EFFECT OF TRADITIONAL STARTER CULTURES ON QUALITY OF CHEESE

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Approval of the Graduate School of Natural and Applied Sciences.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Sciences.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master Science.

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ABSTRACT

EFFECT OF TRADITIONAL STARTER CULTURES ON QUALITY OF CHEESE

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In this study, the physico-chemical changes occurring in white cheese and possible effects of starter culture combinations to the ripening period during 30 days storage were examined.

A total of thirty six lactic acid bacteria were isolated from a cheese made without using starter culture. For identification gram staining, catalase, gas production and coagulation tests were performed. For determination of species API50 CH (BioMérieux) and partial 16S rDNA gene sequence analysis were used.

Four cheeses were produced, one by using commercial starter culture [Lyofast CMS (*Lactococcus lactis* subs. *lactis* and *Lactococcus lactis* subs. *cremoris*)]as control and the other three by using different combinations of

isolates [*Lactococcus lactis* subs. *lactis* (13%) + *Lactobacillus brevis* (40%)+ *Lactobacillus paracasei* (47%); *Lactococcus lactis* subs. *lactis* (36%)+ *Lactobacillus paracasei* (64%); *Lactococcus lactis* subs. *lactis* (24,5%) + *Lactobacillus paracasei* (28,5%) + *Lactobacillus brevis* (47%)].

Cheeses were ripened in 15 % saline solution at 4°C for 30 days. Samples were taken from each treatment and analyzed on 2nd, 15th and 30th days.

Sensory, microbiological and chemical properties of the cheese preparations as pH, acidity, salt, fat, moisture, protein contents during storage period were determined.

In this respect effect of using different starter culture combinations on quality of Turkish white cheese was determined and *Lactococcus lactis* subs. *lactis* (13%) + *Lactobacillus brevis* (40%)+ *Lactobacillus paracasei* (47%) combination was found as the best and can be suggested as an ideal combination for white cheese production.

Key words: White cheese, starter culture, lactic acid bacteria

ÖZ

GELENEKSEL STARTER KÜLTÜRLERİN PEYNİR KALİTESİNE ETKİSİ

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Bu çalışmada, 30 günlük depolama periyodu boyunca beyaz peynirdeki fiziko-kimyasal değişiklikler ve starter kültür kombinasyonlarının olgunlaşma periyoduna etkisi araştırılmıştır.

Starter kültür kullanılmadan yapılan bir peynirden toplam otuz altı laktik asit bakterisi izole edilmiştir. Tanımlama için gram boyama, katalaz, gaz üretim ve pıhtılaşma testleri yapılmıştır. Türlerin belirlenmesi için API50 CH (BioMérieux) ve kısmi 16S rDNA gen sekanslama analizleri yapılmıştır.

Dört peynir üretilmiştir. Bunlardan biri kontrol grubu olarak ticari kültür kullanılarak [Lyofast CMS (*Lactococcus lactis* subs. *lactis* and *Lactococcus lactis* subs. *cremoris)*] diğerleri ise elde edilen izolatların değişik

kombinasyonları kullanılarak [*Lactococcus lactis* subs. *lactis* (13%) + *Lactobacillus brevis* (40%)+ *Lactobacillus paracasei* (47%); *Lactococcus lactis* subs. *lactis* (36%)+ *Lactobacillus paracasei* (64%); *Lactococcus lactis* subs. *lactis* (24,5%) + *Lactobacillus paracasei* (28,5%) + *Lactobacillus brevis* (47%)] yapılmıştır.

Peynirler % 15'lik tuz çözeltisinde 4°C'de 30 gün boyunca olgunlaştırılmıştır. Her bir örnekten numuneler alınarak 2., 15. ve 30. günlerde analiz edilmiştir.

Duyusal, mikrobiyolojik ve pH, asitlik, tuz, yağ, protein, nem miktarı gibi kimyasal özellikleri depolama periyodu boyunca analiz edilmiştir.

Bu kapsamda, değişik starter kültür kombinasyonlarının kullanılmasının Türk beyaz perynirinin kalitesi üzerine etkisi belirlenmiştir. *Lactococcus lactis* subs. *lactis* (13%) + *Lactobacillus brevis* (40%)+ *Lactobacillus paracasei* (47%) kombinasyonu en iyi sonucu vermiştir ve beyaz peynir üretiminde ideal kombinasyon olarak önerilebilir.

Anahtar Kelimeler: Beyaz peynir, starter kültür, laktik asit bakterisi.

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LIST OF ABBREVIATIONS

DİE: Devlet İstatistik Enstitüsü

LAB: Lactic acid bacteria

GRAS: Generally Recognized as Safe

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen

CHAPTER 1

INTRODUCTION

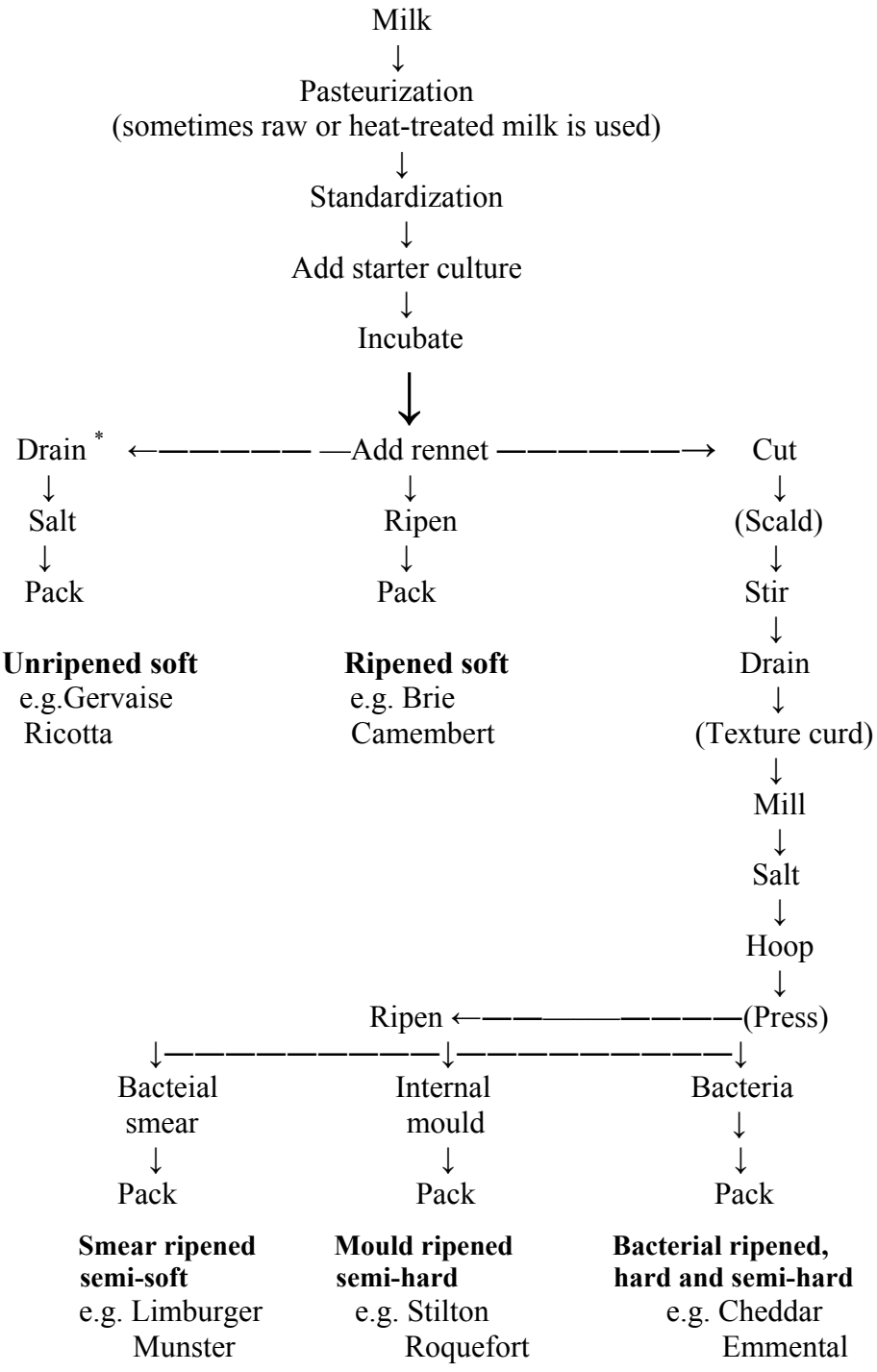
In human nutrition milk and milk products are very important. Milk carries many nutrients that the infant needs for growth and development. For children, adolescent, elderly people pregnant and nursing mothers, milk plays an important role in meeting the requirements of many essential nutrients, and hence milk is considered as a protective food. Milk helps to balance human diet by supplementing good quality protein, calcium and vitamins particularly, vitamin A, riboflavin, niacin and folic acid. In addition milk contains several bio-protective molecule that ensure health security to humans [1]. However, milk is also a suitable media for microorganisms and can spoil easily. Therefore, in order to increase its resistance and to obtain different dairy products, it is processed into different products.

Cheese is the most consumed milk product in the world. It is also as nutritious as milk considering proteins, vitamins and minerals. In addition, digestibility of proteins increases due to the proteolytic activity during cheese ripening. Cheese is also a suitable nutrient for patients who have diabetes or lactose malabsorption, because of the low lactose ratio it contains [2].

1.1 Cheese Production

The basic technology for the manufacture of all types of cheese is similar, relatively small changes in procedures during manufacture resulting in large perceived differences in the final cheese (Table 1.1).

Table 1.1 Procedure for cheese production



Note: Stages in parentheses are not involved in the manufacture of some varieties.

***** Manufacture may involve some light cutting and scalding.

1.2 Cheese Milk

Cheese may be made from the milk of any species, while cows' milk is most commonly used in the US and Western Europe, there is increasing interest in manufacture of goats' and, to a lesser extent, sheep milk cheese. In regions where fresh milk is scarce, cheese has been successfully made from recombined anhydrous milk fat and reconstituted skim milk powder [80].

Especially the following criterion are important in the choice of cheese milk;

- Physical and chemical composition of the milk must be normal
- Protein content (especially casein) must be high
- Microorganism count of raw milk must be low
- Raw milk should not contain inhibitors like antibiotics
- Coagulation test with clotting enzyme must be positive

1.3 Pasteurization

Pasteurization is very important to kill pathogen microorganisms like *Campylobacter* and *Salmonella.* The optimum pasteurization temperature of milk is 72°C for 15 seconds. Overpasteurization produces too soft a curd, and this may or may not be corrected by prior additions of soluble salt $CaCl_2$.
For cheese made from raw milk ripening time must be long and for fresh cheese types only pasteurized milk must be used.

1.4 Standardization

The composition of the milk is important in determining the characteristic of cheese.

a) Standardization of fat ratio
b) Standardization of protein ratio

1.5 Chemical Changes During Curd Formation

Conversion of milk from a fluid to a gel (coagulation) is a basic step common to all types of cheese. Gel formation is a consequence of protein destabilization and may be brought about either by acid proteinases such as chymosin, the active component of rennet, quiescent acidification to a pH value close to the isoelectric point of the proteins, or by a combination of acidification and heating.

1.5.1 Action of Rennet

Rennet coagulation involves two distinct stages, a proteolytic stage in which the casein micelle is destabilized by hydrolysis of $_{K}$-casein to yield para- $_{K}$-casein micelles, and a secondary, calcium mediated, stage in which paracasein micelles undergo limited aggregation. The secondary stage requires quiescent conditions and a temperature in excess of 20 °C.

Hydrolysis of $_{K}$-casein primarily involves cleavage of the peptide bond, Phe_{105}-Met_{106}, which is uniquely sensitive to hydrolysis by acid proteinases. This cleavage yields a para- $_{K}$-casein, common to all caseins and a macropeptide unique to each component.

After addition of rennet, usually 30 minutes later for most cheese types, curd is firm enough to be cutted. After cutting curd is subjected to different treatments according to cheese type.

1.6 Chemical Changes During Cheese Ripening

Cheese is chemically, microbiologically and enzymatically a complex and dynamic system. This makes the process of cheese ripening highly complex. Cheese contains a defined microbiological starter flora and an undefined, highly variable, adventitious flora. The diversity of the microflora involved in cheese ripening adds to the complexity of the process; individual reactions in

that process are catalyzed by different enzymes. The nature of the substrate, which consists essentially caseins, fat and carbohydrate in milk; the variety of agents involved in biochemical transformations; the diversity of modifications undergone by constituents of cheese; and large number of products formed all contribute to flavor development in ripened cheese [42,43,44,45,46,47]. The major biochemical changes involved during cheese ripening are proteolysis, lipolysis, lactose fermentation and production of volatile compounds (Table 1.2) [47,48,49,50,51]. Although lipolysis and lactose metabolism are fundamental processes in cheese making, their contributions to the texture and intensity of flavor of the finished product are somewhat difficult to define for some cheese varieties. Proteolysis, however, plays a direct role in development of the desired texture, aroma, and intensity of background flavor in most matured cheeses [52,53,54].

