INVESTIGATIONS ON AROMA PROFILE OF ARTISANAL YOGHURT STARTER CULTURES

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ABSTRACT

INVESTIGATIONS ON AROMA PROFILE OF ARTISANAL YOGHURT STARTER CULTURES

Yoghurt is an important fermented milk product that is consumed widely around the world as a part of the diet. Yoghurt is produced by lactic acid fermentation using *Streptococcus thermophilus, and Lactobacillus delbrueckii* ssp. *bulgaricus*. The most important criteria in yoghurt production are the selection of artisanal yoghurt starter cultures to achieve the typical aroma and quality of the final product. The typical aroma of yoghurt is identified chiefly by acetaldehyde that is the most important for yoghurt aroma. The aim of this study is to determine artisanal yoghurt starter cultures that provide the most desirable aroma to yoghurt, in order to promote industrial production with such flavor.

In the first part of this study, the influence of different aroma cultures addition on the aroma production properties in yoghurt products was investigated. Nine different aroma cultures were added into the starter culture combinations at two different inoculation ratios. Among all of the combinations, just one yoghurt combination was selected according to the highest acetaldehyde and also the highest sensory scores.

According to the results, two batches of yoghurt were produced: Sample 1 containing only yoghurt starter cultures (*Lactobacillus bulgaricus* Y30 + *Streptococcus thermophilus* Y24), was used as control yoghurt; Sample 2 containing yoghurt starter cultures plus with selected aroma culture (P10), was used as yoghurt with aroma culture. Yoghurt samples were stored at 4 °C for 21 days. The pH, titratable acidity, total solids, fat, protein, syneresis, viscosity, aroma profile, lactic acid and lactose profile, proteolytic activity, microbiological characteristics and sensory characteristics of the samples were investigated during 21 days of storage.

ÖZET

GELENEKSEL YOĞURT STARTER KÜLTÜRLERİNİN AROMA PROFİLİ ÜZERİNE İNCELEMELER

Yoğurt tüm dünyada yaygın olarak tüketilen fermente bir üründür. Yoğurt, süte katılan *Streptococcus thermophilus* ve *Lactobacillus delbrueckii* ssp. *bulgaricus* starter kültürlerinin laktik asit fermentasyonu sonucunda oluşmaktadır. Yoğurt üretiminde en önemli kriter istenilen aroma ve son ürün kalitesini belirleyen yoğurt starter kültürlerinin seçimidir. Yoğurt lezzetinde en önemli bileşik olarak asetadehit gösterilmektedir. Bu çalışmanın amacı, yoğurda en çok istenilen lezzeti veren bileşenlerin incelenmesi ve yoğurdun depolanma süresince yoğurt starter kültürlerinin etkilerinin tespit edilmesidir.

Bu çalışmanın ilk bölümünde yoğurt ürünlerinde aroma üretim özellikleri üzerine katılan farklı aroma kültürlerinin etkisi incelenmiştir. Starter kültür kombinasyonları içerisine iki farklı aşılama oranında, 9 farklı kültür eklenmiştir. Bütün bu kombinasyonlar içerisinde sadece bir yoğurt kombinasyonunda yüksek oranda aroma ve duyusal sonuçlar alınmıştır.

Bu sonuçlara göre, kontrol olarak kullanılan ve sadece yoğurt starter kültürlerinden üretilen (*Lactobacillus bulgaricus Y30* + *Streptococcus thermophilus* Y24) ve kontol yoğurtta kullanılan starter kültürelere ek olarak aroma kültürü (P10) katılmasıyla oluşan 2 grup yoğurt kombinasyonu üretiliştir. Örnekler 21 gün boyunca 4 °C'de depolanmıştır. Örneklerin pH, titre edilebilir asitlik, kuru madde, yağ, protein, su salma, viskozite, uçucu bileşenleri, laktik asit ve laktoz miktar belirlenmesi, proteolitik aktiviteleri, mikrobiyal özellikleri ve duyusal karakteristikleri depolanma süresi boyunca incelenmiştir.

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INTRODUCTION

1.1. Historical Background

Yoghurt is produced from milk by lactic acid fermentation using *Streptococcus thermophilus*, and *Lactobacillus delbrueckii ssp. bulgaricus* that grow synergistically. Lactose is converted into lactic acid by two homofermentative bacteria. They also supply recovery of secondary products which consist of particular flavors of the yoghurt (De Brabandere et al., 1999).

In prehistoric times, yoghurt was discovered by accident in nature. Ancient people did not know fermentation process of yoghurt before yoghurt starter bacteria were discovered. Traditionally, they were making yoghurt from heated milk inoculated with yoghurt from the previous day. The word "yogurt" possibly originated from the Turkish word "jugurt" which first appeared in the 8^{th} century. Yoghurt was first founded in the Middle East by nomadic people who started the fermentation process of the yoghurt. In that region, there was a restricted accessibility of milk because of the desert climate which was as high as 40°C and also because animals were hand-milked. Therefore, cooling of milk was not possible and contamination was inescapable. Under these conditions, it was not possible to preserve milk for a long time. However, fermentation process that included warming the milk over a fire was used as a solution (Trachoo, 2002).

Later, after the refrigeration storage started, people stopped using the old fermentation process in the Middle East. Although the evolution of the yoghurt making process was exactly initiative, the production of yoghurt soon became the established pattern of preservation, and since the early 1900s, defined micro-organisms have been used to prepare these products on a large scale in factories. In the following years, new yoghurt production technologies were developed in many countries while only a few of them had commercial significance. Additionally, yoghurt consumption increased and yoghurt is proved to have health benefits all over the world (Tamime & Robinson, 2007).

1.2. Industrial Manufacture of Yoghurt

Traditionally, yoghurt was made basicly from boiled milk inoculated with the yoghurt from the previous day. Inoculated milk was kept overnight at room temperature. No additional ingredients were added to yoghurt. However, because of today's industrial technology, the process of yoghurt production improved. The traditional and improved process of yoghurt productions are presented with the fundamental steps of manufacturing different types of yoghurts:

I. Preliminary treatment of milk:
   - Filtration: Separation of cellular matter and other contaminants from the milk controlling the existence of antibiotics in the milk that affect the starter bacteria.
   - Milk reception and storage: Temperature of raw milk should be stored about 5°C. Also, quality of milk should be checked and controlled.
   - Standardization of fat and solids-not-fat content: Milk is standardized for the good quality of yoghurt. The solids-not-fat in milk is managed by the standards. Generally, there are some applications for the standardization of milk, such as the removal of part of the fat content from milk, mixing full cream milk with skimmed milk, addition of cream to full fat milk or skimmed milk, and evaporation under vacuum.

II. Homogenization: This treatment decreases the size of fat globules about 1-2µm, by this means, the size reduction is provided regular circulation of milk fat in yoghurt gel. It improves the structure of yoghurt and the stability of the whey separation. The temperature is usually between 55°C and 80°C with the pressure 100-250 bar.

III. Heat treatment: After homogenization, milk is pasteurised under 90-95 °C for about 5-10 minutes using the plate heat exchangers in industrial area (Chandan & O'Rell, 2006). This treatment eliminates pathogens and unwanted microorganisms, inactivates enzymes that denaturate lipid compounds and also inhibits or activates variable production of factors in the direction of yoghurt starter cultures. Afterwards, by the heat treatment, the pH of milk is reduced, whey or serum proteins such as β-lactoglobulin and α-lactalbumin are denatured, and hydrophilicity of casein is increased (Tamime & Robinson, 2007). Consequently, heat treatment affects many factors during yoghurt production.

IV. Inoculation and incubation: The heat-treated milk is cooled to 40-45 °C optimum temperature and after that it is inoculated with the mixed starter culture (*S. thermophilus* and *L. bulgaricus*). The starter culture is activated and the inoculation ratio between the rods and the cocci is well balanced (1:1). The inoculation amount differs between the percentages of 0.5% and 5%, but optimum amount is 2%. Milk is put into suitable containers, then inoculated with starter cultures mix, and finally incubated at 42-43°C for about 3-4 hours. A decrease in pH at 4.5-4.6 is commonly achieved at the end of the fermentation process.

V. Cooling and storage: At the end of the incubation time, yoghurt products are not put in the cold storage right away, because starter cutures keep growing. Generally, yoghurt products are cooled to 5ºC or 10ºC just after fermentation and stored until their distribution to the market. Finally, they are stored at 4ºC between 1-2 days to improve well texture and aroma. Lee and Lucey (2010) presented these fundamental steps of the improved process of yoghurt production (see Figure 1.1.)

