s, P4 20 sec, P3 35 s, P2 100 s, P1 180 s caused deterioration of the curd. So these parameters should be optimized before the application of microwave treatment if the volume and temperature of yogurt were different given above.

Time necessary to reach the pasteurization temperature was longer than the microwave treatment. This can create some problems during industrial applications. On the other hand microwave treatment is instantaneous process and there is no need to reach the pasteurization temperature. Nowadays nearly in every home a microwave oven is present and this enables consumer an easy way to preserve yogurt at a longer time at homes.

It was concluded that four of these methods increased the storage stability of yogurt. Usage of additives in yogurt (nisin and lysozyme) prevented the textural damage. Although both industrial and home scale applications of microwave is possible, initial cost of post heat treatment process is lower than microwave treatment.

10 % pekmez addition gave the satisfactory sweetness needed to produce an acceptable yogurt. Pekmez addition slowed down the fermentation process by inhibiting starter bacteria and as a result reduced the acid production.

Pekmez addition significantly lowered the viscosity and increased the whey syneresis of yogurt. Further tests should be done to prevent this problem possibly by using different stabilizers.

Nisin addition was more effective than microwave treatment in the preservation of pekmez yogurt.

By the use of pekmez new type of a flavoured yogurt was produced which is highly nutritious and having high iron content. By this way market samples will be able to enriched.

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APPENDICES

APPENDIX A

Tables of Post Heat Treatment at 60 °C for 5-10-15 Minute.

Table A.1. Influence of heat treatment of 5-10-15 min. at 60 °C on pH during storage at 4 °C for four weeks.

Storage time (day)	pH							
	c	c	5	5	10	10	15	15
1	4.27	4.31	4.23	4.24	4.22	4.19	4.24	4.24
8	4.22	4.25	4.17	4.17	4.14	4.11	4.15	4.15
15	4.18	4.21	4.14	4.15	4.13	4.08	4.11	4.12
22	4.14	4.14	4.10	4.12	4.11	4.08	4.11	4.10
29	4.10	-	4.09	-	4.09	-	4.10	-

Table A.2. Average pH values of unheated and 5-10-15 min. heated samples.

Storage Time (day)	pH (Average)			
	c	5	10	15
1	4.290	4.235	4.205	4.240
8	4.235	4.270	4.125	4.150
15	4.195	4.145	4.105	4.115
22	4.140	4.110	4.095	4.105
29	4.100	4.090	4.090	4.100

Table A. 3. % pH changes of unheated and 5-10-15 min. heated samples.

Storage Time (day)	pH Change(%)			
	c	5	10	15
1	0.00	0.00	0.00	0.00
8	1.28	1.53	1.90	2.12
15	2.21	2.12	2.38	2.94
22	3.49	2.95	2.61	3.18
29	4.44	3.42	2.73	3.30

Table A. 4. Effect of heat treatment on % Lactic acid during storage.

Storage time (day)	% Lactic Acid							
	c	c	5	5	10	10	15	15
1	1.02	1.02	1.12	1.13	1.11	1.16	1.17	1.17
8	1.2	1.0	1.18	1.17	1.16	1.21	1.19	1.19
15	1.19	1.17	1.21	1.21	1.27	1.25	1.22	1.23
22	1.2	1.2	1.22	1.22	1.26	1.26	1.25	1.26
29	1.31	1.29	1.23	1.25	1.32	1.27	1.25	1.25

Table A. 5. Average values of % lactic acid for duplicate samples.

Storage Time (day)	Average Lactic acid (%)			
	c	5	10	15
1	1.020	1.125	1.135	1.172
8	1.100	1.175	1.185	1.190
15	1.180	1.210	1.260	1.226
22	1.200	1.220	1.260	1.225
29	1.300	1.240	1.295	1.250

Table A. 6. Lactic acid production rate during storage.

Storage time (day)	Lactic acid production rate (% La/week)			
	c	5	10	15
1	0.000	0.00	0.00	0.000
8	0.080	0.050	0.050	0.018
15	0.160	0.085	0.125	0.054
22	0.180	0.095	0.125	0.053
29	0.280	0.115	0.160	0.078

Table A. 7. Effect of heat treatment on whey syneresis of yogurts.