Lipolysis in most varieties of cheese is not extensive, but some hydrolysis occurs during cheese ripening. Degradation of lipids is essential to Cheddar cheese flavor, so that especially the shortchain volatile fatty acids such as butyric, caproic, caprylic, and capric are present. Methyl ketones, ethanol, and 2-butanol also are thought to be important flavor compounds in Cheddar cheese [55]. Lipolytic activity in cheese may come from milk lipase, starter bacteria, adventitious bacteria, or enzyme preparations added to milk. Milk lipase is only active in cheeses made from raw milk. Certain strains of lactobacilli liberate intracellular lipase upon autolysis, and this may account for the lipolytic activity in hard cheese.

Lipolysis and proteolysis are important in Swiss cheese flavor. Many of the flavor characteristics of Swiss cheese depend on free fatty acids produced by fermentation and lipolysis [56, 57, 58, 59] and on peptides and amino acids produced by proteolysis. The contribution of L. *bulgaricus* to lipolysis and proteolysis was studied by Bide et al. [60]. Two of the starter organisms, L. *bulgaricus* and *P. shermanii,* had slight lipolytic activity but not enough to account for the amounts of free fatty acids in Swiss cheese. *Lactobacillus bulgaricus,* however, produced greater quantities of amino acids and peptides than did either P.*shermanii* or *S. thermophilus* [60].

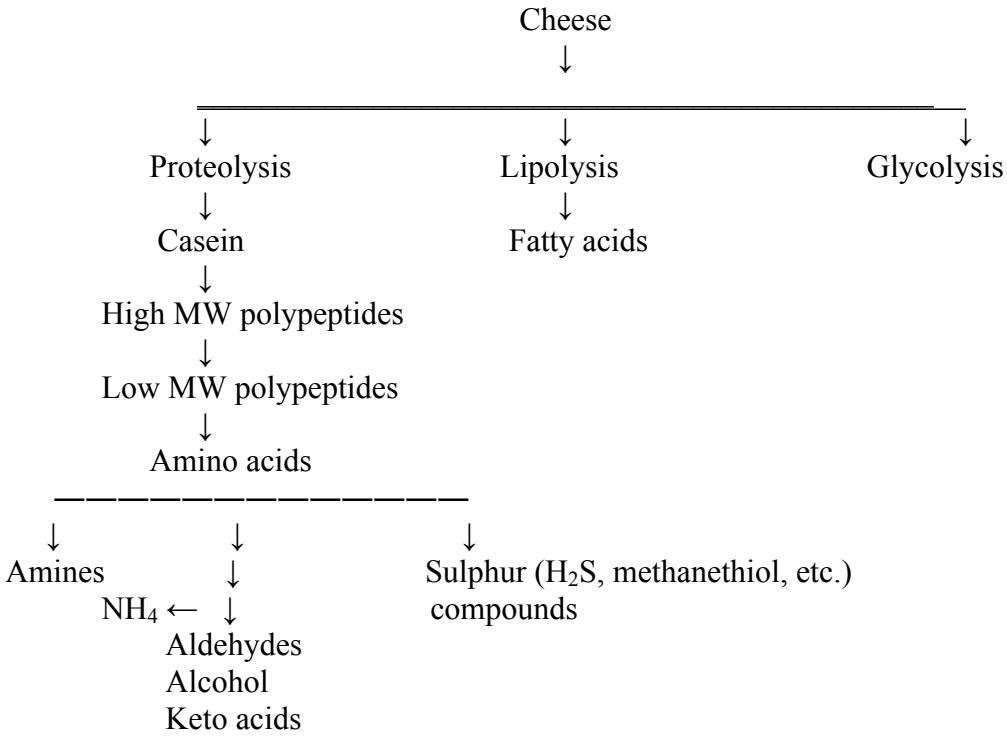
In addition to the starter bacteria adjunct nonstarter lactic acid bacteria contribute to cheese ripening [62]. Adjunct cultures are nonstarter lactic acid bacteria, consisting mainly of *Lactobacillus* sp., which are used in addition to a standard mesophilic starter to improve and to enhance the flavor of cheese [63, 64]. However, for the role of the adjuncts in cheese ripening to be maximized, the intracellular enzymes must be released from the cells into the cheese matrix, which explains much of the attention given to cell autolysis during ripening [65, 66].

There has been considerable interest in using defined strains of nonstarter lactic acid bacteria as adjunct cultures to accelerate and improve flavor and texture development during cheese ripening [67, 68]. Attenuated adjunct cultures with enhanced autolytic properties should provide more controlled and consistent ripening, resulting in improved flavor and texture, particularly in lower fat cheese [69,70]. Attempts have therefore been made to add adjunct cultures that have been modified or attenuated so that they can play an appreciable role during cheese ripening without producing excess lactic acid. Physical methods of sublethal treatments such as freeze shocking (**FS**), heat shocking (**HS**), and spray drying (**SD**) are the most-studied techniques of attenuating adjunct cells [71, 72, 73]. These treatments have led to varying levels of cell viability, modification of the ability to produce acid, and intracellular proteinase or esterase activities [71].

Abdel Baky et al. [74] reported that inoculation of cheese curd with a heat-shocked culture of either *Lactobacillus casei* or *Lactobacillus helveticus* does not greatly affect cheese composition but influences flavor intensity. When comparing the influence of heat shocked *Lb. helveticus*, *Lactobacillus bulgaricus* or *Streptococcus thermophilus* on the ripening and quality of Gouda cheese, Bartels et al. [75] found that *Lb. helveticus* gave the best results. El-Abboudi et al. [76] suggested that the development of typical Cheddar cheese flavor is accelerated by the addition of homogenized thermal-treated cells of *Lb. casei* sp. *casei*. This treatment does not increase gross proteolysis in cheese as measured by soluble nitrogen but accelerates the

breakdown of peptides, thus increasing the amount of amino nitrogen and also reducing bitterness. To date, comparable studies on the impact of HS and FS of bacterial cells on cheese ripening is limited and to some extent contradictory. Although Ezzat and El-Shafei [77] suggested that heat shocked cells are better candidates for the enhancement of flavor in Ras cheese (a Kashkavaltype hard cheese); Aly [78] found no significant differences between the two treatments on low fat cheese quality. Johnson et al. [69] compared the performance of FS, freeze-dried, and SD adjunct cells of *Lb. helveticus* to enhance flavor and body development in reducedfat Cheddar cheese. Cheese flavor intensity is enhanced in all adjunct cheeses, and cheeses made with an adjunct spray dried at high outlet air temperature have the least off-flavor intensity. Other sensory measures are not significantly different among the cheeses in spite of differences in the cellular properties of the adjunct cultures and chemical measures of cheese ripening.

Table 1.2 Chemical changes during cheese ripening



1.7 The Classification of Cheese

- Hard (26-50% moisture)

internally ripened, no added ripening microorganisms

e.g. Parmesan, Cheddar

internally ripened, added ripening bacteria

e.g. Emmental

internally ripened, secondary surface ripening by mould

e.g. Blue Cheshire

- Semi-hard (42-52% moisture)

internally ripened, no added ripening microorganisms

e.g. Lancashire, Edam

internally ripened, ripening mould added

e.g. Stilton, Roquefort

- Semi-soft (45-55% moisture)

surface ripened, ripening bacteria added

e.g. Limburger

- Soft (48-80% moisture)

surface ripened, ripening mould added

e.g. Brie

unripened

e.g. Cottage

- Others

e.g. brines varieties, Whey cheese [80]

1.8 Starter Cultures

Dairy starters are cultures of harmless, active bacteria, grown in milk or whey, which impart certain characteristics and qualities to various milk products (Table 1.3). The culture may be one strain of a microorganism species, called a single-strength culture, or a number of strains and/or species called a multi-strain or mixed-strain culture. Starter cultures are now lyophilized with milk components, nutrients, and energizers and distributed commercially in the dry state or are frozen with liquid nitrogen at - 196ºC and distributed in this state [18].

In White cheese production usually lyophilized and DVS (Direct Vat Set) cultures are used. Lyophilized cultures need much more equipment, professional personnel and have a contamination risk during reproduction [29]. On the other hand DVS cultures provide the cheese manufacturers the following benefits: Cultures are inoculated directly into the milk in the cheese vat; less batch-to-batch variation; cultures tested before use for activity and contamination; more predictable performance; high flexibility; possibility of composing "impossible" mixtures (e.g. mix of thermophilic and mesophilic

strains); improved possibilities of product development at the dairy; less risk of contamination and phage attack; and, no cost of bulk starter preparation [30]. Therefore, DVS cultures are much more preferred. For example in England in 1995 the DVS culture usage ratio was 40% [29].

Table 1.3 Some Bacterial Cultures Used in Fermented Milk Product Manufacture [18]

Culture	Major known function	Product use
Propionic bacterium shermanii	Flavor and eye formation	Emmental and Swiss cheese
Lactobacillus bulgaricus Lactobacillus lactis Lactobacillus helveticus	Acid and flavor	Bulgarian buttermilk, yoghurt, Kefir, Swiss, Emmental and Italian cheese
Lactobacillus acidophilus	Acid	Acidophilus buttermilk
Streptococcus thermophilus	Acid	Emmental, Cheddar and Italian cheese and yoghurt
[1]Lactococcus diacetilactis	Acid and flavor	Sour cream, cheese
[1]Lactococcus lactis [1]Lactococcus cremoris	Acid	Cultured buttermilk, sour cream, cottage cheese
Leuconostoc citrovorum Leuconostoc dextranicum	Flavor	Cultured buttermilk, sour cream, cottage cheese, ripened cream butter
[2]Enterococcus durans [2]Enterococcus faecalis	Acid and flavor	Soft Italian, Cheddar and some Swiss cheese

[1]The genus *Lactococcus* was not included in Bergey's Manual of Systematic Bacteriology. It was established by Schleifer et al. for the lactic streptococci *Streptococcus lactis*, *Streptococcus cremoris* and *Streptococcus diacetilactis.*

[2] The genus *Enterococcus* was not included in Bergey's Manual of Systematic Bacteriology, where some of its species were included in the genus *Streptococcus.* A proposal was made by Schleifer and Kilpper- Bälz to transfer streptococci that had long been informally referred to as enterococci to separate genus *Enterococcus.* In that publication *Streptococcus faecalis* and *Streptococcus durans* were transferred to the new genus as *Enterococcus faecalis* and *Enterococcus durans*, respectively [88].

In the production of different cheese types, according to taste, aroma and texture of cheese, different types of microorganisms are used. For example, cheeses whose curd is scalded during the manufacture stage are made by using thermophilic starters like *Streptococcus salivaris subsp. thermophilus, Lactobacillus delbrueckii subsp. helveticus, Lactobacillus delbrueckii subsp. lactis.* On the other hand, in cheeses that need to be ripened, mesophilic starters like *Lactococcus* species, *Leuconostoc cremoris, Lactobacillus casei*, are used. In ripened cheeses using combination of lactococcus, streptococcus and lactobacillus enables faster acid production, hydrolysis of milk proteins, synthesis of aroma components in consequence of symbiotic relation between them [10]. While for unripened soft cheeses like Mozzarella and Cottage cheese, acid production ability of starter is important, for ripened hard cheeses like Cheddar, Emmental and Gruyere, proteolytic activity is more important [22]. In addition to these, defining the bacteriophage spectrum of starter cultures [23] and decarboxylase activity of enterococci which are thought to be used as starter culture, is also important [24].

1.8.1 Classification of starter cultures

Cheese starter cultures may be classified in a number of ways. The microorganisms themselves, for example, may be classified according to optimal growth temperature. Mesophilic starters comprise *Lactococcus* and *Leuconostoc* and have an optimal growth temperature of ca. 30°C, while thermophilic starters comprise the more widely used *Lactobacillus* species

and *Str. salivarius* ssp. *thermophilus* and have an optimal temperature of 40-45°C (Table 1.4).

In either case, starters may be mixed, in which the number of strains is unknown or defined in which a known number of strains are present. Mesophilic defined cultures may consist of single, paired or multiple strains. In recent years it has been found possible to reduce the number of strains in multiple cultures from the six originally used to two or three without any adverse effect on performance [80]. Commercial suppliers of food starter cultures are given in Table 1.5.