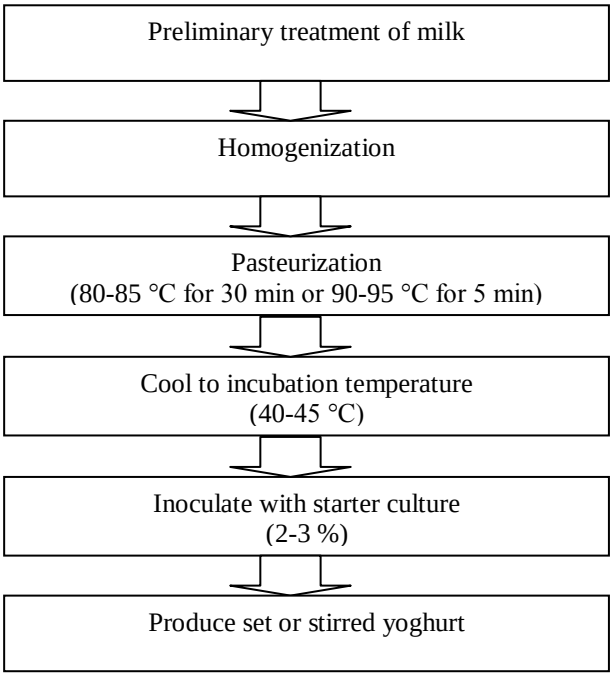

Figure 1.1. Generalized process of improved process as set or stirred yoghurt production. (Source: Lee & Lucey, 2010.)

1.3. The Classification of Yoghurt

Yoghurt is classified into various types such as physical (set, stirred, drinking), chemical (full, medium, low fat), and flavor (plain, fruit, flavored) compositions. Other types can be classified according to manufacturing method such as strained, dried, frozen and constantly pasteurized that are located in the commercial processes (Tharmaraj & Shah, 2003). Set-yoghurt is fermented in a retail container, however for stirred yoghurt fermentation occurs in tanks and the gel is broken before cooling and packaging by stirring. For example, "Ayran" is the stirred yoghurt of low viscosity. Plain yoghurt is traditional type. Fruit yoghurts are obtained by the addition of fruit particles, and, lastly, flavored yoghurt is manufactured by the addition of sweetening and coloring compounds. These classifications are presented at Figure 1.2.

Moreover, in recent years, the consumption of probiotic yoghurt has increased because of the health benefit effects of it on the immune and digestive systems. Probiotic yoghurt consists of positive correlation between two starter cultures as *Lactobacillus acidophilus* and *Bifidobacterium* spp. that are generally (Hassan & Frank, 2001).

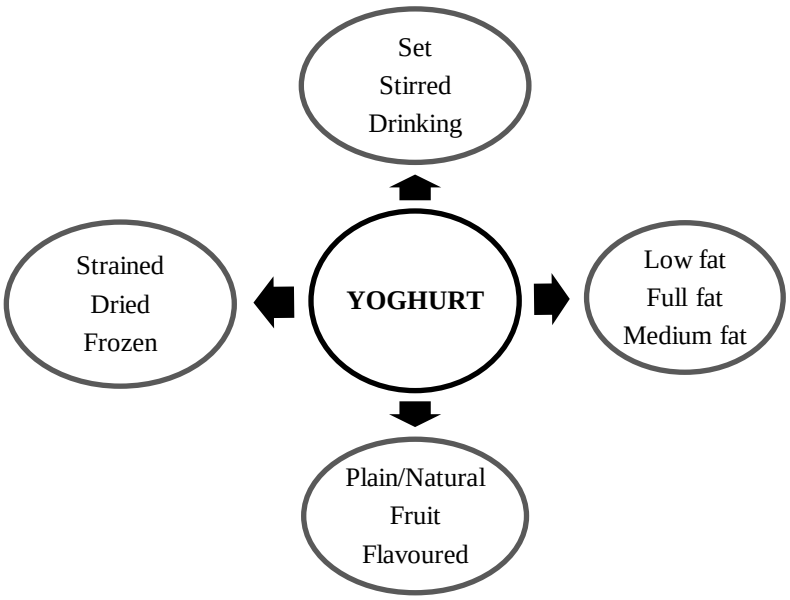

Figure 1.2. The classification of various yoghurt products
(Source: Tamime & Robinson, 2007).

1.4. Yoghurt Starter Cultures

A starter culture can be described as microbial preparation of active cells at least one microorganism added during a fermented dairy production to start attractive alterations (Hassan & Frank, 2001). Starter cultures form the desired body, texture, and flavor in the fermented dairy product and also determine the shelf life (Vedamuthu, 2006).

Yoghurt starter cultures that include lactic acid bacteria (LAB) occupy a central role in the production of yoghurt. They cause rapid acidification through the production of organic acids, alteration of lactose into lactic acid, foundation of texture by the production of exopolysaccharides, and the development of the typical yoghurt aroma (Leroy & De Vuyst, 2004).

Generally, LABs are characterized as the two thermophilic starter bacteria groups: *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. These species are very well known starter microorganisms of yoghurt fermentation. These two lactic acid bacteria strains grow protocooperatively in milk, raise the acidity, coagulate milk proteins, and produce particular yoghurt aroma compounds. Furthermore, yoghurt production can benefit from the use of other starter cultures called adjuncts. They are not essential for the technological processes in themselves, but they are selected to enhance aroma production. Also, they are called particularly flavoring cultures (Coppola et al., 2008).

In literature, some yoghurt products may involve a mixture of different starter cultures. Different starter culture mixes occur such as *L. helveticus, L. jugurti, L. acidophilus* and *Bifidobacterium* spp. for different yoghurt products (Trachoo, 2002). For example, whilst dahi in India is produced using a mixed starter culture containing *S. thermophilus, Lactococcus lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *cremoris* (Tamime & Marshall, 1997).

Table 1.1. Lactic acid bacteria involved in production of fermented milks
(Source: Duboc & Mollet, 2001)

Bacteria	**Various fermented milk products**
Lactobacilli	
L. delbrueckii subsp. delbrueckii	Fermentedmilk drinks, yoghurt
L. delbrueckii subsp. lactis	
L. delbrueckii subsp. bulgaricus	Yoghurt, Bulgarian buttermilk, mozzarella
L. helveticus	Kefir, koumiss, mozzarella
L. acidophilus	Acidophilus milk, kefir
L. johnsonii	Probiotic yoghurt, fermented milk drinks
L. casei	
L. paracasei	Kefir
L. rhamnosus	
L. plantarum	
Streptococci	Yoghurt, dahi, mozzarella
S. thermophilus	
Lactococci	Cultured buttermilk, kefir, dahi
L. lactis subsp. lactis	
L. lactis subsp. cremoris	
L. lactis biovar diacetylactis	

1.4.1. *Lactobacillus bulgaricus*

L. bulgaricus ferments fewer sugars such as glucose, fructose, galactose and lactose to lactic acid and constructs acetaldehyde from lactose in milk, and some strains that produce exopolysaccharide (EPS). This organism produces D (-) - lactic acid up to 1.7% in milk and has an optimum growth temperature of 42-45 ºC (Trachoo, 2002). The average size of *L. bulgaricus* is about 0.5-0.8 μm x 2.0-9.0 μm in diameter, non motile, short rods with rounded ends, and it is in short chains (see Figure 1.3). The cell wall peptidoglycan type is Lys-D-Asp.

L. bulgaricus is more proteolytic than *S. thermophilus* and milk provides a range of amino acids that stimulate the growth of *S. thermophilus* strains. *Lactobacillus bulgaricus* ferment lactose and possesses β-galactosidase enzyme rather than phospho-β-galactosidase.