Storage time (day)	% Whey Syneresis							
	c	c	5	5	10	10	15	15
1	44	44	40	40	41	41	42	40
8	49	44	42	48	44	46	40	46
15	42	44	42	44	40	42	46	42
22	38	42	42	42	40	42	40	46
29	44	38	42	42	40	42	44	44

Table A. 8. Average values of whey syneresis for unheated and heat treated yogurts.

Storage Time (day)	Average whey syneresis (%)			
	c	5 min.	10 min.	15 min.
1	44	40	41	41
8	46.5	45	45	44
15	43	43	41	44
22	40	42	41	43
29	42	42	41	44

Table A. 9. Effect of heat treatment on starter culture population.

Storage Time (day)	Starter culture (cfu)*10^6							
	c	c`	5	5`	10	10`	15	15`
1	380	432	266	274	160	142	82	80
8	258	238	196	180	118	94	56	34
15	1082	862	46	46	96	90	821	80
22	852	554	60	44	50	38	44	74
29	416	290	22	20	26	34	24	38

APPENDIX B

Tables of Post Heat Treatment at 65 °C for 5-10-15 Minute

Table B. 1. Influence of heat treatment of 5-10-15 min. at 65°C on pH during storage at 4 °C for four weeks.

Storage time (day)	pH							
	c	c`	5	5`	10	10`	15	15`
1	4.32	4.31	4.23	4.26	4.23	4.27	4.26	4.23
8	4.27	4.25	4.22	4.22	4.25	4.21	4.26	4.26
15	4.23	4.21	4.23	4.23	4.26	4.25	4.28	4.26
24	4.19	4.14	4.18	4.18	4.25	4.28	4.24	4.33
29	4.11	4.09	4.20	4.20	4.28	4.28	4.25	4.29

Table B. 2. Average pH values of unheated and 5-10-15 min. heated samples.

storage time (day)	c	5	10	15
1	4.29	4.245	4.25	4.245
8	4.235	4.22	4.255	4.26
15	4.195	4.23	4.255	4.27
24	4.140	4.19	4.65	4.285
29	4.10	4.20	4.28	4.27

Table B. 3. Effect of heat treatment on % Lactic acid during storage.

Storage time (day)	% Lactic Acid							
	c	c	5	5	10	10	15	15
1	0.97	0.99	0.98	1.10	1.02	1.06	0.83	1.06
8	1.19	1.01	1.09	1.06	1.04	1.06	1.01	0.92
15	1.19	1.17	1.13	1.04	1.07	1.11	1.00	1.00
24	1.20	1.20	1.13	1.10	1.14	1.07	1.05	1.15
29	1.31	1.29	1.12	1.12	1.17	1.09	1.22	1.03

Table B. 4. Average values of Lactic acid for unheated and heat treated yogurts.

Storage Time (day)	Average Lactic acid (%)			
	c	5 min.	10 min.	15 min.
1	0.980	1.040	1.04	0.945
8	1.100	1.075	1.050	0.965
15	1.180	1.085	1.090	1.000
24	1.200	1.115	1.105	1.100
29	1.300	1.120	1.130	1.125

Table B. 5. Effect of heat treatment on whey syneresis of yogurts.

Storage time (day)	% Whey Syneresis							
	c	c\`	5	5\`	10	10\`	15	15\`
1	44	44	44	45	45	41	44	41
8	50	46	46	44	48	46	44	42
15	50	44	42	42	44	48	44	42
24	42	38	42	42	44	48	48	44
29	42	42	42	42	44	46	46	46

Table B. 6. Average values of whey syneresis for unheated and heat treated yogurts.

Storage Time (day)	Average whey syneresis (%)			
	c	5 min.	10 min.	15 min.
1	44.0	44.5	44.5	42.5
8	48.0	45.0	47.0	43.0
15	46.0	42.0	46.0	43.0
24	39.0	42.0	45.0	46.0
29	42.0	42.0	45.0	46.0

Table B. 7. Effect of heat treatment on starter culture population.