Table 1.4 A Few Basic Characteristics of Starter Culture Bacteria [18]

L. *lactis* and L. *cremoris*	E.*faecalis* and E.*durans*
Gram + coccus	Gram + oval-shaped coccus
Growth at 10°C	Growth at 10°C
No growth at 45°C	Growth at 45°C
Reduce litmus strongly	Reduce litmus strongly
Non-salt tolerant < 6.5%	Salt tolerant >6.5%
No spores	No spores
Non-thermoduric	Thermoduric
	Growth at pH 9.6
S. *thermophilus*	*Leuconostoc citrovorum* *Leuconostoc dextranicum*
Gram + coccus	Gram + coccus
No growth at 10°C	Growth at 10°C
Growth at 45°C	No growth at 45°C
Reduce litmus milk	Reduce litmus milk weakly
Non-salt tolerant < 6.5%	Non-salt tolerant <6.5%
No spores	No spores
Thermoduric	Non-thermoduric
No growth at pH 9.6	
L.*bulgaricus*	L.*acidophilus*
Gram + rod, medium or long	Gram + rod, long
No growth at 10°C or 20°C	No growth at 10°C
Growth at 45°C	Growth at 45°C
Reduce litmus strongly	Does not reduce litmus milk
Non-salt tolerant < 6.5%	Non-salt tolerant <6.5%
No spores	No spores
Thermoduric	Non-thermoduric

Table 1.5 Commercial suppliers of food starter cultures [90]

Company	Country
Alce	Italy
ASCRC	Australia
Centro Sperimentale del Latte	Italy
Chr. Hansen	Denmark
CSK	The Nederlands
Danisco	Denmark
Degussa	Germany
DSM	The Nederlands
Gewürzmüller	Germany
NZDRI	New Zealand
Quest International	The Nederlands
Rhodia	France
Lallemand	Canada

1.8.2 Lactic Acid Bacteria

Lactic acid bacteria (LAB) comprise a heterogeneous group of non-sporulating Gram-positive organisms which ferment sugars and produce lactic acid. Their ability to lower pH by producing acid from sugar leads to the development of desirable organoleptic properties, prevents the growth of pathogens and ensures the stability and safety of the final product. Thus, lactic acid production acts as a selective trait which allows LAB to predominate [5].

LAB live in habitats where pH of medium is low. There are eight genera that produce lactic acid: *Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Carnobacterium, Enterococcus and Sporolactobacillus.* All lactic acid bacteria are anaerobes, however they are facultatively anaerobes, they can grow in the presence of oxygen. Some strains produce H_2O_2 through flavoprotein oxidase systems, and eliminate H_2O_2 by their catalase or peroxidase. Lactic acid bacteria use lactose as their main source of carbon to produce energy. Lactic acid bacteria that only produce lactic acid as an end product are called homofermentative ; those that also produce acetic acid, ethanol and carbon dioxide are termed heterofermentative. The differences observed in fermentation products are determined by the presence or absence of the enzyme aldolase, one of the key enzymes in glycolysis. Heterofermentors lack aldolose, and cannot break down fructose biphosphate to triose phosphate, however they have transketolase to produce lactic acid, acetaldehyde, ethanol, and CO_2 [6].

Lactic acid bacteria can grow in most or our common food raw materials. They constitute part of their natural microflora, can be used in spontaneous fermentation, and can also be added as starters. LAB need some sugar for fermentation, either naturally present or added. Except for some fruits, the pH of raw materials is seldom low enough to inhibit the growth of these bacteria. The members of genus *Lactobacillus* are also found among the gastrointestinal microflora [7].

There are numerous application areas for use of lactic acid bacteria both in industry and human health, including preservation of foods and use as probiotics (Table 1.7);

- Food industry uses salt, sugar, smoke, nitrite, sulphite, the parabens, organic acids, benzoic and sorbic acid to preserve foods. Chemical preservation is disfavoured by consumers as the demand for more natural and safer foods is increasing. Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and/ or their antibacterial products. LAB play an important role in food fermentations, causing the characteristic flavor changes and exercising a preservative effect on fermented products. They are associated with many different foods and they are generally considered to be harmless or even an advantage for human health. In United States they are afforded GRAS (Generally Recognized as Safe) status. The general conclusion is that the pathogenic potential of lactobacilli is quite low. This conclusion is based on the widespread consumption of these microorganisms in fermented foods, their presence as normal colonizers in the human body, failure to isolate these bacteria's primary pathogens, and lack of negative side effects of these bacteria when in high levels to immunocompromised humans. There are some exceptions however. The genus *Streptococcus* includes many human and animal pathogens while *Streptococcus thermophilus* is nonpathogenic and is used in manufacture of yoghurt and several cheese types.

The preservation of food is obtained by competitive growth, metabolic products- lactic acid, H_2O_2, etc- and bacteriocins- protein containing antimicrobial products- of microorganisms. Most bacteriocins produced by LAB have a narrow antibacterial spectrum, but some have a broad action range. LAB that produce broad-spectrum bacteriocins offer great potential in biopreservation. Nisin is licensed for use as a food additive over 45 countries and pediocin A has a possible antibotulinal effect [8].

- There are alternative ways of using lactic acid bacteria, most notably the species *L. acidophilus* (Table 1.6). The researches show that *L. acidophilus* can also be used as probiotic or living organism, which upon in certain numbers (10^7-10^9 cfu/day), exert health benefits beyond inherent basic nutrition [9].

The beneficial actions these bacteria provide include:

- Manufacturing B vitamins such as niacin (B-3), folic acid, biotin and pyridoxine,
- Providing the enzyme lactase, allowing digestion of milk and milk-based foods. Sweet acidophilus milk (which is made by inoculating milk with *L. acidophilus* bacteria) is consumed by individuals who suffer from lactose maldigestion and intolerance, a condition that effect approximately 75 % of world's population. Maldigestion and intolerance occurs when enzymes (lactase) cannot break down lactose or milk sugar in the intestine. Failure to digest lactose results in discomfort, cramps and diarrhea,
- Enhancing protein digestion and absorption, particularly of proteins found in cultured milk/ yoghurt,
- Acting as anti-carcinogenic factors with anti-tumor potential (by binding to mutagens, deactivating carcinogens, inhibiting carcinogen binding enzymes of colonic microbes and influencing secondary bile salt concentration),
- Acting to prevent hypertension (peptidase action on milk protein yield tripeptides which inhibit angiotensin 1 converting enzyme),
- Enhancing bowel function,
- Helping to control high cholesterol levels,
- Helping to control the spread of undesirable microorganisms.

For example, *Candida albicans,* a normally non-threatening yeast that resides within our guts, can spread aggressively and be potentially degrading to our health when the friendly bacteria's control is compromised due to such causes as the administration of antibiotics. It is therefore generally recommended

that individuals supplement with probiotics immediately following a course of antibiotics.

Table 1.6 Microorganisms Used as Probiotic [39]

Lactobacillus species	*Lactobacillus bulgaricus, Lactobacillus cellebiosus, Lactobacillus dellbrueckii, Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus brevis, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus helveticus, Lactobacillus salivarus*
Bifidobacterium species	*Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium thermophilum*
Bacillus species	*Bacillus subtilis, Bacillus pumilus, Bacillus lentus, Bacillus licheniformis, Bacillus coagulans*
Pediococcus species	*Pediococcus cerevisiae, Pediococcus acidilactici, Pediococcus pentosaceus*
Streptococcus species	*Streptococcus thermophilus, Streptococcus intermedius*
Bacteriodes species	*Bacteriodes capillus, Bacteriodes suis, Bacteriodes ruminicola, Bacteriodes amylophilus*
Propionibacterium species	*Propionibacterium shermanii, Propionibacterium freudenreichii*
Leuconostoc species	*Leuconostoc mesenteroides*
Mold	*Aspergillus niger, Aspergillus oryzae*
Yeast	*Saccharomyces cerevisiae, Candida torulopsis*

Table 1.7 Metabolic products of lactic acid bacteria which exhibit antimicrobial properties [61]

Product	Main target organisms
Organic acids	
Lactic acid	Putrefactive and Gram-negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia, some yeasts and fungi
Hydrogen peroxide	Pathogens and spoilage organisms, especially in protein-rich foods
Low-molecular metabolites	
Reuterin (3-OH-propionaldehyde)	Wide spectrum of bacteria, moulds and yeasts
Diacetyl	Gram-negative bacteria
Fatty acids	Different bacteria
Bacteriocins	
Nisin	Some LAB and Gram-positive bacteria, notably endospore-formers
Other	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

1.8.3 Role of Starter Microorganisms in the Manufacture of Cheese

During pasteurization in addition to pathogens also beneficial microorganisms, which are involved in ripening of cheese, are killed. So to compensate this loss addition of starter culture is a technological must. In modern practice bacteria of the group commonly referred to as lactic acid bacteria (LAB) are added to milk as starter cultures, the key role being the production of lactic acid by fermentation of lactose. Lactic acid is responsible for the fresh acidic flavour of unripened cheese and is of importance in the formation and texturizing of the curd. In addition, starters play other essential roles: the production of volatile flavour compounds such as diacetyl and aldehydes, and the synthesis of proteolytic and lipolytic enzymes involved in the ripening of cheese and the suppression of pathogenic and some spoilage microorganisms. Therefore, starter cultures used in manufacture of cheese are very important in defining the quality of cheese.

Acid production in milk and flavor development during ripening are both related with proteolytic activity of the starter. Proteolytic activity of LAB aims to produce amino acids for their self development. Although LAB show low proteolytic activity when compared with *Bacillus, Pseudomonas, Enterococcus* , this activity has an important role in cheese ripening. LAB have proteases bound their cell wall which enables them to hydrolyze big protein molecules into small peptides. With the help of peptidases localized outside the cell wall these peptides forms oligopeptides. Oligopeptides which are not longer than 6 amino acid are taken into cell and are hydrolyzed into amino acids. Peptidases are still active in ripened cheeses. Especially they become active after the lysis of LAB [26]. While for ripened hard cheeses starter cultures with high proteolytic activity are used, for fresh cheeses consumed unripened proteolytic strains are not used. Also for semi hard Salted White cheese which is ripened for 2-3 months, low proteolytic activity is required. If the proteases are not in balance with peptidases and are found in cheese at higher ratios, it can cause bitterness and texture defects. However not only the proteases are responsible for bitterness. In addition to them,

process parameters and enzymes (rennet etc.) added to milk for casein coagulation can cause bitterness [27].

Species of four genera, *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus* are most widely used as starters, the use of a fifth genus, *Pediococcus*, having been proposed more recently.

1.8.4 Characteristics of Starter Cultures Used in White Cheese Production

Starter to be used;

- should have high acid production ability,
- should produce good taste and smell in desired dose and combination,
- should not have high proteolytic activity in order to avoid fast ripening and bitterness,
- should have high antagonistic activity to inhibit pathogens,
- should be resistant to phages,
- should have resistance against antibiotics,
- should grow at cheese production temperature,
- should be resistant to certain salt concentration [40].

In Turkey the most produced and consumed cheese type is Salted White cheese. According to DİE, in 1996 total cheese production in Turkey was 200.802 tons and 153.635 tons (76.5 %) of these was white cheese [3]. Major important characteristics of Salted White cheese are that, it is cut in 7x7x7 cm size, kept in salted water (acidity is 0.27 and NaCl concentration is 14% or 16 %) for about 5 hours and get ready to consumption after 2 months ripening period in which they are kept in salted water filled sealed cans at 3ºC-4ºC [11]. The texture is firm and smooth. The contents for moisture and fat-in-dry-matter contents are 51.5-57.1 % and 46.2-53.3 %, respectively [41].

In order to produce best quality Turkish White cheese lots of technological and microbiological studies were conducted, but there is not still an ideal

starter combination [12]. In 1970's acid (*Lactococcus lactis subsp. lactis, L. lactis subsp. cremoris*) and aroma (*L. lactis subsp. biovar. diacetylactis, Leuconostoc cremoris)* developer quartet coc combinations were suggested by European firms that produce commercial starter culture. Also Üçüncü (1971) who worked with different commercial starters found this combination suitable for Turkish White cheese. This combination gives the best result than the other suggested combinations for hard and ripened cheeses. However, CO_2 production rate of aroma developers [13] and diacetyl that they produce does not contribute aroma of White cheese [14] and these strains were defined as unnecessary in starter combinations. Acid developer lactococci are still today the most suitable and commonly used starters in White cheese production. But their sensitivity to high salt concentrations makes it necessary to search for alternatives [15].