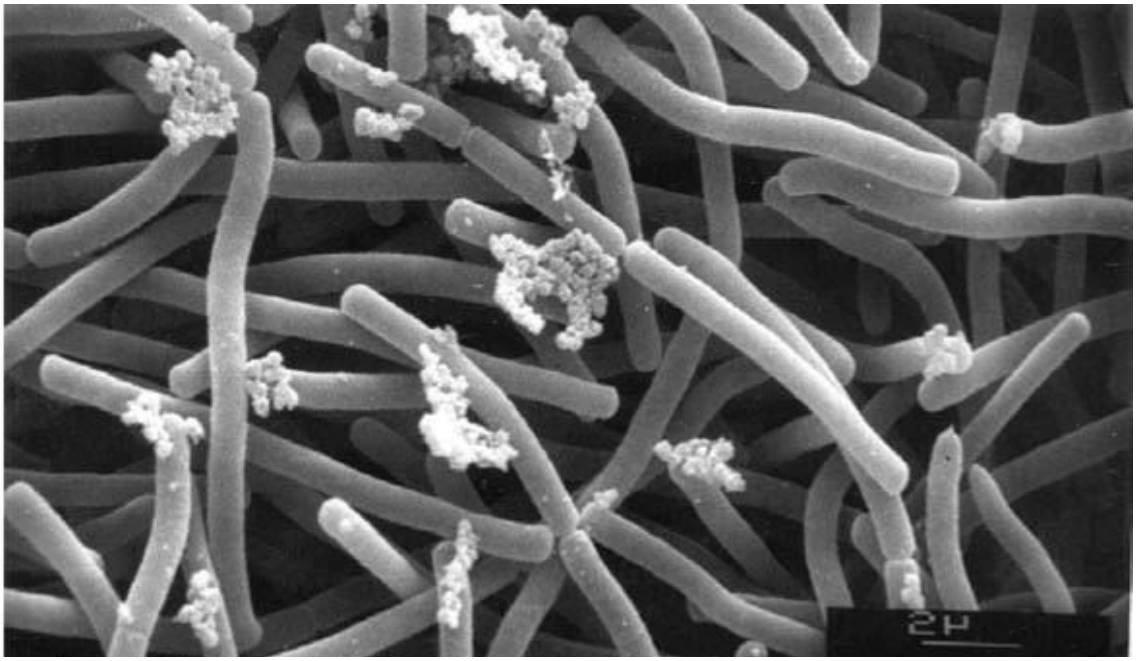

Figure 1.3. An illustration showing the microbial cell morphology of *L. bulgaricus.* (Source: Tamime & Robinson, 2007)

1.4.2. *Streptococcus thermophilus*

S. thermophilus can ferment glucose, fructose, lactose and saccharose from lactose in milk. It also produces L (+)-lactic acid (0.7-0.8 %), acetaldehyde, and diacetyl from lactose in milk. This organism is gram-positive and anaerobic homofermentative lactic acid. A number of strains involve B vitamins and several amino acids and produce EPS. Although it shows susceptibility to antibiotics, it can grow in the existence of bile salts. This organism is thermotolerant and the optimum growth temperature for *S. thermophilus* varies between 37-42 °C. The average size of *S. thermophilus* is about 0.7-0.9 μm in diameter. It is non motile, spherical, short-rod shaped (see Figure 1.4). It occurs

in pairs and chains. The cell wall peptidoglycan type is Lys-Ala2-3 (Rasic & Kurmann, 1978).

In general, the coagula in milk of *S. thermophilus* cultures are weak due to low acid production and urease activity. Urea is divided into carbondioxide by the enzyme urease.

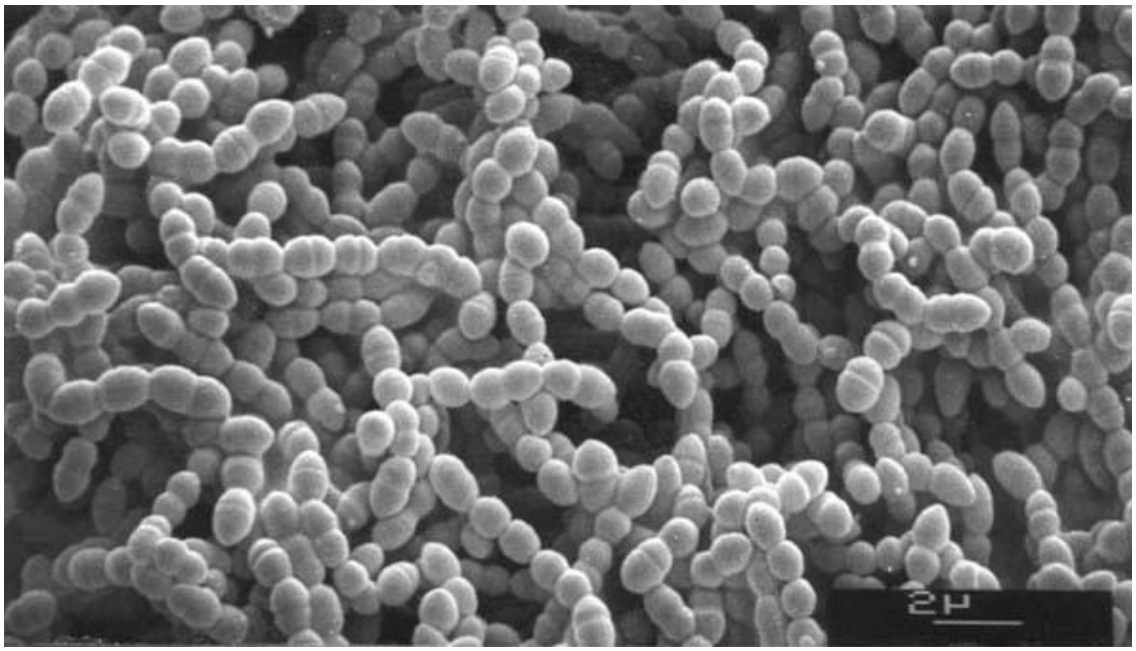

Figure 1.4. Illustrated the cell morphology of *S. thermophilus.*
(Source: Tamime & Robinson, 2007)

1.5. Fermentation of Yoghurt

Yoghurt is produced with a mixed culture of *S. thermophilus* and *L. bulgaricus* in a 1:1 ratio. The growth association between the two organisms was first reported by Orla-Jensen (1931). This relationship among the yoghurt starter cultures used to be named as symbiosis. *S. thermophilus* strains are normally used in association with *L. bulgaricus*. The combination of the two lactic acid bacteria is called "rod & coccus." The coccus is primarily responsible for the initial acid production at a higher rate than that produced when growing alone. Therefore, coccus grows faster than the rod. Yoghurt fermentation occurs at two parts:

- First, the growth of *S. thermophilus* is induced by proteolytic activity of *L. bulgaricus*. *L.* bulgaricus grows up gradually due to its microaerophilic at the end of the first part. The high lactic acid concentration increases during the growth of *S. thermophilus*. Accordingly, this growth slows down (Vedamuthu, 1991).
- Second part initiates when *S. thermophilus* produces enough formic acid. This synergistic effect between two starter cultures occurs after desirable acidity of final yoghurt fermentation (Hamdan & Kunsman, 1971). According to Rasic and Kurmann (1978), when yoghurt starter cultures are used as single strains, lactic acid and acetaldehyde production is lower than mixed cultures. Additionally, *L. bulgaricus* has more proteolytic enzymes than *S. thermophilus*.

Tamime et al. (1977) reported that the activity of *S. thermophilus* and *L. bulgaricus* shows difference in the rate of acid development between the mixed starter and the single strains. The rate of acid development is greater when mixed yoghurt cultures of *S. thermophilus* and *L. bulgaricus* are used compared with the single strains (see Figure 1.5).

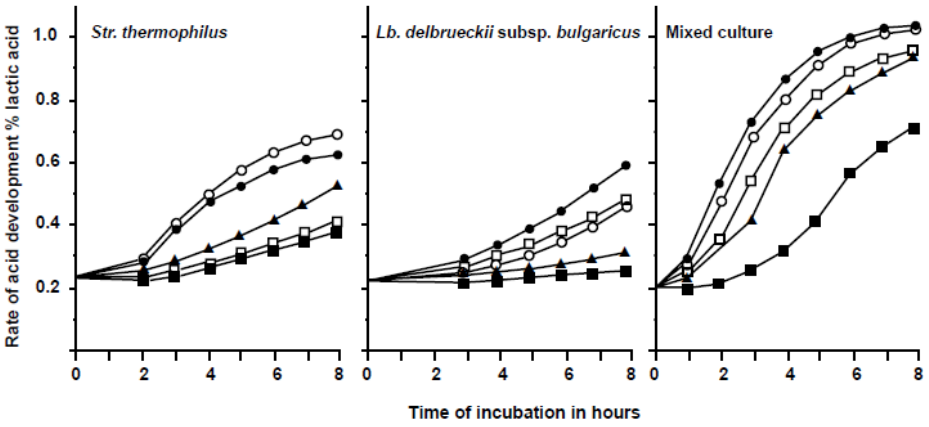

Figure 1.5. Comparison of rates of acid development of mixed and single cultures during incubation hours (Source: Tamime & Robinson, 2007).