Storage Time (day)	Starter culture (cfu)*10^6							
	c	c`	5	5`	10	10`	15	15`
1	1020	1040	86	96	59	76	52	38
8	1100	1256	54	57	59	100	16	8.6
15	1000	1260	68	40	64	39	11	2.8
24	752	366	46	45	30	48	8	-
29	412	216	38	35	-	21	5.2	$<10^5$

APPENDIX C
Tables of Nisin Treatment at different concentration

Table C. 1. Effect of nisin treatment on pH at different depth during storage

Storage Time (day)	pH			
	c	Top	Middle	Bottom
1	4.58	4.54	4.56	4.57
4	4.50	4.52	4.51	4.50
7	4.38	4.47	4.44	4.46
10	4.31	4.39	4.37	4.38
18	4.20	4.36	4.34	4.32
24	4.15	4.22	4.20	4.20
30	4.10	4.22	4.29	4.26
32	4.09	4.24	4.24	4.30
34	4.05	4.16	4.18	4.19
40	4.02	4.29	4.29	4.33

Table C. 2. Effect of nisin teratment on titratable acidity at different depth during storage

Storage Time (day)	% Titratable Acidity			
	c	Top	Middle	Bottom
1	0.954	0.954	0.954	0.972
7	1.150	1.062	1.080	1.134
15	1.180	1.068	1.116	1.144
20	1.200	1.080	1.098	1.152
22	1.224	1.060	1.134	1.170
25	1.260	1.076	1.134	1.180
30	1.300	1.044	1.134	1.116

Table C. 3. Effect of nisin addition on the % increase in the titratable acidity at different depth during storage

Storage Time (day)	% Increase in Titratable Acidities			
	c	Top	Middle	Bottom
1	0	0	0	0
7	20.50	11.32	13.21	16.98
15	24.00	11.95	16.98	17.49
20	26.00	13.21	15.09	18.52
22	28.30	11.11	18.87	20.37
25	32.10	12.79	18.70	21.40
30	36.31	9.43	18.70	14.81

Table C. 4. Effect of nisin addition on the count of starter culture at different depth during storage

Storage Time (day)	MRS (log cfu)			
	control	Top	Middle	Bottom
1	8.38	7.97	8.26	8.35
6	8.36	7.78	7.96	8.15
12	8.50	7.58	8.17	8.18
18	8.30	7.08	7.83	8.01
24	8.20	6.70	7.30	7.58
32	8.20	6.47	7.40	7.35

Table C. 5. Effect of nisin addition on pH at different concentration during storage

Storage Time (day)	pH							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	4.32	4.31	4.39	4.38	4.39	4.39	4.36	4.39
8	4.27	4.25	4.20	4.22	4.26	4.28	4.27	4.27
15	4.23	4.21	4.34	4.30	4.30	4.32	4.31	4.29
22	4.19	4.14	4.35	4.41	4.30	4.39	4.37	4.36
29	4.11	4.09	4.38	4.40	4.39	4.38	4.39	4.38

Table C. 6. Average pH change during storage

Storage Time (day)	Avearge pH			
	c	50 RU	100 RU	150 RU
1	4.375	4.385	4.390	4.375
8	4.235	4.210	4.270	4.270
15	4.195	4.320	4.310	4.300
22	4.140	4.380	4.345	4.365
29	4.100	4.390	4.385	4.385

Table C. 7. Effect of nisin addition at different concentration on titratable acidity during storage.

Storage Time (day)	% Titratable Acidity							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	0.975	0.990	0.954	1.026	0.981	0.927	0.972	0.981
8	1.19	1.010	1.017	1.017	1.098	1.071	1.080	0.999
15	1.19	1.170	1.044	1.125	1.062	1.035	1.089	1.080
22	1.20	1.200	1.071	1.026	1.026	1.008	1.080	1.062
29	1.31	1.290	0.954	1.053	1.080	1.071	1.098	1.071

Table C. 8. Average titratable acicity during storage

Storage Time (day)	Average Titratable Acidity (%)			
	c	50 RU	100 RU	150 RU
1	0.9990	0.9900	0.9540	0.9765
8	1.1000	1.0170	1.0710	1.0395
15	1.1800	1.0440	1.0485	1.0845
22	1.2000	1.0485	1.0260	1.0710
29	1.3000	1.0530	1.0755	1.0845

Table C. 9. Effect of nisin addition at different concentration on % syneresis during storage.

Storage Time (day)	Syneresis							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	42	43	42	40	40	40	34	30
8	40	43	42	36	42	38	43	38
15	40	40	40	40	36	39	38	38
22	40	40	38	38	36	40	36	37
29	40	44	36	38	38	40	36	36

Table C. 10. Average % synersis during storage

Storage Time (day)	Syneresis %			
	c	50 RU	100 RU	150 RU
1	42.5	41.0	40.0	40.0
8	41.5	40.5	40.0	40.5
15	40.0	40.0	37.5	38.0
22	40.0	38.0	37.0	36.5
29	40.0	37.0	37.0	36.0

Table C. 11. Effect of nisin addition at different concentration on viscosity during storage.