In other studies conducted to find out starter culture combinations in White cheese production, white cheeses produced without using starter cultures were examined. First time Özer (1964) suggested that fecal streptococci (*Enterococcus faecalis, E. faecium, E. durans*) could be combined with lactobacillus. Also Yorgancıoğlu (1986) suggested *E. faecalis* and *E. faecium* which have high acid production rate, are resistant to salt but have low proteolytic activity. *L. lactis subs. lactis, E. faecalis, E. durans* and *Lactobacillus plantarum* combination was found to be successful when compared with commercial Lactococcus, Enterococcus and Lactobacillus combinations in lactic acid production and inhibition of other microorganisms aspects [19]. Although using enterococci as starter culture is not usual, it is known that it plays an important role in the ripening of cheddar cheese [20] and *E. faecalis* is used as starter culture in Cheddar cheese, Mozzarella, Provolone production [11,20]. It is also important that these bacteria are resistant to high salt concentrations which is an asset for Salted White cheese, can adapt bad conditions easily and produce antimicrobial substance. However, the most important disadvantage of them is that, some strains of these bacteria produce enterotoxin rarely and most strains produce biogen amine related to their amino acide decarboxylase activity [21].

Lactococcus lactis subsp. lactis and *Lactococcus lactis subsp. cremoris* are used in Cottage and Cream cheese production as starter cultures because of their limited metabolic products, limited production of acetate and diacetyl as secondary metabolites and low proteolytic and lipolytic activity [25]. Also in White cheese production these microorganisms are used by taking characteristics of cheese into account.

Evrensel and Aynalı (1996) defined that, in cheese production *Lc. lactis ssp. lactis, Str. salivaris ssp. thermophilus, Streptococcus durans* and *Lb. delbrueckii spp. bulgaricus* played an important role in acid production while *Brevibacterium linens, Propioniobacterium shemanii, Leuconostoc spp., Lc. Lactis spp. diacetylactis* played an important role in taste and aroma production [31].

Kirov and Chamakov (1972) produced White cheese by using 3 different culture combinations including yoghurt culture (*Lc. delbrueckii spp. bulgaricus* and *Str. salivarius spp. thermophilus*), *Enterococcus* species and *Lc. lactis ssp. lactis* + *Lc. lactis spp. cremoris* + *Streptococcus durans* and specified that cheeses produced by using yoghurt culture and *Streptococcus durans* were more harder and had much better aroma [32].

Torres and Chandan (1981) defined that cheeses produced by using lactic acid bacteria, yoghurt culture and lipase enzyme were found to have similar composition after 12 weeks ripening period. However, it was also found that in cheeses ripened by lipase, fatty acid ratio increased continuously and proteolytic activity was maximum in cheeses produced by using yoghurt culture. Cheeses produced by using lactic acid bacteria were found to be much more acceptable when compared with cheeses produced by using lipase and yoghurt culture [33].

Kehagias et al. (1995) produced White cheese from cow and sheep milk by using mesophilic (*Lc. lactis ssp. lactis* and *Lc. lactis spp. cremoris)* and thermophilic starter cultures (*Str. salivarius spp. thermophilus* and *Lb. debrueckii spp. bulgaricus*) and specified that the highest pH and water

soluble protein ratio found in cheeses produced by using thermophilic starters. It was also defined that, these cheeses were the least approved ones in sensorial aspect [34].

Uysal (1996) produced 3 types of White cheese by using raw milk (control), pasteurized milk and starter culture which contains $5x10^{10}$ cfu/g *Lc. lactis ssp. lactis* and *Lc. lactis ssp. cremoris* in different ratios (1 package or 1/2 package for 1200 liter milk) and found out that dry substance ratio was 39.19-38.91% , 38.80-38.48%, 38.42-38.26% , fat ratio was 20.01-20.00 %, 20.00-19.88 %, 19.88-19.75%, total nitrogen ratio was 2.60-2.39 %, 2.53-2.30 %, 2.47-2.16%, water soluble nitrogen ratio was 0.36-0.46 %, 0.36-0.60 %, 0.38-0.77 % and ripening ratio was 13.88-19.85% 15.11-26.76%, 16.74- 36.24% respectively during 90 days ripening period. As a result it was specified that according to starter culture ratio added to milk, proteolytic activity also increased.

Davide et al. (1994) defined that in Kesogort cheese production (made in Philippines) usage of 0.5-2 % yoghurt culture increased taste and aroma and gave a better texture. Furthermore it was found that, best quality cheese was obtained with addition of 1.5 % yoghurt culture [36].

Kıvanç et al. (1992) examined cheeses produced by using yoghurt culture and without culture from pasteurized milk for 3 months period for alteration of microorganisms in industrial and hygienic aspects and defined that in yoghurt culture used cheeses number of industrially important lactic acid bacteria was high while the number of hygienically important pathogen microorganisms were low when compared with the cheeses produced without using starter culture [37].

Giori et al. (1985) examined effect of pH and heat on the proteolytic activity of *Lc. lactis spp. cermoris, Lc. lactis spp. lactis, Streptococcus faecium, Streptoccus faecalis, Lactobacillus plantarum, Lactobacillus casei* and defined that *Lactobacillus* had the maximum proteolytic activity at 15 ºC and 45 °C [38].

1.9 Objective of the Study

Given the great potential economic value of lactobacilli, one of the main objectives of microbiologists is to develop a clear picture of the microflora present in the various dairy products, and the way in which it changes during processing. For example, during cheese manufacture and ripening, complex interactions occur between individual components of cheese microflora, and identification of these bacteria is essential for understanding their individual contribution to cheese manufacture. This allows the development of a more targeted approach to starter/adjunct selection for the improvement of cheese quality [79].

In our country in the production of dairy products starter cultures are being used from 1970's. Although in the production of cheese, butter and yoghurt starter cultures are being used, there is not still any commercial starter culture production. In plants using starter cultures, there are problems about culture usage. The most important problem is that, there are only a few researches about suitability of imported cultures to Turkish White cheese. In such cases it is unavoidable to have different quality cheeses in market. In addition to this, different operating procedures, usage of different raw materials and marketing cheeses before completion of ripening period are other problems in Turkey [28]. As a matter of fact, Kaptan and Büyükılıç (1983) analysed 72 white cheeses sold in Ankara considering chemical, sensorial and microbiological aspects and concluded that they are not in White Cheese Standarts [4].

In this study different microorganisms were isolated from traditional cheeses produced from unpasteurized milk without using starter culture. In order to find out best starter culture suitable for Turkish White cheese, by using different combinations white cheeses were produced. Best combination was determined by analysing sensory, microbiological and chemical properties of cheese as pH, acidity, salt, fat, moisture, protein contents during 30 days storage period.

CHAPTER 2

MATERIALS AND METHODS

2.1 Bacterial Strain Isolation

Bacterial strains were isolated from a cheese made from unpasteurized milk without using a starter culture. Cheese was obtained from İncek village. It was çökelek. Its texture and aroma was good but it was a little bit salty.

10 g sample was taken from cheese and homogenized in sterile 90 ml of 0.1 % peptone water. Serial 8 fold dilutions in sterile 0.1 % peptone water were prepared for bacterial isolation. Dilutions were plated on MRS and M 17 Agar containing cycloheximide (50 μg/ml) by pour plating method. Plates were incubated at 30°C for 48h. After activating single colonies in MRS and M 17 Broth at 30°C for 24h single colony isolation is performed by streaking to obtain pure cultures.

2.2 Storage

Single colonies were activated in MRS and M 17 Broth at 30°C for 24h and stored in slant, 20 % glycerol at 1/1 ratio and microbank. Glycerol and microbanks were stored at - 80°C while slants were stored at refrigerator.

2.3 Identification

For identification gram staining, catalase, gas production and coagulation tests were performed. For determination of species API50 CH (BioMérieux) was used. Cultures were also sent to DSMZ (Deutsche Sammlung von

Mikroorganismen und Zellkulturen) for partial 16S rDNA gene sequence analysis.

In DSMZ genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR product was carried out. Purified PCR products were sequenced using the CEQ^{TM}DTCS-Quick Start Kit (Beckmann Coulter). Sequence reactions were electrophoresed using the CEQ^{TM}8000 Genetic Analysis System.

The resulting sequence data from strains was put into the alignment editor ae2, aligned manually and compared with representative 16S rRNA gene sequences of organisms belonging to the *Firmicutes*. For comparison 16S rRNA sequences were obtained from the EMBL data base, RDP or DSMZ's own database.

2.3.1 Gram Staining

Gram staining was performed and slides were examined under light microscope.

2.3.2 Catalase Test

Cultures grown for 24 h on the maintenance medium were tested for catalase production with 3% (w/v) H_2O_2. 1 ml of H_2O_2 solution was poured over the surface of an agar culture. Effervescence caused by the liberation of free oxygen as gas bubbles, indicated the presence of catalase in the culture under test. As positive control E. coli was used.

2.3.3 Gas Production Test

Gas production test was performed with durham tubes. Gas production is defined as the presence of gas in the inverted Durham tube with a

corresponding effervescence produced when the tube is gently shaken. Durham tube containing maintenance broths (either MRS or M17) were inoculated for 48 h at 30˚C.

2.3.4 Coagulation Test

For coagulation test, microorganisms were activated in maintenance broths (MRS or M17) at 30˚C for 24h, centrifuged (15000 rpm / 5min / 4°C) and washed with sterile potassium phosphate buffer (0.01 M/ pH 7) twice in order to remove broth and incubated in 10 % skim milk powder at 30°C for 24h. Curd formation and pH decrease were observed.

2.3.5 Identification of Species

Colonies which are gram positive, catalase negative were chosen. Also curd formation and pH reduction capability of microorganisms were evaluated. After choosing the ones which were thought to be the best quality starter, identification at species level was performed by using API50 CH (BioMérieux). In order to be sure, cultures were also sent to DSMZ for 16S rDNA gene sequence analysis.

2.4 Milk

Raw milk was supplied from Mühye village near Birlik Mahallesi, Çankaya and transported to METU within ice bags and pasteurized at 72°C for 5 min.

2.5 Commercial Starter Culture

A commercial lactic starter culture (for white cheese) of *Lactococcus lactis* subs. *lactis* and *Lactococcus lactis* subs. *cremoris* (Lyofast CMS) was used

for control group. It was provided from Mayasan Ltd. Şti. (İstanbul) in powder form.