1.6. Starter Functions in Yoghurt Fermentation

The major functions of starters during the yoghurt fermentation are listed in order of lactic acid production, proteolytic activity, aroma production, and exopolysaccharide production (Vedamuthu, 2006). These are presented below with many details.

1.6.1. Lactic Acid Production

Lactic acid production is the most important function of starters from lactose in milk during fermentation. Lactic acid initiates some reactions such as aroma production and texture formation. Lactic acid is produced by homofermentative and heterofermentative ways in D (-) and L (+) forms. During the fermentation of yoghurt, *S. thermophilus* grows faster than *L. bulgaricus*. Therefore, L (+) lactic acid is produced first hour fermentation followed by D (-) lactic acid. Lactic acid concentration of yoghurt usually contains 45–60% L (+) lactic acid and 40–55% D (-) lactic acid. The ratio of L(+) : D(-) lactic acid could be utilized to evaluate the quality of yoghurt (Tamime & Robinson, 2007).

Lactic acid production affects coagulation of milk beginning at pH 5–5.2. At this point, the pH decreases under the isoelectric point of casein, casein micelles first become unstable by converting calcium and phosphate complex, and continue until forming curd and yoghurt gel at pH 4.6–4.7 (Tamime & Robinson, 2007).

The characteristic aroma of yoghurt that is sharp and acidic is provided by the lactic acid production during the growth of *S. thermophilus and L. bulgaricus*. Garvie (1978) and Hemme et al. (1981) stated that *S. thermophilus* possesses enzyme lactate dehydrogenase (LDH) and produces mainly L (+) lactic acid. Moreover, *L. bulgaricus* produces D (-) lactic acid (Gasser et al., 1970; Kandler, 1983). Thus, both lactic acid isomers are concurrently produced by starters in yoghurt. Lactic acid also has a preservative effect as of inhibitory to many spoilage and pathogenic bacteria, and the decreased pH is an additional stabilizing factor (Chandan & O'Rell, 2006).

1.6.2. Proteolytic Activity

The starter cultures are responsible for proteolysis which is the hydrolysis of milk proteins (caseins and serum proteins). Mostly, *L. bulgaricus* appears to be capable of the proteolytic system, but *S. thermophilus* exhibits very weak proteolysis in milk. Therefore, proteolytic system of *L. bulgaricus* arouses the growth of *S. thermophilus* and releases the peptides from the caseins and serum proteins. The proteolytic activity of the two yogurt bacteria may be important for impact flavor compounds, the physical structure of yoghurt, and composition (Wouters et al., 2002).

On the other hand, starter cultures can not produce essential free amino acids. Therefore, they obtain suitable sources of nitrogen and carbon from milk proteins and peptides in their growth medium. Generally, the free amino acid amount of cow's milk generally does not exceed 10 mg 100 ml^{-1} (Zourari et al., 1992).

Although yoghurt starter cultures show weakly proteolytic activity during fermentation, they cause favorably hydrolysis of milk proteins in yoghurt. Proteins and peptides in yoghurt are digested by proteolytic activity of starter cultures that can lead to the development of bitterness. *L. bulgaricus* produces bitter peptides from casein as a result of extensive protein degradation by proteolysis (Tamime & Robinson, 2007).

1.6.3. Production of Flavor Compounds

Yoghurt starter cultures have an important role for the production of flavor compounds effecting special yoghurt aroma. Flavor compounds can derive from conversions of lactose, caseins (milk proteins), amino acids, lipids, citric acid, and free fatty acids. These compounds are divided into the following four types: non-volatile acids (lactic, pyruvic, oxalic or succinic), volatile acids (butyric, acetic, formic or propionic), carbonyl compounds (acetaldehyde, acetone, diacetyl or acetoin) and various compounds (amino acids) (Tamime & Robinson, 2007). Whole aroma and flavor of yoghurt are basically due to the production of carbonyl compounds are shown in Table 1.2. Yoghurt flavor consists of acetaldehyde, acetone, acetoin and diacetyl. The typical yoghurt flavor compound is acetaldehyde which is a key carbonyl compound (Trachoo, 2002). High level of acetaldehyde is important because it generates typical and desirable natural yoghurt for consumers. This compound is formed of the protein degradation which is

produced from threonine converted into glycine by threonine aldolase enzyme (Chandan & O'Rell, 2006).

Mixed cultures produce greater amount of acetaldehyde due to the protocooperative growth of the yoghurt starter cultures. Single culture of *L.bulgaricus* plays the most important role for high level of acetaldehyde production. A summary of these results can be seen in Table 1.2.

Table 1.2. Production of carbonyl compounds (μg/g) by yoghurt starter cultures (Source: Tamime & Robinson, 2007; Routray & Mishra, 2011)

Organism	Acetaldehyde	Acetone	Acetoin	Diacetyl
S. thermophilus	1.0–13.5	0.2–5.2	1.5–7.0	0.1–13.0
Lb. delbruekii subsp. bulgaricus	1.4–77.5	0.3–3.2	Trace–2.0	0.5–13.0
Mixed cultures	2.0–41.0	1.3–4.0	2.2–5.7	0.4–0.9

Even though, *L. bulgaricus* has a more important role, the protocooperative growth of the yoghurt starter cultures impact the amount of acetaldehyde that is greater in mixed cultures (Carminati et al., 2010).

1.6.4. Production of Exopolysaccharides (EPSs)

EPSs are long-chain polysaccharides consisting of branched, repeating units of sugars (Welman et al., 2003). They can be classified into two groups: (1) Homopolysaccharides composed of one monosaccharide such as dextran, α- D - glucans, β - D - glucans and fructans, (2) heteropolysaccharides composed of different types of monosacharides such as D-glucose, D-galactose, rhamnose, and mannose (Duboc et al., 2001; Welman et al., 2003). In general, the amount of EPS production can increase up to 40 mg 100 ml^{-1} (Cerning, 1995).

Exopolysaccharides are produced by yoghurt starter cultures. They provide special body and the smooth texture in yoghurt. They are economically important in that

they give functional and beneficial health effects on yoghurt (Welman et al., 2003). EPS-producing starters have many factors that affect the texture of yoghurt, such as the growth medium, the temperature, the level of acidity in the growth medium and the strain variation (Tamime & Robinson, 2007). They can also decrease susceptibility for syneresis during yoghurt storage. EPS-producing lactic acid cultures shows higher viscosity and lower degree of syneresis compared with non-EPS-producing cultures (Bouzar et al., 1996; Folkenberg et al., 2005).

Starter cultures including EPS producers affect texture and stability of yoghurt products. There is a high consumer demand for smooth and creamy texture of yoghurt products in yoghurt industry. EPSs can be used alternatively in industry for this reason. Furthermore, even though EPS materials do not produce taste by themselves, EPSs from starters impart an improved perception of taste in the yoghurt product (Duboc et al., 2001).

1.7. Yoghurt Aroma

1.7.1. Aroma Compounds

Yoghurt aroma is the most important factor determining the acceptance and preference by consumers in yoghurt industry. It basically occurs through the production of non-volatile or volatile acids and carbonyl compounds (Tamime & Robinson, 2007). More than 90 different aroma compounds, such as carbohydrates, alcohols, aldehydes, ketones, acids, esters have been classified in yoghurt (Ott et al., 1997) (see Table 1.3). Particularly, carbonyl compounds have important roles for the final and desired yoghurt aroma due to their high amount (Kaminarides et al., 2007). Aroma compounds are carbonyl compounds such as acetaldehyde, ethanol, acetone, acetoin, diacetyl and 2-butanone. However, among them, acetaldehyde is considered as the major flavor compound for the typical yoghurt aroma (Ott et al., 1997; Beshkova et al., 1998).

Furthermore, in the literature, yoghurt aroma has a balance between the important volatile compounds. For instance, the ratio between acetaldehyde and diacetyl is 1:1, which gives a typical yogurt flavor (Bottazzi & Dellaglio, 1967). Another balanced ratio among acetaldehyde and acetone is 2.8:1, which plays an important role in yogurt aroma. This ratio results in the desired “fullness” flavor. While there is a lot of discussion on

these volatiles' concentration which are needed to produce an optimal yoghurt flavor, literature has not provided an agreed opinion (Boelrijk et al., 2003).