Storage Time (day)	Viscosity (cpu)							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	5880	5720	6040	5760	5640	-	5260	5560
8	5060	5060	5160	-	5080	5020	5100	5140
15	5240	5300	5220	5280	5020	-	5320	5520
22	5320	5320	5000	5000	5120	5460	5400	5800
29	5000	5200	5200	5200	5100	5100	4960	-

Table C. 12. Effect of nisin addition at different concentration on viscosity during storage.

Storage Time (day)	Viscosity			
	c	50 RU	100 RU	150 RU
1	5800	5900	5640	5410
8	5060	5160	5050	5120
15	5270	5250	5020	5420
22	5320	5000	5290	5600
29	5100	5220	5100	4960

Table C. 13. Effect of nisin addition at different concentration on number of total starter culture during storage.

Storage Time (day)	MRS Count (log cfu)							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	8.365	8.36	8.196	8.167	8.009	7.939	8.013	7.886
8	8.462	8.544	8.301	8.431	8.220	8.399	8.267	8.176
15	8.279	8.305	8.029	8.117	8.033	8.033	7.903	7.903
22	8.201	8.103	7.929	8.000	8.187	8.00	7.477	6.800
29	8.301	8.004	7.748	7.748	7.176	7.544	7.398	6.903

Table C. 14. Effect of nisin addition at different concentration on the starter culture count during storage.

Storage Time (day)	log cfu MRS			
	c	50 RU	100 RU	150 RU
1	8.360	8.180	7.970	7.950
8	8.500	8.370	8.320	8.225
15	8.300	8.070	8.030	7.400
22	8.177	7.970	8.100	7.280
29	8.177	7.500	7.400	7.230

Table C. 15. Effect of nisin addition at different concentration on count of <u>S. thermophilus</u> during storage.

Storage Time (day)	M17 (log cfu)							
	c	c	50 RU	50RU	100RU	100RU	150RU	150 RU
1	8.128	8.152	7.934	8.225	8.004	8.013	7.380	6.954
8	8.161	8.243	7.204	7.041	7.431	7.401	6.301	6.903
15	8.107	8.176	7.430	-	7.643	7.548	7.845	7.477
22	8.143	-	7.362	7.176	7.050	7.556	6.300	6.301
29	7.414	7.489	5.903	5.899	5.778	5.602	5.000	5.176

Table C. 16. Effect of nisin addition at different concentration on the <u>S. thermophilus</u> count during storage.

Storage Time (day)	log cfu M17			
	c	50 RU	100 RU	150 RU
1	8.15	8.01	8.10	7.23
8	8.20	7.43	7.15	6.70
15	8.14	7.64	7.43	6.70
22	8.09	7.55	7.27	6.30
29	7.47	6.69	6.90	6.17

APPENDIX D

Tables of Microwave Treated Yogurt at different Power Setting and Time Combination

Table D. 1. Time-temperature profile of yogurt with initial temperature around 20 °C.

Time (sec)	Temperature °C				
	P5	P4	P3	P2	P1
0	22.0	21.0	24.5	22.0	22.0
10	29.0	29.0	30.0	31.0	24.0
30	46.0	47.0	42.0	36.5	26.0
40	53.0	53.0	48.0	41.0	27.0
50	61.0	60.0	55.0	45.0	28.0
60	68.0	65.0	62.0	51.0	29.0

Table D. 1. Time-temperature profile of yogurt with initial temperature around 40 °C.

Time (sec)	Temperature °C				
	P5	P4	P3	P2	P1
0	43	40	41	38	40
10	52	46	48	46	41
20	58	57	54	51	42
30	65	60	60	57	44
40	80	59	65	62	45
60	84	83	76	72	45

Table D. 3. Effects of microwave treatment on pH.