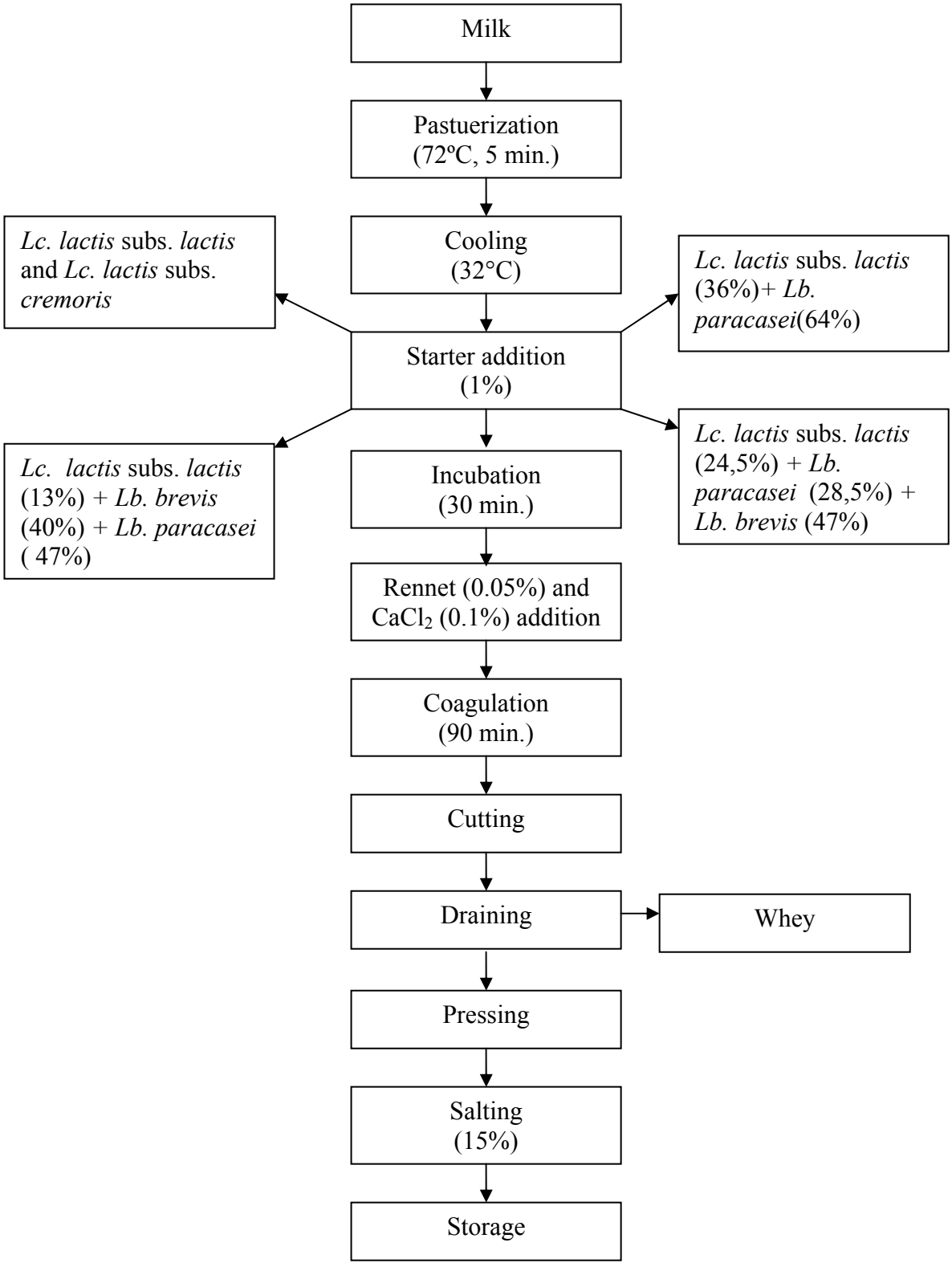
2.6 Rennet Enzyme

Rennet enzyme (Trakya A.Ş.) was used for the coagulation of milk. The power of enzyme is 1/8000. It was added to milk at 0.05% ratio.

2.7 Manufacture of White Cheese

Starter cultures were activated in maintenance broths (either MRS or M 17) at 30˚C for 24h. After activation they were centrifuged (15000 rpm / 5min / 4°C) in order to obtain pellet. Pellet was inoculated in 10% skim milk powder (30˚C, 24h). Milk was pasteurized (72 °C for 5 min), and cooled down to 32°C.. At this temperature, starter culture, activated in skim milk powder, was added at 1% ratio (30 ml starter culture for 3000 ml milk). Four cheeses were produced, one by using commercial starter culture [Lyofast CMS (*Lactococcus lactis* subs. *lactis* and *Lactococcus lactis* subs. *cremoris)* ($1.4x10^{10}$CFU/ml)]as control and the other three by using different combinations of isolates [*Lactococcus lactis* subs. *lactis* ($1.5x10^{8}$ CFU/ml)(13%) + *Lactobacillus brevis* ($4.3x10^{8}$ CFU/ml)(40%) + *Lactobacillus paracasei* (5.2 $x10^{8}$ CFU/ml)(47%); *Lactococcus lactis* subs. *lactis* (36%)+ *Lactobacillus paracasei*(64%)*; Lactococcus lactis* subs. *lactis* (24,5%) + *Lactobacillus paracasei* (28,5%) + *Lactobacillus brevis* (47%)*]*. For the coagulation of the milk and the elimination of the whey, 30 min after the starter culture addition, rennet enzyme (0.05%) and $CaCl_2$ (0.1%), for a firm structure, were added. The milk was coagulated for 90 min. The coagulum was cut and pressed (under a 10 kg weight), overnight cheese was salted in 15 % saline solution. Cheeses were ripened in this solution in plastic containers at 4°C for 30 days.

Table 2.1 Flow Chart of White Cheese Production Using Different Starter Culture Combinations



2.8 Analysis of Cheese

2.8.1 Sensory Analysis

For sensory analysis of cheeses Hedonic Type scale was used [81]. Evaluation was done by a group containing 4 people. Example of the Hedonic Type scale is given below.

Table 2.2 Hedonic Type Scale

Name:…….. Sample No:…….. Date:……

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Notes to specify:……..

2.8.2 Microbiological Analysis

2.8.2.1 Sampling Procedure

10 g sample was taken from cheeses and homogenized in sterile 90 ml of 0.1 % peptone water. Serial 8 fold dilutions in sterile 0.1 % peptone water were prepared for bacterial analysis. Two measurements were carried out and average values are represented.

2.8.2.2 Total Aerobic Count

Plate Count Agar was used for total aerobic count. Plates were incubated at 30°C for 72 h.

2.8.2.3 *Lactobacillus* Enumeration

MRS agar was used for the enumeration of *Lactobacillus*. Plates were incubated at 30°C for 24 h.

2.8.2.4 *Lactococcus* Enumeration

M 17 agar was used for the enumeration of *Lactococcus*. Plates were incubated at 30°C for 24 h.

2.8.2.5 Yeast and Mould Enumeration

Potato Dextrose Agar was used for yeast and mould enumeration. Plates were incubated at 25°C for 5 days.

2.8.2.6 Coliform Bacteria Enumeration

Violet Red Bile Agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 h.

2.8.3 Chemical Analysis

2.8.3.1 Total Solid Content

Total solid content of the cheeses were determined by using oven drying method. The difference in weight before and after drying for 4 hours at 100ºC gives the results of solid content.

2.8.3.2 Fat Content

3 g cheese sample was weighed into a butyrometer vessel and filled with 10 ml H_2SO_4 (d: 1.55 g /cm^3). 1 ml amyl alcohol was added. Butyrometer vessel was completed to the level of 35% with H_2SO_4 solution and centrifuged in Gerber centrifuge for 10 min. The oil level was read from butyrometer vessel.

2.8.3.3 Fat Content in Total Solid

Fat content in total solid was determined by dividing fat content to total solid.

2.8.3.4 Ash Content

Samples were dried in oven for 1 h and burned in ash oven at 550ºC until all black color was disappeared. After cooling in desiccator, they were weighed. The difference in weight before and after burning process gives the ash content.

2.8.3.5 Salt Content

5 g cheese sample was crushed in porcelain mortar with the help of hot distilled water and watery part was transferred into an erlenmayer flask. Same process was repeated 5-6 times to enable all salt transferred to water. Then water level was completed to 500 ml with distilled water at room temperature. 25 ml of this solution was transferred into another erlenmayer flask, 1-2 drops of K_2CrO_4 (5%) was added and titrated with 0.1 N $AgNO_3$ until tile red color was occurred.

$$\% \text{ Salt} : \frac{G \times 0.00585 \times 100}{P}$$

G: Consumed 0.1 N $AgNO_3$ amount (ml)

P: Cheese amount included in titration (0.25 g)

2.8.3.6 Salt Content in Total Solid

Salt content in total solid was determined by dividing salt content to total solid.

2.8.3.7 Acidity

For determination of titratable acidity 3 g cheese was weighed and crushed with 10 ml water in porcelain mortar. This solution was transferred into an erlenmayer flask, 5 drops phenolphthalein was added and titrated with 0.1 N NaOH to the first permanent color change to pink.

$$\% \text{ Acidity} : \frac{\text{NaOH amount} \times 0.009 \times 100}{\text{Cheese amount}} \quad \text{(for 0.1 N NaOH)}$$

2.8.3.8 pH

Results were measured with a pH-meter.

2.8.3.9 Protein Content

Protein content was determined by Kjeldahl method [82].

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Bacterial Identification

After gram staining, all isolates found to be gram positive. In 36 isolate 33 were rod shaped and 3 of them were coccus shaped.

From the rod shaped isolates 3 of them were found to be catalase positive and eliminated.

Only four strains produced gas which means except them all others were homofermantative.

As a result of coagulation test, from 33 isolate 7 of them were chosen. In coagulation test, isolates were inoculated in skim milk powder (10%, 30°C, 24 h) and curd formation and pH decrease were observed in comparison with reference culture (Lyofast CMS). After 24 h incubation period pH of commercial starter culture was 4.70 whereas it was minimum 2.95 for sample number 4 which might be resulted from viable cell count ratio or type of microorganism. For evaluation of starter cultures firm curd structure and pH decrease ratios were used. The pH results are presented in Table 3.1.

Table 3.1 pH decrease during curd formation after 24h

Sample No:	**pH**
Negative Control (no microorganism)	6.03
Positive Control (commercial starter culture)	4.70
1	4.39
2	3.05
3	3.14
4	2.95
5	3.08
6	4.18
7	4.30

3.1.1 Species Identification

3.1.1.1 Identification by API

For this purpose API 50 CH (BioMérieux) was used. Results were evaluated in METU Medical Center by using BioMérieux software.

Table 3.2 The biochemical profile of four strains

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Strain 1	24 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	48 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Strain 2	24 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-
	48 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	+	+	+
Strain 3	24 h	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
	48 h	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Strain 4	24 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-
	48 h	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-
		0	GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	MDG	NAG	AMY	ARB	ESC	SAL	CEL	MAL	LAC	MEL	SAC	TRE	INU	MLZ	RAF	AMD	GLYG	XLT	GEN	TUR	LYX	TAG	DFUC	LFUC	DARL	LARL	GNT	2KG	5KG

Note: + sign shows the usage of sugars.

As can be seen from the tables sugar usage characteristics of microorganisms were different. For strain 2 Arbutine, Melezitose, β Gentiobiose, D-Tagatose, 2 ceto-gluconate and 5 ceto-gluconate fermentations were characteristic as compared with strain 1.

For strain 3 D-Mannose, Amygdaline, Salicine, Cellobiose, Melezitose, β Gentiobiose and 2 ceto-gluconate fermentations were not observed as compared with strain 2.

For strain 4 L-Sorbose, Sorbitol, Inuline and D-Turanose fermentations were characteristic.

The software results are presented in table 3.6.

Table 3.3 Species identification results

Strain No:	**Species**
1	*Lactococcus lactis lactis*
2	*Lactobacillus brevis*
3	*Lactobacillus collinoides*
4	*Lactobacillus paracasei*
5	*Lactobacillus brevis*
6	*Lactobacillus brevis*
7	*Lactobacillus brevis*

Perez et al. indicated that 23,2 % of the isolates were incorrectly identified by API as compared with protein fingerprinting [89]. So in order to be sure, cultures were also sent to DSMZ for 16S rDNA gene sequence analysis.

3.1.1.2 Genetic Identification

Strains 1, 2, 3 and 4 were sent to DSMZ for 16S rDNA analysis. Results were same with API 50CH except for strain 3. According to results of DSMZ strain 3 was found to be *Lactobacillus brevis* not *Lactobacillus collinoides.*

The difference might be resulted from the recent LAB taxanomy. Because API does not take into account the recent progress in LAB taxonomy [89].

3.2 Cheese Manufacture

Each cheese was produced by using 3 liter milk. Four samples were produced, one by using commercial starter culture as control and the other three by using different combinations of isolates. Starter culture combination ratios are presented in Table 3.7.

Table 3.4 Starter culture combination ratios

Sample No:	**Starter culture combination ratios**
1 (Control)	Lyofast CMS (*Lactococcus lactis* subs. *lactis* and *Lactococcus lactis* subs. *cremoris)*
2	*Lactococcus lactis* subs. *lactis** (13%) + *Lactobacillus brevis* (40%) + *Lactobacillus paracasei* (47%)
3	*Lactococcus lactis* subs. *lactis** (36%)+ *Lactobacillus paracasei*(64%)
4	*Lactococcus lactis* subs. *lactis** (24,5%) + *Lactobacillus paracasei* (28,5%) + *Lactobacillus brevis* (47%)

***** *Lactococcus lactis* subs. *lactis* isolated from cheese, not the commercial strain.

The most important role of the starters used during manufacturing of cheese is processing casein derivates, which were hydrolyzed by rennet as big peptides, into smaller peptides and free amino acids by proteinase and peptidases [49].

Studies related to proteinase system of lactobacilli are not as much as lactococcus. However, casein hydrolyzing, cell wall binded proteinases were also determined in lactobacilli as in lactococcus [64].

Hickey et al. (1983) claimed that, *Lactobacillus casei* and *Lactobacillus plantarum* had more proteolytic activity when compared with *Lactococcus lactis subs. cremoris* [84].

3.3 Microbiological Analysis

In order to define the microbiological structure of cheeses during ripening, 4 produced cheeses were analysed in microbiological aspects.

3.3.1 Total Aerobic Count

The maximum number of total aerobic count was found to be $3.1x10^{10}$ (sample 1) and minimum $1.8x10^{7}$ (sample 3) at second day. This number decreased maximum to $2.1x10^{6}$ (sample 4) during 30 days ripening period. This may be explained by the high pH value with the effect of salt and storage temperature.