Table 1.3. List of aroma compounds that have been identified in plain yoghurt (Source: Cheng, 2010)

Carbonyl compounds	***Alcohols***	***Sulfur compounds***	***Heterocyclic compounds***
Acetaldehyde	Methanol	Dimethyl sulfide	Furan
Acetone	Ethanol	Methional	Furfural
Propanal	1-Propanol	***Nitrogen compounds***	Pyrazine
2-Propanone	2-Propanol	N,N-dimethylformamide	Pyrrole
Butanal	1-Butanol	Lactamide	Benzothiazole
2-Butanone	2-Butanol	N-ethyl-benzenamine	Furfuralcohol
Diacetyl	Cyclobutanol	***Hydrocarbons***	***Terpene***
Acetoin	1-Pentanol	Heptane	L-limonene
Pentanal	***Acids***	Methylcyclohexane	***Esters***
Hexanal	Acetic acid	Nonane	Methyl formate
Heptanal	Propionic acid	***Aromatic compounds***	Methyl acetate
Octanal	Butyric acid	Benzene	Ethyl acetate
Nonanal	Pentanoic acid	Toluene	Butyl acetate
Decanal	Isovaleric acid	Ethylbenzene	Diehyl phthalate
Benzaldehyde	Hexanoic acid	Propylbenzene	

1.7.2. Flavor Formation

The formation of flavors occurs by different metabolic pathways through the specific enzymes. Flavor formation in yoghurt occurs in three main pathways involved in glycolysis (the conversion of lactose and citrate), lipolysis and proteolysis that Figure 1.6 is modified from Van Hylckama Vlieg et al. (2007).

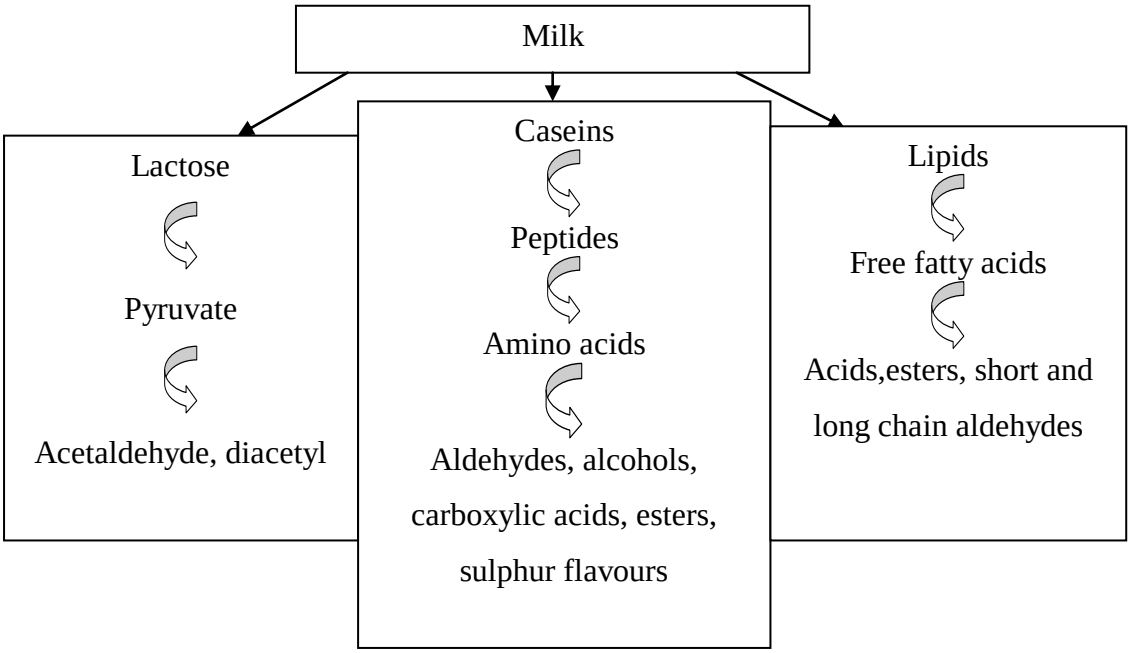

Figure 1.6. Flavor formation steps in fermented dairy products. The three main pathways involved in flavor formation in fermented foods are glycolysis, lipolysis and proteolysis. (Source: Cheng, 2010)

The first major pathway glycolysis is the transformations of lactose, lactate and citrate by the starter cultures such as *Lactococci* and *Lactobacilli*. This pathway produces major aromatic compounds in yoghurt that is carbonyl compounds such as acetaldehyde, diacetyl, acetoin, and ethanol. Another pathway is proteolysis that is conversion of caseins to large peptides by protease enzymes. Peptides with an undesired such as bitter taste may accumulate and these may be effectively removed by peptidases. Finally, volatile flavour compounds are produced from amino acids by amino acid convertases (Van Hylckama Vlieg et al., 2007). Another major pathway is lipolysis which is caused by the formation of free fatty acids. Short-chain fatty acids are strong precursors of flavor compounds such as odd-carbon methylketones, secondary alcohols, esters and lactones (Cheng, 2010).

Acetaldehyde, (CH_3CHO), which is an organic volatile compound is the most essential flavor of yoghurt. It gives fruity like green apple or nutty flavor in yoghurt due to the symbiotic growth both *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Bottazzi & Dellaglio, 1967; Ott et al., 1997).

Additionally, acetaldehyde was firstly investigated by Pette and Lolkema (1950). They claimed that acetaldehyde is a major compound of yoghurt aroma. Later, Hamdan et

al. (1971) indicated that “high concentrations of acetaldehyde are necessary to produce a desirable flavor in yogurt”. In the literature, good flavored yoghurt results are showed as desirable levels 23–40 mg/kg and at least 8–10 mg/kg of acetaldehyde (Ott et al., 2000).

Acetaldehyde can be derived from several precursors such as glucose, pyruvate, amino acids (threonine and glycine), and nucleotides (DNA) (see Figure 1.7). The possible metabolic pathways for acetaldehyde production are defined in the following steps: (Tamime & Robinson 2007).

I. Pyruvate is converted to Acetyl-Coenzyme A by the pyruvate formate lyase or the pyruvate dehydrogenase.
II. Acetaldehyde can be produced by acetate conversion as part of the acetate utilization.
III. The most important pathway for acetaldehyde production is appeared by activity of threonine aldolase (TA), which converts threonine to acetaldehyde and glycine. Threonin aldolase enzyme activity has been detected in yoghurt starter cultures both *L. bulgaricus* and *S. thermophilus*. When the growth temperature reaches from 30◦C to 42◦C, threonine aldolase activity decreases in *S. thermophilus*. But, enzyme activity remains identical in *L. bulgaricus*. As yoghurt is manufactured at a higher temperature, it is expected to be mainly produced by *L. bulgaricus* in view of the fact that *L. bulgaricus* probably produce acetaldehyde. Furthermore, acetaldehyde production during fermentation could be associated to the threonine aldolase activity of serine hydroxymethyltransferase enzyme, because this enzyme possesses threonine aldolase activity for acetaldehyde production (Chaves et al., 2002).
IV. DNA to 2- deoxyribose-5-phosphate is degraded by deoxyribose aldolase enzyme. 2- deoxyribose-5-phosphate is further broken down to acetaldehyde.