Storage time (day)	pH						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	4.30 4.22	4.18 4.14	4.14 4.10	4.09 4.07	4.11 4.08	4.09 4.10	4.14 4.10
8	4.22 4.22	4.11 4.11	4.12 4.12	4.04 4.04	4.06 4.10	4.08 4.05	4.02 4.05
15	4.21 4.21	4.14 4.09	4.11 4.12	4.10 4.07	4.10 4.10	4.12 4.12	4.09 4.08
22	4.18 4.06	4.15 4.14	4.11 4.15	4.08 4.03	4.08 4.05	4.12 4.07	4.09 4.09
29	4.11 4.06	4.09 4.15	4.14 4.13	4.06 4.10	4.09 4.09	4.08 4.16	4.11 4.09

Table D. 4. Average pH values of microwave treated sample.

Storage Time (day)	Average pH						
	c	P1 10	P2 40	P3 10	P3 20	P5 5	P5 10
1	4.260	4.165	4.120	4.080	4.095	4.095	4.120
8	4.220	4.110	4.120	4.040	4.080	4.065	4.035
15	4.190	4.115	4.115	4.085	4.120	4.120	4.085
22	4.120	4.095	4.130	4.055	4.065	4.095	4.090
29	4.085	4.120	4.135	4.080	4.130	4.120	4.100

Table D. 5. Effects of microwave treatment on titratable acidity.

Storage time (day)	Lactic acid (%)						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	0.77 0.77	0.92 0.92	0.909-	0.95-0.95	- 0.96	1.16 0.91	1.12 1.22
8	0.90 0.96	-	0.86 0.83	1.04 0.94	0.92 0.85	0.87 0.94	0.96 0.85
15	1.00 1.10	0.73 0.86	0.88 0.85	0.93 0.87	0.93 0.87	0.82 0.90	0.87 0.92
22	1.10 1.26	0.84 0.82	0.89 0.82	0.97 0.9	0.93 0.84	0.84 0.89	1.04 0.87
29	1.15 1.31	0.9 0.95	0.95 0.95	0.95 1.95	0.92 1.10	1.03 1.03	0.95 0.98

Table D. 6. Effects of microwave treatment on titratable acidity.

Storage Time (day)	Average Lactic acid %						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	0.770	0.918	0.895	0.950	0.963	1.035	1.169
8	0.930	-	0.842	0.985	0.886	0.905	0.904
15	1.050	0.795	0.865	0.930	0.900	0.855	0.895
22	1.180	0.828	0.857	0.936	0.882	0.865	0.954
29	1.230	0.927	0.945	0.950	1.026	1.026	0.963

Table D. 7. Effect of microwave treatment on Syneresis.

Storage time (day)	% Syneresis						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	40 44	36 36	38 38	34 38	42 38	44 34	40 42
8	44 38	40 36	42 42	44 48	42 44	42 42	44 44
15	42 42	38 38	44 38	42 35	42 38	44 38	42 42
22	41 37	40 40	38 38	41 42	40 38	38 38	44 42
29	40 36	38 44	42 38	40 40	40 44	42 44	43 43

Table D. 8. Average Syneresis (%) of microwave treated samples.

Storage Time (day)	Average Syneresis (%)						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	42.0	36.0	38.0	36.0	40.0	39.0	41.0
8	41.0	38.0	42.0	46.0	43.0	42.0	44.0
15	42.0	38.0	42.0	38.5	40.0	41.0	42.0
22	39.0	40.0	38.0	41.5	39.0	38.0	43.0
29	37.5	41.0	40.0	40.0	42.0	43.0	43.0

Table D. 9. Effect of microwave treatment on viscosity of yogurt.

Storage Time (day)	Viscosity (cp)						
	C	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	6900 6980	6080 6360	6220 5880	5940 5900	6440 5900	4980 6020	4800 5260
8	5220 5220	4840 4400	4020 3900	4640 5680	5560 5280	4840 5900	4840 5840
15	5560 4600	4440 5340	4820 5260	5260 5240	5240 4660	4800 4640	5560 5460
22	5540 6200	5660 5540	6000 5760	5640 5780	5100 5940	5540 5720	6160 6160
29	6000 6000	5800 5800	5720 5280	4360 4940	5340 5520	5080 5000	5580 5700

Table D. 10. Average viscosity (cp) values of microwave treated yogurts.

Storage time (day)	Average Viscosity (cp)						
	C	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	6940	6220	6050	5920	6170	5500	5030
8	5220	4620	4020	5160	5420	5370	5340
15	5870	4890	5040	5250	4950	5630	5510
22	5220	5600	5880	5710	5520	5630	6160
29	6000	5800	5500	4650	5430	5040	5640

Table D. 11. Effect of microwave treatment on MRS count of yogurt.