Table 3.5 Total aerobic count (CFU/g)

Day	**Sample No**			
	1	**2**	**3**	**4**
2	$3.1x10^{10}$	$7.2x10^{9}$	$1.8x10^{7}$	$1.1x10^{9}$
15	$3.6x10^{9}$	$1.5x10^{9}$	$4.5x10^{7}$	$4.5x10^{6}$
30	$8.1x10^{7}$	$6.3x10^{7}$	$9.3x10^{6}$	$2.1x10^{6}$

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods total aerobic count was found to be between $1.3x10^8$- $7.8x10^9$ cfu/g [86].

3.3.2 *Lactobacillus* Enumeration

The maximum number of *Lactobacillus* count was found to be $5.2x10^{10}$ (sample 1) and minimum $1.8x10^7$(sample 3) at second day. This number decreased maximum to $9.0x10^6$ (sample 3) during 30 days ripening period.

Table 3.6 *Lactobacillus* enumeration (CFU/g)

Day	Sample No			
	1	**2**	**3**	**4**
2	$5.2x10^{10}$	$5.4x10^9$	$1.8x10^7$	$4.5x10^9$
15	$2.7x10^8$	$3.2x10^8$	$4.5x10^7$	$2.7x10^7$
30	$9.9x10^7$	$1.1x10^7$	$9.0x10^6$	$2.1x10^7$

Lactobacilli starters are normally present at levels of 10^9 bacteria/g, contribute to the lactic fermentation, and are involved at the beginning of ripening. For example, in Emmental cheeses, lactobacilli ferment galactose excreted by *Streptococcus thermophilus,* achieve acidification process, and contribute to primary proteolysis. Their numbers decrease rapidly during ripening, at a rate depending to some degree on the sensivity of the starters to salt, on the water activity, and on the autolysis power of the strains [83].

3.3.3 *Lactococcus* Enumeration

The maximum number of *Lactococcus* count was found to be $4.6x10^{10}$ (sample 1) and minimum $2.1x10^7$ (sapmle 3) at second day. This number decreased maximum to$1.8x10^6$ (sample 4) during 30 days ripening period.

Table 3.7 *Lactococcus* enumeration (CFU/g)

Day	Sample No			
	1	**2**	**3**	**4**
2	$4.6x10^{10}$	$3.8x10^{9}$	$2.1x10^{7}$	$4.2x10^{8}$
15	$4.1x10^{9}$	$4.5x10^{6}$	$1.4x10^{7}$	$3.6x10^{6}$
30	$4.2x10^{7}$	$7.2x10^{6}$	$9.0x10^{6}$	$1.8x10^{6}$

3.3.4 Mould and Yeast Enumeration

The maximum number of mould and yeast count was found to be $1.1x10^{6}$ (sample 1) and minimum $2.4x10^{5}$ (sample 2) at second day. This number decreased maximum to$1.6x10^{4}$ (sample 4) during 30 days ripening period.

Table 3.8 Mould and yeast enumeration (CFU/g)

Day	Sample No			
	1	**2**	**3**	**4**
2	$1.1x10^{6}$	$2.4x10^{5}$	$3.6x10^{5}$	$9.0x10^{5}$
15	$3.5x10^{4}$	$1.2x10^{4}$	$3.6x10^{4}$	$3.6x10^{4}$
30	$5.4x10^{4}$	$1.8x10^{4}$	$5.0x10^{4}$	$1.6x10^{4}$

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods mould and yeast enumeration was found to be between $1.0x10^{1}$- $2.0x10^{7}$ cfu/g [86].

3.3.5 Coliform Bacteria Enumeration

Coliform was not seen in any sample which shows us there is no contamination.

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods coliform bacteria enumeration was found to be between $1.0x10^1$- $4.2x10^7$ cfu/g [86].

3.4 Chemical Analysis

3.4.1 Total Solid Content

At the end of ripening period total solid content was found maximum 41.60 % (sample 1) and minimum 34.80 % (sample 4). There is an increase in total solid content in sample 2 between 2. and 30. day whereas there is a slight increase in other samples.

Kurdal and Gürtunca (1996) found total solid content in cheeses sold in Bursa between 42.46- 48.76% [85].

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods total solid content was found to be 33.13-38.59 % [86].

Berker (1988) found the total solid content at 1., 15. and 30. days as 45.67, 46.15 and 44.75 % respectively in white cheeses produced with traditional methods [87].

3.4.2 Fat Content

At the end of ripening period fat content was found maximum 22.80 % (sample 1) and minimum 19.25 % (sample 3). In sample 2 and 3 fat content increased slightly during ripening period whereas it decreased in sample 4.

Kurdal and Gürtunca (1996) found fat content in cheeses sold in Bursa between 16.40- 23.30% [85].

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods fat content was found to be 16.04-21.15 % [86].

3.4.3 Fat Content in Total Solid

At the end of ripening period fat content in total solid was found maximum 56.89 % (sample 4) and minimum 53.75 % (sample 3). Because of the increasing solid content there is a decrease in all samples in fat content in total solid.

3.4.4 Ash Content

At the end of ripening period ash content was found maximum 10.34 % (sample 3) and minimum 6.45 % (sample 2). Ash content increased in samples 1 and 3 whereas it decreased in samples 2 and 4.

Kurdal and Gürtunca (1996) found ash content in cheeses sold in Bursa between 4.73- 5.93 % [85].

3.4.5 Salt Content

At the end of ripening period salt content was found maximum 9.82 % (sample 4) and minimum 7.72 % (sample 2 and 3). There is an increase in salt content in samples 1, 2 and 4.

Kurdal and Gürtunca (1996) found salt content in cheeses sold in Bursa between 3.70- 5.46 % [85].

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods salt content was found to be 4.21-5.26 % [86].

Berker (1988) found the salt content at 1., 15. and 30. days as 4.25, 4.60 and 4.29 % respectively in white cheeses produced with traditional methods [87].

3.4.6 Salt Content in Total Solid

At the end of ripening period salt content in total solid was found maximum 28.21 % (sample 4) and minimum 18.95 % (sample 2). With increasing salt content, salt content in total solid also increased.

3.4.7 Acidity

At the end of ripening period acidity was found maximum 0.36 % (sample 1) and minimum 0.12 % (sample 4). There is a decrease in samples 1, 2 and 4.

3.4.8 pH

At the end of ripening period pH was found maximum 4.62 (sample 3) and minimum 4.57 (sample 1 and 2). There is a decrease in pH in all samples during 30 day ripening period.

Tayar (1995) stated that, in white cheeses produced in three different plants with traditional methods pH was found to be between 4.38-5.94 [86].

Berker (1988) found the pH value at 1., 15. and 30. days as 5.1, 4.8, 4.7 respectively in white cheeses produced with traditional methods [87].

3.4.9 Protein Content

At the end of ripening period protein content was found maximum 14.66 % (sample 2) and minimum 13.73 % (sample 3). Protein content decreased in samples 1, 3 and 4 while it increased in sample 2.

Kurdal and Gürtunca (1996) found protein content in cheeses sold in Bursa between 14.73- 16.95 % [85].

Table 3.9 Changes in physicochemical parameters of samples throughout ripening

Samples	Ripening period (days)	Total solid (%)	Fat (%)	Fat in total solid (%)	Ash (%)	Salt (%)	Salt in total solid (%)	Acidity (%)	pH	Protein (%)
1	**2**	**39.06**	**22.80**	**58.37**	**5.88**	**4.68**	**11.98**	**0.60**	**5.21**	**14.78**
	15	**41.05**	**22.65**	**55.17**	**7.31**	**9.82**	**23.92**	**0.39**	**4.98**	**14.07**
	30	**41.60**	**22.80**	**54.80**	**10.00**	**7.95**	**19.11**	**0.36**	**4.57**	**14.50**
2	2	37.70	22.50	59.68	10.71	5.85	15.51	0.36	5.01	13.70
	15	39.12	23.25	59.43	13.63	4.68	11.96	0.18	4.68	14.63
	30	40.73	22.75	55.85	6.45	7.72	18.95	0.18	4.57	14.66
3	**2**	**33.30**	**19.00**	**57.05**	**10.00**	**8.19**	**24.59**	**0.18**	**4.86**	**13.81**
	15	**34.78**	**18.87**	**54.25**	**16.27**	**5.61**	**16.12**	**0.18**	**4.80**	**13.51**
	30	**35.81**	**19.25**	**53.75**	**10.34**	**7.72**	**21.55**	**0.18**	**4.62**	**13.73**
4	2	34.69	21.00	60.53	13.72	6.08	17.52	0.18	4.89	14.85
	15	33.28	19.50	58.59	16.66	5.61	16.85	0.15	4.76	13.72
	30	34.80	19.80	56.89	8.33	9.82	28.21	0.12	4.60	13.95

3.5 Sensory Analysis

Evaluation was done by a group containing 4 people. Texture was evaluated by looking to the hardness and smooth structure of cheeses. Results are given in Table 3.13.

Table 3.10 Sensory analysis of samples

Characteristics	Sample 1	Sample 2	Sample 3	Sample 4
Color	7	7	7	8
Texture	5	9	6	9
Taste and Aroma	7	8	6	8
Strange Taste and Aroma	9 (no)	9 (no)	9 (no)	9 (no)
Salt Content	5 (salty)	6 (a little salty)	4 (salty)	4 (salty)
General Acceptance	6	8	6	8

Note: Maximum grade is 9. Data are the average values of 4 people.

CHAPTER 4

CONCLUSIONS

In this study, the physico-chemical changes occurring in White Cheese and possible effects of starter culture combinations to the ripening period during 30 days storage period were examined. Although there are many studies on starter culture combinations in different types of cheeses, studies specific to Turkish White Cheese were limited.

In this study 4 samples by using different combinations [*Lactococcus lactis* subs. *lactis* + *Lactococcus lactis* subs. *cremoris* (1), *Lactococcus lactis* subs. *lactis* + *Lactobacillus brevis* + *Lactobacillus paracasei* (2), *Lactococcus lactis* subs. *lactis* + *Lactobacillus paracasei* (3), *Lactococcus lactis* subs. *lactis* + *Lactobacillus paracasei* + *Lactobacillus brevis* (4)] were produced and ripened at + 4°C for 30 days. At the 2^{nd}, 15^{th} and 30^{th} days microbiological, physical, chemical and sensory properties were examined. Below mentioned results were obtained.

1) During ripening period there was a decrease in the number of lactic acid bacteria. However decrease in ratio was smaller in sample 3 and 4 which shows that the bacteria used during manufacture of those samples were more resistant to salt and acidity. Although there was an increase in salt content in samples 1, 2 and 4, there was a decrease in salt content in sample 3. The slight decrease in the number of microorganisms in sample 3 may be related with this salt content.

2) In any of the samples no coliform was observed which indicates that the pasteurization was sufficient and no contamination occurred during the manufacturing process.

3) In samples 1 and 2 total solid, fat and protein contents were found to be higher than the others.

4) In samples 3 and 4 acidity development was slower.

5) The highest salt content was found in sample 4.

6) In sample 1 and 3 the ash content was higher than the others at the end of 30 days ripening period.

7) In the sensory analysis samples 2 and 4 were the most appreciated ones.

There are studies recently evaluated the suitability of probiotic cultures as adjunct cultures in various cheeses: *Lb. acidophilus* and *Lb. casei* in Argentinian Fresco cheese, *Lb. paracasei* in cheddar cheeses, and *Lb. acidophilus* in goat's milk cheeses [83].

Tittsler et al. claimed that the homofermentative L. *casei* and L. *plantarum* generally improved flavor, whereas the heterofementative L. *brevis* and L. *fermenti* generally produced undesirable flavors [64]. However according to physico-chemical analysis (total solid, fat and protein content) sample 2 was the nearest one to the sample 1 which was produced by using commercial culture. In sensory analysis sample 2 and 4 were the most appreciated ones which shows that independent from analytical results taste and aroma is also very important in choosing the right starter culture.

In summary, *Lactococcus lactis* subs. *lactis* (13%) + *Lactobacillus brevis* (40%)+ *Lactobacillus paracasei* (47%) combination seems to be the best and can be suggested as an ideal combination for White Cheese production.

REFERENCES

1) http://www.love4cow.com/humanhealth.htm

2) Demirci, M., 1990, Peynirin beslenmedeki yeri ve önemi. Gıda, 15 (5) 285-289.