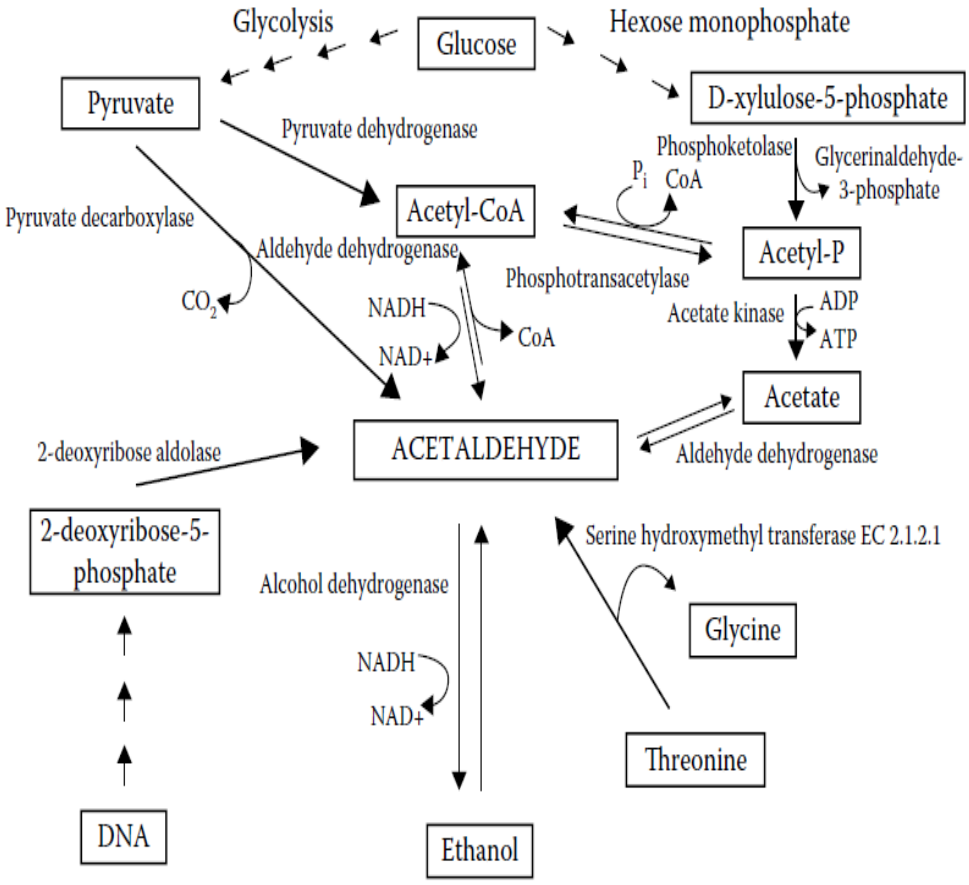

Figure 1.7. Different metabolic pathways of acetaldehyde production
(Source: Chaves et al., 2002)

Diacetyl, (2,3-butanedione, CH3COCOCH3), is a diketone which is an essential flavor compound for yoghurt products. It can be defined as buttery aroma and taste. It can be produced by fermentation of citric acid to pyruvate in milk by specific citrate-utilizing lactic acid bacteria (Vedamuthu, 2006). The precursor for diacetyl is further pyruvate. It was found that diacetyl ranged from 0.2 mg/kg to 3 mg/kg for the typical flavor and aroma of yoghurt (Rasic & Kurmann, 1978; Georgala et al., 1995; Ulberth, 1991). *S. thermophilus* strains are responsible for the formation of diacetyl in yoghurt, but also in some studies, *L. bulgaricus* is founded as a producer of diacetyl. Diacetyl production is shown in Figure 1.8 as fermentation of citrate.

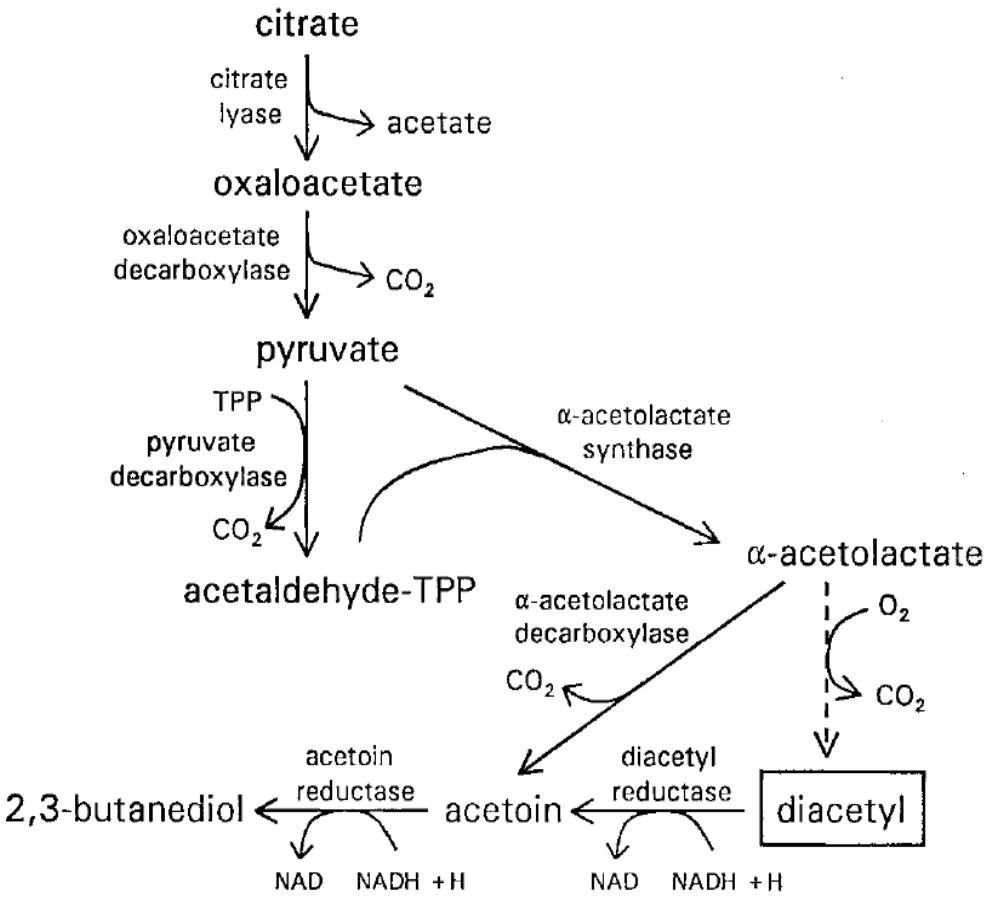

Figure 1.8. Pathway for the transformation of citrate to diacetyl
(Source: Marsili, 2002).

Acetoin ($C_4H_8O_2$) is a common flavor in dairy products. It gives a mild creamy and buttery flavor along with diacetyl. Furthermore, acetoin is significantly weaker than diacetyl flavor, thus it leads to decrease sharpness flavor of diacetyl (Cheng, 2011).

Butanone ($CH_3COC_2H_5$) is a ketone (methyl ethyl ketone (MEK). It has a sharp and sweet odor and it gives sweaty taste in yoghurt aroma. Acetone is also known as CH_3COCH_3. It gives a sweet, fruity aroma to effect the aroma and flavor qualities of yoghurt (Georgala et al., 1995).

Moreover, these volatile compounds change in yoghurt during storage. The amount of acetaldehyde decreased during storage. Also, during yoghurt production, the level of acetaldehyde becomes at level of acidification pH 5.0, then reaches a maximum at pH 4.2 and stabilizes at pH 4.0 (Hruskar et al., 2005).

Several aroma compounds can be changed in yoghurt during storage. These are summarized as follows:

1. The concentrations of acetaldehyde and diacetyl declined in sheep's milk yoghurt. However, acetone and ethanol amounts did not change during the fermentation hours and shelf life of yoghurt (Tamime & Robinson, 2007).
2. The concentration of acetaldehyde decreased in yoghurts during 10 days of storage at 4 °C, but the diacetyl and ethanol contents increased (Hruskar et al., 2005).

1.7.3. Analysis of the Volatile Compounds

Gas chromatography (GC) is used for the identification and quantification of volatile compounds in yoghurt. Volatiles can be detected by a variety of detectors including flame ionization (FID), thermal conductivity (TCD), electron capture (ECD), flame photometric (FPD), photoionization (PID), and mass spectrometry (MS) (Fisher & Scott, 1997). Rapid measurement techniques for volatiles, such as steam distillation, direct extraction, simultaneous steam distillation and solvent extraction static headspace and dynamic headspace/purge and trap (Beshkova et al., 1998; Cheng, 2010). Also, flavor profiling of yoghurt products according to the procedure submitted by Ulberth (1991) and Ott et al. (1999) used rapid method as static headspace gas chromatography (HS-GC) sampling to quantify diketones in acidic and mild yoghurts. These techniques can alter the composition of sensitive aroma compounds (Mortazavian et al., 2009).

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals

The chemicals and their catalog codes are given in the Appendix A1.

2.1.2. Media

MRS and M17 media were used for activation and enumeration of *Lactobacillus bulgaricus, Streptococcus thermophilus* and aroma culture. The single strains were grown in reconsituted skim milk (10% skim milk powder and 1% yeast extract). Evaporated milk used for yogurt production (14-17% total solids) was supplied from Or-Köy Dairy Plant, Urla, İzmir.