Storage Time (day)	MRS count (10^{*6})						
	C	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	14 21	43 60	60 75	57 25	33 64	195 35	22 111
8	141 158	43 81	250 105	69 132	113 34	126 126	120 120
15	125 146	42 85	72 119	54 48	98 46	65 61	122 166
22	164 184	47 41	13 10	42 105	18 31	35 32	37 20
29	189 191	16 17	9 7	22 21	19 19	36 40	68 68

Table D. 12. Average number of starter bacteria count during storage.

Storage Time (day)	MRS (log cfu)						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	8.24	7.72	8.83	7.69	7.60	8.06	7.83
8	8.18	7.79	8.25	7.87	8.00	8.10	8.08
15	8.13	7.81	7.98	7.86	7.71	7.80	8.16
22	8.24	7.65	7.06	7.38	7.86	7.55	7.46
29	8.29	7.22	6.90	7.28	7.27	7.58	8.13

Table 13. Effect of microwave treatment on M17 count of yogurt.

Storage Time (day)	M17 count (10^{*6})						
	c	P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	95 120	23 29	50 50	5 16	15 10.2	35 35	22 23
8	32 34	19 17	12 18	47 21	43 3	41 42	75 58
15	34 38	23 36	1.4 1.5	2.7 2.7	9 12.5	12 11	3 3
22	22 21	40 41	3.8 4.6	5.8 5.2	18 48	4.4 5	3.8 5.4
29	17 19	1.6 1.5	1-	1-	1 -	2.7 3	3 3

Table 14. Average number of M17 count during storage.

Storage time (day)	M17 (log cfu)						
		P1 10	P1 40	P3 10	P3 20	P5 5	P5 10
1	8.03	7.93	7.69	7.2	7.	7.54	7.34
8	7.52	7.25	7.20	7.53	7.58	7.62	7.82
15	7.55	7.46	6.13	6.43	7.03	7.08	6.46
22	7.34	6.60	6.57	6.74	6.52	6.67	6.66
29	7.23	6.20	5	5	5	6.43	5.50

APPENDIX E
Tables of Lysozyme Treated Yogurt at Different Concentration

Table E. 1. pH of yogurt from top, middle and bottom of the glass during storage.

Storage time (day)	pH			
	control	Top	Middle	Bottom
1	4.51	4.56	4.52	4.51
4	4.48	4.54	4.50	4.49
8	4.45	4.54	4.47	4.46
15	4.43	4.53	4.55	4.50
18	4.42	4.52	4.51	4.50
29	4.41	4.53	4.52	4.49

Table E. 2. Titratable acidity of yogurt from top, bottom and middle of the glass during storage

Storage time(day)	Lactic acid (%)			
	control	Top	Middle	Bottom
1	0.918	0.918	0.918	0.990
4	0.927	0.954	0.954	1.008
8	1.008	0.990	0.990	1.062
11	1.134	0.990	1.008	1.080
15	1.152	0.990	1.008	1.099
18	1.200	1.044	1.062	1.098
29	1.250	1.008	1.098	1.098

Table E. 3. Number of starter bacteria on the top, middle and bottom of the glass

Storage Time (day)	MRS (log cfu)			
	c	Top	Middle	Bottom
1	8.17	7.19	7.24	8.14
8	7.25	6.00	6.20	6.70
11	8.23	5.47	6.34	6.50
15	7.6	5.63	6.36	6.60
29	7.08	4.69	5.20	5.360

Table E. 4. Effect of lysozyme treatment at different concentration on the pH of the yogurt

Storage time (day)	pH							
	control		300 mg/L		600 mg/L		1200 mg/L	
1	4.55	4.56	4.55	4.57	4.58	4.59	4.57	4.56
8	4.49	4.47	4.50	4.50	4.52	4.54	4.52	4.51
15	4.45	4.49	4.53	4.53	4.41	4.58	4.52	4.56
22	4.40	4.46	4.53	4.52	4.55	-	4.53	4.52
29	4.39	4.42	4.51	-	4.56	4.55	4.55	4.54

Table E. 5. Average pH values of lysozyme treated yogurt

Storage Time (day)	Average pH			
	c	300 mg/L	600 mg/L	1200 mg/L
1	4.555	4.560	4.585	4.565
8	4.480	4.500	4.53	4.515
15	4.470	4.530	4.550	4.540
22	4.430	4.520	4.550	4.530
29	4.410	4.51	4.560	4.550