3) Anonymous, 2000, Süt ve Süt Mamülleri, 8. Beş Yıllık Kalkınma Özel İhtisas Komisyonu Raporu, DPT Müsteşarlığı, Ankara.

4) Anonymous, 1989, TS 591, Beyaz Peynir Standardı, Türk Standartları Enstitüsü, Ankara.

5) Ozkaya, F.D., Xanthopoulos, V., Tunail, N., Litopoulou-Tzanetaki, E., 2001. Technologically important properties of lactic acid bacteria isolates from Beyaz cheese made from raw ewes' milk. J.Appl. Microbiology. 91: 861-870.

6) Madigan M. T., J. M. Martinko, J. Parker (1997) Biology of microorganisms. Prentice Hall International, Inc.

7) Hughes D. B., and D. G. Hoover (1991). Bifidobacteria: Their potential for use in American Dairy Adjuncts. Food Technlogy. 45, 4, 74-83.

8) Stiles M. E. (1996) Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70, 331-345.

9) Sanders M. E. (1999) Probiotics. Food Technol. 53, 67-75.

10) Yaygın H. ve Kılıç, S. 1993. Süt endüstrisinde saf kültür. Altındağ matbaacılık, İzmir, 108 p.

11) Tekinşen, O. C. ve Atasever M. 1994. Süt ürünleri üretiminde starter kültür. Selçuk Üniversitesi Veteriner Fakültesi Yayın Ünitesi, Konya, 150 p.

12) Tunail, N., 1980. Laktik asit bakterilerinin starter olarak seçilmeleri. Kükem Kongresi Bildirisi, Kükem Dergisi, 3; 89-91.

13) Tunail, N. 1978. Starter olarak kullanılan laktik asit bakterileri ile beyaz peynirlerimizden izole edilen bazı bakterilerin önemli fizyolojik özellikleri üzerine araştırmalar. A.Ü. Ziraat Fakültesi, Ziraat Mikrobiyolojisi Kürsüsü, Ankara.

14) Tunail, N., Acar, J. ve Aşkın, O. 1982. Gıdaların mikrobiyolojik özellikler açısından standardizasyonu. Türkiye 3. Gıda Kongresi Bildirisi. Gıda Teknolojisi Derneği Yayın No: 4. 248-261.

15) Yorgancıoğlu, A. 1986. Salamura beyaz peynirlerden N-grup streptokoklar ile D-grup fekal streptokokların izolasyonları, identifikasyonları ve starter olarak önemli özelliklerinin araştırılması. A.Ü. Fen Bil. Enstitüsü, Gıda Bilimleri ve Teknolojisi Anabilim Dalı, Ankara.

16) Üçüncü, M. 1971. Çeşitli starterlerle işlenen beyaz peynirlerin nitelikleri üzerine araştırmalar. Doktora tezi, Ankara, 177 p.

17) Özer, İ., 1964. Türkiye salamura beyaz peynirlerinin olgunlaşmasında rol oynayan laktik asit mikroflorası üzerinde araştırmalar. A.Ü. Veteriner Fakültesi Yayınları, No:170. Ankara Üniversitesi Veteriner ve Ziraat Fakültesi Basımevi, Ankara.

18) Kosikowski, F., 1982. Cheese and Fermented Milk Foods. F.V. Kosikowski and Associates, Newyork, p. 16-17.

19) Gürsel, A., Tunail, N., Gürsoy, A., Ergül, E. ve Aydar, L.Y. 1994. Yerli ve ithal fekal ve laktik grup streptokoklar ile laktobasil içeren starter kombinasyonlarının beyaz peynir üretiminde kullanılması. Kükem Dergisi, 17; (1); 1-14.

20) Tamime, A.Y. 1983. Microbiology of "Starter Cultures". Dairy Microbiology, Volume 2, The Microbiology of Milk Products Ed. Robinson R.K., Applied Science Pubshers Ltd., 113-156, Alsevier Science Publishing CO., INC.

21) Tunail, N. 1999. Biology of *Enterococcus* spp. microflora of the intestine. Academic Press, 1365,1373.

22) Law, B.A. 1984. Flavour development in cheeses. Ch. 7 in Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk. Ed. by Davies, F.L. and Law, B.A. Elsevier Appl. Sci. Publishers, 187-208, London and Newyork.

23) Tunail, N., Demirtaş, D. ve Durlu-Özkaya, F. 2000. Yoğurt fabrikalarında görülen bakteriyofaj problemi ve çözüm önerileri. Süt ve Süt Ürünleri Sempozyumu Tebliğler Kitabı, 146-153, 22-24 Mayıs, Tekirdağ.

24) Durlu-Özkaya, F., Alichanidis, E., Litopoulou-Tzanetaki, E., Tunail, N. 1999. Determination of biogenic amine content of Beyaz cheese and biogenic amine production ability of some lactic acid bacteria. Milchwissenshaft, 54; (12); 680-682.

25) Marshall, V.M.E. and Law, B.A. 1984. The physiology and growth of dairy lactic acid bacteria. Ch. 3 in Advance in the Microbiology and Biochemistry of Cheese and Fermented Milk. Ed. by Davies, F.L. and Law, B.A. Elsevier Appl. Sci. Publishers, 187-207, London and Newyork.

26) Hugenholtz, J., van Sinderen, D., Kok, J. and Konings, W.N. 1987. Cell wall-associated proteases of *Streptococcus cremoris* Wg2. Appl. and Env. Microbiology, 53; (4); 853-859.

27) Coşkun, H., 2000. Peynirlerin olgunlaşmasında starterlerin rolü. Süt Mikrobiyolojisi ve Katkı Maddeleri. VI. Süt ve Süt Ürünleri Sempozyumu Tebliğler Kitabı, 47-56, Tekirdağ.

28) Koçak, C., Yetişmeyen A. ve Atamer, M., 1994, Süt Endüstrisinde Starter Kültürler. Ankara Üniv., Ziraat Fak., Yayınları No:1362, Derleme:62, Ankara.

29) Özenen, Z.L., 1995, Süt sanayinde büyük kolaylık: DVS kültürleri. Dünya Gıda Derg. 2, 27-28.

30) http://www.indiadairy.com/newproducts.html

31) Evrensel, S.S. ve Aynalı, N.,1996. Süt endüstrisinde starter kültür kullanımı. Gıda Sanayi, 47, 31-35.

32) Kirov, N. and Chamakov, K., 1972. A starter for the production of white pickled cheese. Khraniteina Promishlenast. (Abs).21 (2) 31-32.

33) Torres, N. and Chandan R.C., 1981. Latin American White Cheese. A rewiew. J.Dairy Sci., 68, 2160-2164.

34) Kehagias, C., Koulouris, A., Samona, S., Molliou and Kaumoutsos G., 1995. Effect of various starters on the quality of cheese in brine. Food Microbiology, 12, 413-419.

35) Uysal, H., 1996. Değişik miktarlarda kültür kullanılarak üretilen Beyaz peynirlerde proteoliz düzeyi üzerine bir araştırma. Ege Üniv. Ziraat Fak. Derg., 33 (1) 107-114.

36) Davide, C.L., Sarmaga, I.G. and Reforma, C.P., 1994. Characteristics and consumers acceptability of a new soft cheese with yogurt starter bacteria (Abs). Philipline Agriculture, 77 (3), 295-305.

37) Kıvanç, M., Sert, S. and Özdemir, S., 1992. Pastörize sütten starter kullanmadan veya yoğurt kültürü katılarak yapılmış Beyaz peynirlerin mikrobiyolojik analizleri. Gıda Sanayi, 6 (2) 50-59.

38) Giori, G.S., Valdez, G.F., Ruiz Holgado A.P. and Oliver, G., 1985. Effect of pH and temperature on the proteolytic activity of lactic acid bacteria. J. Dairy Sci., 68, 2160-2164.

39) Yılmaztekin, M., 2001. Beyaz peynir üretiminde *Lactobacillus acidophilus* ve *Bifidobacterium bifidum*'dan yararlanma olanakları üzerine bir araştırma. Yüksek Lisans Tezi, Harran Üni. Fen Bilimleri Enstitüsü, Şanlıurfa.

40) Halkman, A.K., 1991. Tarım Mikrobiyolojisi. A.Ü. Zir. Fak. No:1214, 1-10, Ankara.

41) Abd El- Salam, M.E., Alichanidis, E., & Zerfiridis, G.K., 1993. Domiati and Feta type Cheeses. In P. F. Fox (Ed), Cheese; Chemistry, Physics and Microbiology. Vol 2, 301-335.

42) Davies, F. L. 1987. Genetic manipulation of dairy cultures. Dairy Ind. Int. 52:14.

43) Forss, D. A. 1979. Reviews of progress of dairy science: mechanisms of formation of aroma compounds in milk and milk products. J. Dairy Res. 46: 691.

44) Harper, W.J. 1959. Chemistry of cheese flavors. J. Dairy Sci. 42: 207.

45) Marth, E. H. 1963. Microbiological and chemical aspects of Cheddar cheese ripening. A review. J. Dairy Sci. 46:869.

46) Morris, H.A. 1978. Cheese ripening research- trends and perspectives. J. Dairy. Sci. 61: 1198.

47) Reiter, B., T.F. Fryer, M.E. Sharpe and R.C. Lawrence 1966. Studies on cheese flavor. J. Appl. Bacteriol. 29:231.

48) Law, B.A. 1984 Flavor development in cheeses. Page 187 in Advances in the microbiology and biochemistry of cheese and fermented milk. F.L. Davies and B. A. Law. Elsevier Appl. Sci. Publ., London, Engl.

49) Mills. 0. E., and T. D. Thomas. 1981. Nitrogen sources for the growth of lactic streptococci in milk. N.Z. J. Dairy Sci. Tcchnol. 15:43.

50) Mitche.U. *G.* E. 1981. The production of selected compounds in a Swiss-type cheese and their contribution to cheese flavom. Aust. J. Dairy Technol. 3621.

51) Reiter, B., T. F. Fryer, A. Pickeriug, H. R. Chapman, R. C. Lawrence, and M. E. Sharpe. 1967. The effect of the mimbial flora on the flavor and free fatty acid composition of Cheddar cheese. J. Dairy Res. 34:257.

52) Fox, P. F. 1987. *Cheese:* an overview. Page 1 *in* Cheese: chemistry, physics and microbiology. Vol. 1. *Cheese.* P. F. Fox, ed. Elsevier Appl. Sci. Publ., NewYork, *NY.*

53) Fox P. F. 1988-1989. Acceleration of cheese ripening. Food Biotechnol. 2:133.

54) Fox, P. F. 1989. Proteolysis during cheese manufacture and ripening. J.Dairy Sci. 72:1379.

55) Day, E. A., R. Bassette. and M. Keeney. 1960. Identification of volatile carbonyl compounds from Cheddar cheese. J. Dairy Sci. 43:463.

56) Biede, S. L., and E. G. Hammond. 1979. Swiss cheese flavor. I. Chemical analysis. J. Dairy Sci. 62:227.

57) Biede, S. L., and E. G. Hammond. 1979. Swiss cheese flavor. II. Organoleptic analysis. J. Dairy Sci. 62:238.

58) Krett. 0. J. and J. B. Stine. 1951. The role of lowerfatly acids in Swiss cheese. J. Dairy Sci. 34:476.

59) Kristoffersen, T., and I. A. Gould. 1960. Cheddarcheese flavor. II. Changes in flavor quality and ripening products of commercial Cheddar cheese during controllsd curing. J. Dairy Sci. 43:1202.

60) Biede, S. L., P. V. Padsen, E. G. Hammond, and B. A. Glatz. 1979. The flavor of Swiss cheese. Dev. Ind. Microbiol. 20:203.

61) Holzapfel, W.H., Geisen, R., Schillinger, U., 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol. 24, 343– 362.

62) Fox, P. F., J. Law, P.L.H. McSweeney, and J. Wallace. 1993. Biochemistry of cheese ripening. Pages 523–556 *in* Cheese: Chemistry, Physics and Microbiology, Vol. 1. P. F. Fox, ed. Chapman & Hall, London.

63) Fox, P. F., P.L.H. McSweeney, and C. M. Lynch. 1998. Significance on non-starter lactic acid bacteria in Cheddar cheese. Aust J. Dairy Technol. 53:83–89.

64) Khalid, N. M., and E. H. Marth. 1990. Lactobacilli their enzymes and role in ripening and spoilage of cheese. J. Dairy Sci. 73:2669–2684.

65) Chapot-Chartier, M. P. 1996. Autolysis of lactic acid bacteria.Lait 76:91–109.

66) Lortal, S., R. Lemee, and F. Valence. 1997. Autolysis of thermophilic lactobacilli and dairy propionibacteria: a review. Lait 77:133–150.