2.2. Methods

2.2.1. Selection of Yoghurt Isolates

All microorganisms were selected from the Izmir Institute of Technology, Food Engineering Department Starter Culture Collection. Also, among isolate *S. thermophilus* (Y24), and isolate *L. bulgaricus* (Y30) had potential to be used as starter cultures in dairy industry regarding their high technological and organoleptic characteristics. The yoghurt isolates that decrease pH to 4.60-4.70 up to 5 h, produce high amounts of acetaldehyde and EPS were selected. *S. thermophilus* and *L. bulgaricus* were activated separately using MRS broth (pH 6.2), and M17 broth (pH 6.9). Inoculum of 1%, taken from stock

cultures, was transferred to M17 or MRS broths depending on the culture and then incubated at 42 °C for 16 h.

2.2.2. Screening of Aroma Cultures

Nine different aroma cultures were added into the starter culture combinations at two different inoculation ratios. Then, they were screened for their organoleptic properties based on their appearance, consistency with spoon, consistency in mouth, odor, flavor, and overall acceptability by our research group. Consequently, yoghurts having the best organoleptic properties were chosen for further yoghurt production according to acetaldehyde production and sensory analysis (see Table 3.1 and Table 3.2).

2.2.3. Yoghurt Analysis

2.2.3.1. Coagulation of Yoghurt Isolates

Yoghurt isolates were inoculated in 10% skim milk and 1% yeast extract until coagulation of the milk. *S. thermophilus, L. bulgaricus* and aroma culture (P10) were activated separately using M17 broth (pH 6.9) and MRS broth (pH 6.2). Strains were taken from stock cultures and were transferred to M17 or MRS broths depending on the culture and then incubated at 42 °C for 24h. Yoghurt isolates were inoculated in 10% skim milk and 1% yeast extract. Then, they were incubated at 42 °C for 6h until their formation of coagulation (curd).

2.2.3.2. Yoghurt Production

Evaporated milk was inoculated with 2% of *S. thermophilus* and *L. bulgaricus* strains in 1:1 ratio in 250 ml plastic cups (as control). The inoculated samples were incubated at 42°C until pH 4.50-4.60 (approximately 3-4 h). Yoghurt samples were immediately cooled and stored at 4 °C for 21 days. The yoghurt samples were stored at 4°C for 21 days and the following analyses were conducted. The combinations of

selected isolates were investigated during fermentation hours and storage days. Sample numbers were assigned for each different combination in Table 2.1.

Table 2.1. Sample numbers given to each different combination of yoghurt isolates.

Samples	Combination of Isolates
Sample 1 (control)	Y30+Y24
Sample 2 (with aroma culture)	Y30+Y24+P10

2.2.4. Chemical and Physical Analysis

2.2.4.1. pH and Titratable Acidity of Yoghurt Samples

Yoghurt samples were held at 42°C until pH decreases 4.60-4.70. Fermentation was terminated at this pH. The pH of samples was determined by a pH meter during fermentation hours (3-4h), post fermentation (cooling time) hours and, storage days (Hanna HI 221, Germany). The yoghurts were cooled at 4°C, stored at the same temperature during all period of post-acidification for 21 days. Measurements were done in triplicate.

For determination of titratable acidity, 10 g yoghurt was weighed and diluted with distilled water to 100 g. 1-2 drops of phenolphatlein solution were added as indicator. Afterwards, samples were titrated by using standardized (F=1.1336) 0.1 N NaOH solutions until the appearance of first pink color. All measurements were done in triplicate.

2.2.4.2. Total Solids, Fat and Protein Contents of Milk and Yoghurt Samples

Total solids of milk and yoghurt samples at day 1 were measured gravimetrically by direct forced air oven drying at 100°C ± 1°C and during 4h (AOAC 990.20, 2006). Firstly, dishes were pre-dried at 100°C ± 1°C and were weighed, then were cooled the room temperature in a dessicator. Three g of milk and yoghurt sample were added on the weighed dish for 4 h at 100°C and then were weighed again. The difference in weight before and after drying gives the results of total solids content of milk and yoghurt samples. Total solids content of milk were weight of dried milk residue expressed as % of original samples weight (Deibel et al., 2009).

Fat content of milk and yogurt samples at day 1 were determined by butyrometers using the Gerber method. Into Gerber butyrometer vessel were put 10 ml H_2SO_4 (d: 1.82 g/ml). Ten ml of samples were added into vessels and then, 1ml isoamyl alcohol was added, and was closed with a lock stopper. Butyrometer contents were mixed to dissolve curd in Gerber centrifuge for 10 min at 1100 rpm and release fat. The oil level was read as percentage oil in yoghurt from butyrometer vessel (AOAC 2000.18, 2006).

Protein content of yogurt samples at day 1 was assessed by Kjeldahl method (991.20) of the AOAC (2006) using 6.38 as the nitrogen conversion factor. The fat, protein and total solid contents of the samples were determined in duplicate.

2.2.4.3. Syneresis of Yoghurt Samples

Syneresis of yoghurt samples during 21 days of storage was determined in duplicate (Wacher-Rodarte, Galvan et al. 1993). Each sample (10 ml) was centrifuged (Hettich-Universal 320R, Tuttlingen, Germany) at 5000 rpm for 20 min at 4 ºC. The clear supernatant was decanted and measured. Syneresis was based on the volume of clear supernatant per 100 ml yoghurt. All measurements were done in duplicate (Horiuchi et al., 2009).

2.2.4.4. Apparent Viscosity of Yoghurt Samples

The apparent viscosity measurements were determined by a viscometer Haake Viscotester 550 (Thermo Inc. Germany) LV4 spindle at a speed of 100 rpm in circulating water bath at 10 °C during 21 days of storage in duplicate. Yoghurt samples were stirred for 20 s clockwise and 20 s counter-clockwise. Concentric cylinder MV-DIN sensor was used for analyses. About 60 ml of sample was put into the cylindrical container. Apparent viscosity (μ) was calculated at mPa.s. All measurements were done in duplicate.

2.2.4.5. Analysis of Aroma Compounds

In yoghurt samples, acetaldehyde, ethanol, acetone and diacetyl were determined by static headspace method using a gas chromatograph system (GC) (Agilent 6890N, USA) with an automated headspace sampler (Agilent 7694, USA). Identification and quantification of aroma compounds were detected with (FID) detector (Agilent 5973Nms, USA). Aroma compounds were separated on an Agilent HP-5 (30 m 0.25mm, 0.25 μm) column.

Aliquots of 10g of yoghurt samples were weighed into 20 ml headspace vials (Agilent, USA), which was sealed with 20-mm aluminum crimp caps (Agilent, USA) with dark gray septa (Agilent, USA) and shaken homogeneously. The sample was equilibrated at 80°C for 20 min to achieve volatilization of volatile compounds present in yoghurt. The operating conditions for the headspace sampler and the GC/FID system under the following conditions: injector temperature 250°C, carrier gas helium at constant flow mode, a flow rate of 1 ml/min, oven temperature program initially held at 35°C for 6 min and then programmed from 35°C to 250°C at elevation rate of 30°C/min held at 250°C for 3 min. Total run time was 16.17 min. The interface line to FID was set at 300 °C. Yoghurt samples were placed in a headspace vial an aliquot of the closed airspace above the water phase was sampled directly to the gas chromatographic column with split injection. The column temperature was programmed to facilitate the separation of compounds which were then detected with the flame ionization detector (FID).

The stock standard solutions were prepared with 10000 mg/L acetaldehyde (Fluka, Spain), 4000 mg/L ethanol (Merck, Germany), 1000 mg/L acetone (Merck, Germany), 500 mg/L diacetyl (Merck, Germany) in deionized water. Six calibration

points were chosen and standard solutions were prepared which contain 1 µl, 2 µl, 5 µl, 10 µl, 20 µl, and 40 µl stock standard solutions. Calibration curves were calculated by least-square regression from these six points. The R^2 values for the linear calibration curves were about 0.999.