Table E. 6. Effect of lysozyme treatment at different concentration on the titratable acidity of yogurt

Storage time (day)	Titratable Acidity (%)							
	control		300 mg/L		600 mg/L		1200 mg/L	
1	0.918	0.882	1.026	1.008	0.909	0.936	0.927	0.810
8	0.937	0.91	0.972	0.99	0.891	0.890	0.784	0.930
15	-	0.999	0.981	0.908	0.891	0.890	0.936	0.909
22	1.100	1.100	0.990	1.008	0.918	-	0.990	0.918
29	1.152	1.200	1.008	1.008	0.953	0.963	0.963	0.936

Table E. 7. Average titratable acidity of lysozyme treated yogurt

Storage Time (day)	Average lactic Acid %			
	c	300 mg/L	600 mg/L	1200 mg/L
1	0.890	1.017	0.923	0.870
8	0.923	0.981	0.891	0.860
15	0.999	0.943	0.891	0.923
22	1.100	0.999	0.918	0.954
29	1.176	1.008	0.957	0.955

Table E. 8. Effect of lysozyme treatment on syneresis of yogurt

Storage Time(day)	Syneresis (%)							
	control		300 mg/L		600 mg/L		1200 mg/L	
1	42	38	40	40	44	40	44	42
8	40	36	38	40	36	34	44	40
15	44	36	38	38	38	42	42	42
22	40	44	42	42	44	43	46	-
29	36	37	38	44	46	42	46	46

Table E. 9. Effect of lysozyme treatment on syneresis of yogurt

Storage Time (day)	Syneresis (%)			
	c	300 mg/L	600 mg/L	1200 mg/L
1	40	40	42	43
8	38	40	35	42
15	40	38	40	42
22	42	42	43	46
29	35	42	43	46

Table E. 10. Effect of lysozyme treatment at different concentration on Viscosity of yogurt

Storage Time (day)	Viscosity (cp)							
	control		300 mg/L		600 mg/L		1200 mg/L	
1	5960	5980	5480	5380	5400	5380	5080	5420
8	5620	5620	5740	6000	5240	-	5020	5220
15	5900	-	5660	6200	5600	5640	5200	5800
22	5740	5540	5020	5020	4920	4480	4500	-
29	5900	5370	4780	5420	4500	-	4420	-

Table E. 11. Effect of lysozyme treatment on Viscosity of yogurt

Storage Time (day)	Viscosity (cp)			
	c	300 mg/L	600 mg/L	1200 mg/L
1	5970	5430	5390	5250
8	5620	5870	5240	5120
15	5900	5930	5620	5500
22	5200	5020	4700	4500
29	5640	4600	4500	4420

Table E. 12. Effect of lysozyme treatment at different concentration on the number of starter bacteria

Storage Time (day)	MRS count (cfu*10^6)							
	control		300 mg/L		600 mg/L		1200 mg/L	
1	106	150	14	35	18	43	11	6
8	11	37	10	2	12	4	36	15
15	40	42	56	10	1.2	0.7	0.65	0.32
22	1	2	14	9	0.7	0.07	2	-
29	96	93	-	0.01	0.5	0.05	-	0.0018

Table E. 13. Average number of starter bacteria

Storage time (day)	MRS count (log cfu/ml)			
	control	300 mg/L	600 mg/L	1200 mg/L
1	8.10	7.40	7.50	6.95
8	7.40	6.78	6.89	6.40
15	7.60	7.52	5.98	5.50
22	5.20	7.08	4.84	6.30
29	7.97	4.85	4.70	3.25

APPENDIX F
Tables of Pekmez Yogurts

Table F. 1. pH profile of 5-10-15 % pekmez yogurt.

Fermentation Time (day)	pH			
	c	5 % Pekmez	10 % Pekmez	15 % Pekmez
0	6.35	6.32	6.28	6.24
1	6.23	6.30	6.29	6.38
2	5.53	5.83	6.06	6.15
3	4.51	4.89	5.53	6.02
4	4.26	4.44	4.98	5.80
4.75	4.00	4.19	4.50	5.14

Table F. 2. pH of pekmez yogurts.