67) Drake, M. A., T. D. Boylston, K. D. Spence, and B. G. Swanson. 1996. Chemical and sensory effects of a lactobacilus adjunct in Cheddar cheese. Food Res. Int. 29:381–387.

68) El-Soda, M., A. A. Hantira, N. Ezzat, and H. K. El-Shafei. 1992. Accelerated ripening of Ras cheese using freeze-shocked mutant strains of *Lb. casei*. Food Chem. 44:179–184.

69) Johnson, J., M. Etzel, C. Chen, and M. Johnson. 1995. Accelerated ripening of reduced-fat Cheddar cheese using four attenuated *Lactobacillus helveticus* CNRZ-32 adjuncts. J. Dairy Sci. 78:769–776.

70) Madkor, S. M., P. S. Tong, and M. El-Soda. 1998. Effect of added freeze-shocked adjunct lactobacilli on ripeinig indices and flavor development in Cheddar cheese. J. Dairy Sci. 81 (Suppl. 1):27. (Abstr.)

71) El Soda, M. 1993. Control and enhancement of flavor in cheese in microbiology of flavor in cheese. Pages 219–246 *in* Microbiology and Biochemistry of Cheese and Fermented milk. B. Law, ed. Blackie Academic Professional, London.

72) El Soda, M., S. M. Madkor, and P. S. Tong. 1999. Evaluation of commercial adjunct for use in cheese ripening: 1. Enzymatic activities and autolytic properties in buffer system. Milchwissenschaft 54:85–89.

73) Fox, P. F., J. M. Wallace, S. Morgan, C. M. Lynch, E. J. Niland, and J. Tobin. 1996. Acceleration cheese ripening. Antonie van Leeuwenhoek 70:271–297.

74) Abdel Baky, A., A. El-Neshawy, A. Rabie, and M. Ashour. 1986. Heat-shocked lactobacilli for accelerating flavor development of Ras cheese. Food Chem. 21:301–313.

75) Bartels, H., M. Johnson, and N. Olson 1987. Accelerated ripening of Gouda cheese. 1. Effect of heat-shocked thermophilic lactobacilli and streptococci on proteolysis and flavor development.Milchwissenschaft 42:83–88.

76) El-Abboudi, M., S. Pandian, G. Trepanier, R. Simard, and B. Lee. 1991. Heat-shocked lactobacilli for acceleration of Cheddar cheese ripening. J. Food Sci. 56:948–949.

77) Ezzat, N., and H. El-Shafei. 1991. Acceleration ripening of Ras cheese using freeze and heat-shocked *Lactobacillus helveticus*. Egyptian J. Dairy Sci. 19:347–358.

78) Aly, M. 1994. Flavor-enhancement of low-fat kashkaval cheese using heat-or freeze-shocked *Lactobacillus delbrueckii* var. *helveticus* cultures. Die Nahrung 38:504–510.

79) Beresford T.P., Fitzsimons N.A., Brennan N.L., Cogan T.M., Recent advances in cheese microbiology, Int. Dairy J. 11 (2001) 259-274.

80) Varnam, A.H., Sutherland J.P., 1996. Milk and Milk Products. Chapman & Hall, p 276-303.

81) Nelson, J.A. and Trout, G.M., 1951. Judging Dairy Products. The Olsen Publishing Co. Milwaukee 12, Wis, USA. p 480.

82) Kırdar, S., 2001. Süt ve Ürünlerinde Analiz Metotları Uygulama Kılavuzu. Süleyman Demirel Üniversitesi, Isparta.

83) Coeuret, V., Dubernet, S., 2003. Isolation, characterizsation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Lait 83 (2003) 269-306.

84) Hickey, M. V., Hillier, A. J.,1983. Peptidase activities in lactobacilli. Aust. J. Dairy Technol., 38; 118-121.

85) Kurdal, E., Gürtunca, B., 1996. Bursa il merkezinde tüketime sunulan beyaz peynirlerin kimysal ve mikrobiyolojik nitelikleri üzerine araştırma. Süt Tekn. Der., 1(3) 22-27.

86) Tayar, M., 1995. Beyaz peynirlerin olgunlaşma süresince kimyasal ve mikrobiyolojik özelliklerindeki değişmeler. Gıda. 20 (2) 97-101.

87) Berker, A., 1988. Salamura beyaz peynirlerde olgunlaşma esnasında görülen mikrobiyolojik ve kimyasal değişiklikler. Uludağ Üniv. Vet. Fak. Derg. (1-2-3). 93-99.

88) Sneath, H., Bergey's Manual of Determinative Bacteriology-9.Williams and Wilkins.

89) Perez, G., 2000. Protein fingerprinting as a complementary analysis to classical phenotyping for the identification of lactic acid bacteria from Tenerife cheese. Lait 80 (2000) 589-600.

90) Hansen, E., 2002. Commercial bacterial starter cultures for fermented foods of the future. Int. J. Food Micr. 78 (2002) 119-131.

APPENDIXES

APPENDIX A

CHEMICALS AND SUPPLIERS

Chemicals	**Supplier**
MRS Agar	Merck
MRS Broth	Merck
M 17 Agar	Merck
M 17 Broth	Merck
Plate Count Agar	LAB M
Potato Dextrose Agar	LAB M
Violet Red Bile Agar	Difco
Skim Milk Powder	Fluka
Glycerol	Merck
Rennet Enzyme	Trakya Peynir Mayası
Starter Culture	Lyofast
Calcium Chloride	Marmara Industrial Chemicals

APPENDIX B

COMPOSITION OF CULTURE MEDIA

1. MRS Agar

Formula (grams per liter)

Peptone from casein	10.0
Meat extract	10.0
Yeast extract	4.0
D (+) Glucose	20.0
di-Potassium hydrogen phosphate	2.0
Tween 80	1.0
di-Ammonium hydrogen citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.2
Manganese sulfate	0.04
Agar-agar	14.0

2. MRS Broth

Formula (grams per liter)

Peptone from casein	10.0
Meat extract	8.0
Yeast extract	4.0
D (+) Glucose	20.0
di-Potassium hydrogen phosphate	2.0
Tween 80	1.0
di-Ammonium hydrogen citrate	2.0
Sodium acetate	5.0
Magnesium sulfate	0.2
Manganese sulfate	0.04

3. M 17 Agar

Formula (grams per liter)

Peptone from soymeal	5.0
Peptone from meat	2.5
Peptone from casein	2.5
Meat extract	5.0
Yeast extract	2.5
D (+) Lactose	5.0
Ascorbic acid	0.5
Na-β-glycerophosphate	19.0
Magnesium sulfate	0.25
Agar-agar	12.75

4. M 17 Broth

Formula (grams per liter)

Peptone from soymeal	5.0
Peptone from meat	2.5
Peptone from casein	2.5
Meat extract	5.0
Yeast extract	2.5
D (+) Lactose	5.0
Ascorbic acid	0.5
Na-β-glycerophosphate	19.0
Magnesium sulfate	0.25

5. Plate Count Agar

Formula (grams per liter)

Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Agar No. 2	12.0

6. Potato Dextrose Agar

Formula (grams per liter)

Potato extract	4.0
Dextrose	20.0
Agar No. 1	15.0

7. Violet Red Bile Agar

Formula (grams per liter)

Bacto- Yeast extract	3.0
Bacto- Peptone	7.0
Bacto- Bile Salts No.3	1.5
Bacto- Lactose	10.0
Sodium Chloride	5.0
Bacto- Agar	15.0
Bacto- Crystal violet	0.002

8. Skim Milk Powder

Analysis

Water	< 5 %
Ash	< 10 %
Total nitrogen	~ 5.3 %
Lipid content	< 1.5 %
Reducing sugars (as lactose monohydrate)	~ 55 %

APPENDIX C

RESULTS OF SENSORY ANALYSIS

Name: Sanem **Sample No:1** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Name: İrfan **Sample No:1** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: Funda **Sample No:1** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: Sinem **Sample No:1** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	[2-1]
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	[2-1]
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1

Name: Sanem **Sample No:2** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: İrfan **Sample No:2** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Name: Funda **Sample No:2** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Name: Sinem **Sample No:2** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	[7-6]	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1

Name: Sanem **Sample No:3** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1

Name: İrfan **Sample No:3** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: Funda **Sample No:3** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	[5-4-3]	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	[2-1]
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: Sinem **Sample No:3** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Name: Sanem **Sample No:4** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	2-1
General Acceptance	Very good	Good	Average	Not good
	9-8	7-6	5-4-3	2-1

Name: İrfan **Sample No:4** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	[5-4-3]	2-1
General Acceptance	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1

Name: Funda **Sample No:4** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Texture	Very good	Good	Average	Not good
	[9-8]	7-6	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	[9-8]	7-6	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	[2-1]
General Acceptance	Very good	Good	Average	Not good
	9-8	[7-6]	5-4-3	2-1

Name: Sinem **Sample No:4** **Date:10.08.2005**

Color	Very good	Good	Average	Not good
	9-8	7-6	**5-4-3**	2-1
Texture	Very good	Good	Average	Not good
	9-8	**7-6**	5-4-3	2-1
Taste and Aroma	Very good	Good	Average	Not good
	9-8	**7-6**	5-4-3	2-1
Strange Taste and Aroma	No	Very few	Sensible	A lot
	9-8	**7-6**	5-4-3	2-1
Salt Content	Normal	A little salty	Salty	Very much salty
	9-8	7-6	5-4-3	**2-1**
General Acceptance	Very good	Good	Average	Not good
	9-8	**7-6**	5-4-3	2-1

APPENDIX D

RESULTS OF MICROBIOLOGICAL ENUMERATION

Table D.1 Values of replicates for Total Aerobic Count (CFU/g)

Day	Sample No							
	1		2		3		4	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
2	2.9×10^{10}	3.2×10^{10}	7.2×10^{9}	7.3×10^{9}	1.9×10^{7}	1.6×10^{7}	1.2×10^{9}	1.0×10^{9}
15	3.6×10^{9}	3.5×10^{9}	1.3×10^{9}	1.7×10^{9}	4.6×10^{7}	4.4×10^{7}	4.5×10^{6}	4.4×10^{6}
30	8.0×10^{7}	8.2×10^{7}	6.3×10^{7}	6.3×10^{7}	9.2×10^{6}	9.4×10^{6}	2.1×10^{6}	2.1×10^{6}

Table D.2 Values of replicates for *Lactobacillus* enumeration (CFU/g)

Day	Sample No							
	1		2		3		4	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
2	5.0×10^{10}	5.4×10^{10}	5.6×10^{9}	5.2×10^{9}	1.8×10^{7}	1.8×10^{7}	4.6×10^{9}	4.4×10^{9}
15	2.5×10^{8}	2.8×10^{8}	3.1×10^{8}	3.2×10^{8}	4.7×10^{7}	4.3×10^{7}	2.6×10^{7}	2.8×10^{7}
30	9.9×10^{7}	9.8×10^{7}	1.2×10^{7}	1.0×10^{7}	9.1×10^{6}	8.9×10^{6}	2.1×10^{7}	2.1×10^{7}

Table D.3 Values of replicates for *Lactococcus* enumeration (CFU/g)

Day	Sample No							
	1		2		3		4	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
2	4.9×10^{10}	4.2×10^{10}	3.9×10^{9}	3.7×10^{9}	2.0×10^{7}	2.2×10^{7}	4.1×10^{8}	4.3×10^{8}
15	4.0×10^{9}	4.2×10^{9}	4.5×10^{6}	4.4×10^{6}	1.7×10^{7}	1.1×10^{7}	3.6×10^{6}	3.5×10^{6}
30	4.4×10^{7}	4.0×10^{7}	7.2×10^{6}	7.2×10^{6}	9.0×10^{6}	8.9×10^{6}	2.1×10^{6}	1.5×10^{6}

Table D.4 Values of replicates for Mould and Yeast enumeration (CFU/g)

Day	Sample No							
	1		2		3		4	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
2	1.1×10^{6}	1.1×10^{6}	2.4×10^{5}	2.3×10^{5}	3.6×10^{5}	3.5×10^{5}	9.1×10^{5}	8.9×10^{5}
15	3.8×10^{4}	3.2×10^{4}	1.3×10^{4}	1.1×10^{4}	3.6×10^{4}	3.6×10^{4}	3.6×10^{4}	3.5×10^{4}
30	5.3×10^{4}	5.5×10^{4}	1.9×10^{4}	1.7×10^{4}	5.0×10^{4}	4.9×10^{4}	1.5×10^{4}	1.7×10^{4}