Storage Time (day)	pH							
	c	c	P	P	P+N	P+N	P+M	P+M
1	4.44	4.38	4.69	4.18	4.24	4.22	4.31	4.28
8	4.35	4.33	4.37	4.36	4.32	4.35	4.32	4.31
15	4.30	4.30	4.35	4.32	4.36	4.34	4.28	4.29
22	4.24	4.24	4.28	4.41	4.34	4.35	4.40	4.38
29	4.17	4.19	4.38	4.37	4.35	4.36	4.38	-

Table F.3. Average pH of pekmez yogurts.

Storage Time (day)	pH (Average)			
	c	P	P+N	P+M
1	4.41	4.44	4.23	4.25
8	4.34	4.37	4.34	4.32
15	4.30	4.34	4.35	4.29
22	4.24	4.35	4.35	4.39
29	4.18	4.38	4.36	4.38

Table F. 4. Titratable acidity of pekmez yogurts.

Storage Time (day)	% Lactic Acid							
	c	c	P	P	P+N	P+N	P+M	P+M
1	0.860	0.930	0.660	1.044	0.945	0.945	0.972	0.783
8	0.970	0.980	0.972	0.945	0.963	0.945	0.981	0.972
15	1.017	1.017	0.981	0.940	0.920	0.930	1.010	1.010
22	1.120	1.180	1.008	0.945	0.981	-	0.936	0.936
29	1.170	1.190	1.008	1.062	1.017	0.950	0.860	1.116

Table F. 5. Average titratable acidity of pekmez yogurts.

Storage Time (day)	% Lactic Acid (Average)			
	c	P	P+N	P+M
1	0.890	0.850	0.9450	0.878
8	0.960	0.960	0.954	0.980
15	1.017	0.961	0.925	1.010
22	1.140	0.981	0.981	0.960
29	1.180	1.035	0.984	0.988

Table F. 6. Syenersis of pekmez yogurts.

Storage Time (day)	% Syneresis							
	c	c	P	P	P+N	P+N	P+M	P+M
1	44	40	44	42	46	42	-	43
8	42	42	46	44	46	46	46	44
15	44	42	48	47	44	46	46	45
22	44	44	46	48	46	48	46	45
29	44	44	46	46	44	46	46	-

Table F. 7. Average syneresis of pekmez yogurts.

Storage Time (day)	% syneresis (Average)			
	C	P	P+N	P+M
1	42	43	44	40
8	42	45	46	45
15	43	47	45	45
22	44	47	47	45
29	44	46	45	46

Table F. 8. Viscosity of pekmez yogurts.

Storage Time (day)	Viscosity (cp)							
	C	C	P	P	P+N	P+N	P+M	P+M
1	3460	3840	3400	3960	3540	3720	3820	3000
8	4000	4140	3280	3340	3420	3240	3420	3580
15	4800	5060	3620	3460	3920	3360	3800	3860
22	4580	5100	3410	3430	3660	3240	3900	3980
29	3600	4100	2940	3200	2900	3240	3140	-

Table F. 8. Average viscosity of pekmez yogurt.

Storage Time (day)	Average Viscosity (cp)			
	C	P	P+N	P+M
1	3650	3680	3630	3410
8	4070	3310	3330	3500
15	4930	3540	3640	3830
22	4840	3990	3450	3940
29	3850	3070	3070	3140

Table F. 9. Number of sterter bacteria of pekmez yogurts.

Storage Time (day)	Starter Bacteria (log cfu)							
	c	c	P	P	P+N	P+N	P+M	P+M
1	7.40	7.80	6.60	6.47	5.80	5.8	6.49	-
8	7.50	7.90	7.00	6.90	6.49	6.47	7.00	6.60
15	7.00	7.60	7.30	7.39	6.00	7.00	7.23	-
22	7.58	7.58	6.90	6.63	5.02	5.12	5.80	-
29	7.50	7.50	5.84	6.14	4.84	-	6.60	6.69

Table F. 10. Average number of starter bacteria of pekmez yogurts.

Storage Time (day)	Avergae Starter Bacteria count (log cfu)			
	c	P	P+N	P+M
1	7.60	6.50	5.80	6.49
8	7.70	7.00	6.48	6.80
15	7.30	7.30	6.50	7.23
22	7.58	6.76	5.08	5.80
29	7.50	5.84	4.84	6.69

P: pekmez P+N: Pekmez+Nisin P+M: Pekmez+Microwave

VITA

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