2.2.4.6. Determination of Lactose and Lactic acid

The amount of lactose and lactic acid in yoghurt samples were determined by high performance liquid chromatography (HPLC). They were separated on an Aminex HPX-87H (300× 7-8 mm) column. The dilute sulphuric acid (0.005 mol/L^{-1}) was used as mobile phase at a flow rate of 0.6 mL/min^{-1} and separated lactic acid and lactose by the refractive index detector. It was filtered by Nucleopore 0.45 µm syringe membrane filters for HPLC grade water. Lactic acid and lactose extracts were obtained an aliquot of 5g of yoghurt in 35 mL 0.005 mol/L^{-1} H_2SO_4, and centrifuging at 5000 rpm for 10 min at 4°C and then supernatant was filtered through 0.45 µm polycarbonate membrane filters before chromatographic analysis. Lactic acid and lactose were separated at 0.6 ml/min^{-1}, at 65°C. The sample injection volume was 50 µL and filtrates were injected 50 µl. The standard working solutions of L (+) lactic acid were used. Analyses were performed on hours of fermentation and on days 21 of storage. All measurements were made in triplicate (Serra et al., 2009).

2.2.4.7. Determination of Proteolytic Activity

The proteolytic activity of starter cultures during fermentation of yoghurt was determined by measuring the free amino acid content in filtrates of yogurt samples by using the o-phthaldialdehyde (OPA) method. Apparently, the supernatants of samples were prepared by centrifugation at 5000 rpm for 10 min at 4 °C. All the supernatants thus obtained were filtered through 0.45 µm syringe membrane filter. 400 µl of the filtrate, 3 ml of OPA reagent was added, vortexed for 5 s, followed by measuring absorbance at 340 nm within 2 min using spectrophotometer. The readings of the 0 h samples as well as the reagent blank were deducted from the corresponding readings of yogurt samples to obtain the amount of free amino acids released as a consequence of the proteolytic activity of the starter cultures during storage (Donkor et al., 2007).

2.2.5. Lactic Acid Bacteria Counts

The *S. thermophilus*, *L. bulgaricus* and aroma culture counts were determined using M17 (pH 6.9) and MRS (pH 6.3) agars pour plate method, respectively, at days 1, 7, 14, and 21. The yoghurt samples (1 ml) were decimally diluted in 9 ml sterile peptone water (0.1%) and 1 ml aliquot dilutions were pour plated and incubated anaerobically at 42 ºC 48 h for *L. bulgaricus* and aerobically at 42ºC 48 h for *S. thermophilus.* Anaerobic conditions were created using AnaeroGen in plastic anaerobic jars (Oxoid). After the incubation, the plates with colony forming units (CFU) ranging from 30 to 300 were selected for enumeration. After the colony counting, the numbers were expressed in logarithmic scales (log $CFUg^{-1}$). Two measurements were carried out and average values were reported.

2.2.6. Sensory Profile Analysis of Yoghurt Samples

The yoghurt samples were analyzed at days 1, 7 and 14 by a 10-member trained sensory panel. Yoghurt samples at days were coded and presented to panelists at 8 °C in 100 ml commercial yoghurt containers under typical daylight room conditions. Panelists were selected between the graduate students and faculty in the Food Engineering Department at İzmir Institute of Technology. They were trained twice in a group discussion with reference samples having good and bad quality attributes. Panelists independently assessed all sample for appearance, consistency on the spoon, consistency in the mouth, odor, flavor, and overall acceptability using a hedonic scale of 1-5 (with 5 being the highest attribute score). Samples were randomly ordered at the beginning of the panel and each panelist received the samples in the same order. Sensory evaluation sessions were conducted in duplicate.

2.2.7. Data Analysis

Results of the total solids, fat, and protein contents, pH, titratable acidity, syneresis, apparent viscosity, lactic acid bacteria counts, aroma compounds, lactic acid and lactose amounts, proteolytic activity, sensory scores were analyzed by one-way

analysis of variance using MINITAB® release 14 (Minitab Inc., State College, USA) and Tukey significance test. Significance was accepted at $P<0.05$.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Screening of Yoghurt Samples

In the first part of this study, 18 yoghurt samples, which were consisted of *L. bulgaricus* (Y30) + *S. thermophilus* (Y24) as added at different combinations of 9 aroma cultures, were produced according to two different inoculation ratios of starter cultures. Y30 + Y24 combination were produced as a control sample (at ratio 1:1). Then, they were screened for their organoleptic properties based on their appearance, consistency on spoon, consistency in mouth, odor, flavor, and overall acceptability by our research group.

Firstly, yoghurt samples were selected by highest sensory evaluations that are showed in Table 3.1. Panelists scored samples from 1 to 5 according to personal liking in sensory profile analyses. According to the results, samples sensory profile points were determined between; 4.70 to 2.90. Sensory profiles of control yoghurt sample 1 and sample P10 were closed each other. Actually, P10 had higher acetaldehyde content (35.26 ± 0.67 mg/L) than the other yoghurt combinations (see Table 3.2). According to these results, P10 aroma culture was used at production of sample 2 for investigation of aroma profiles and physical, chemical changes during shelf life. Also, Sample 2 was compared with control yoghurt sample 1 during storage days.

Table 3.1. Sensory scores for yoghurt samples

Yoghurt Samples	Appearance	Consistency on spoon	Consistency in mouth	Odor	Flavor	Overall Acceptance
Sample 1	4.65 ± 0.35 ^{c}	4.55 ± 0.07 ^{d}	4.55 ± 0.07 ^{c}	4.60 ± 0.14 ^{b}	4.55 ± 0.21 ^{c}	4.70 ± 0.00 ^{c}
P1	3.85 ±0.49 ^{b}	3.80 ± 0.28 ^{c}	3.85 ± 0.07 ^{bc}	4.20 ± 0.00 ^{b}	3.80 ± 0.14 ^{bc}	3.80 ± 0.14 ^{bc}
P2	4.35 ± 0.35 ^{bc}	4.30 ± 0.14 ^{cd}	4.40 ± 0.28 ^{c}	4.55 ± 0.21 ^{b}	4.45 ± 0.35 ^{c}	4.50 ± 0.42 ^{c}
P3	2.90 ± 0.14 ^{a}	3.15 ± 0.21 ^{b}	3.20 ± 0.42 ^{a}	3.30 ± 0.28 ^{a}	2.95 ± 0.07 ^{a}	3.05 ± 0.21 ^{a}
P4	3.35 ± 0.07 ^{a}	3.20 ± 0.00 ^{b}	3.25 ± 0.35 ^{a}	3.55 ± 0.35 ^{ab}	3.05 ± 0.35 ^{a}	3.10 ± 0.42 ^{a}
P5	3.65 ± 0.21 ^{b}	3.60 ± 0.42 ^{bc}	3.25 ± 0.21 ^{ab}	3.55 ± 0.49 ^{ab}	4.10 ± 042 ^{a}	3.25 ± 0.21 ^{ab}
P6	3.50 ± 0.00 ^{ab}	3.90 ± 0.14 ^{cd}	3.65 ± 0.35 ^{b}	3.45 ± 0.21 ^{ab}	3.80 ± 0.42 ^{a}	3.30 ± 0.14 ^{ab}
P7	4.55 ± 0.21 ^{c}	4.40 ± 0.00 ^{d}	4.15 ± 0.07 ^{bc}	4.10 ± 0.14 ^{b}	2.95 ± 0.21 ^{bc}	4.00 ± 0.00 ^{bc}
P8	4.30 ± 0.42 ^{bc}	4.30 ± 0.14 ^{cd}	4.10 ± 0.14 ^{bc}	4.15 ± 0.21 ^{b}	3.35 ± 0.21 ^{bc}	3.95 ± 0.21 ^{bc}
P9	4.20 ± 0.42 ^{bc}	4.20 ± 0.14 ^{cd}	4.00 ± 0.28 ^{bc}	4.00 ± 0.14 ^{b}	3.80 ± 0.42 ^{bc}	3.95 ± 0.35 ^{bc}
P10	4.30 ± 0.00 ^{bc}	4.35 ± 0.07 ^{d}	4.10 ± 0.00 ^{bc}	4.10 ± 0.00 ^{b}	4.10 ± 0.42 ^{bc}	4.00 ± 0.42 ^{bc}
P11	3.30 ± 0.42 ^{ab}	3.25 ± 0.07 ^{bc}	3.50 ± 0.28 ^{ab}	3.95 ± 0.07 ^{ab}	3.45 ± 0.35 ^{b}	3.35 ± 0.35 ^{a}
P12	4.10 ± 0.00 ^{bc}	4.10 ± 0.14 ^{cd}	3.85 ± 0.07 ^{bc}	4.05 ± 0.21 ^{b}	3.85 ± 0.07 ^{bc}	3.80 ± 0.14 ^{bc}
P13	3.95 ± 0.21 ^{bc}	3.85 ± 0.35 ^{cd}	3.60 ± 0.28 ^{ab}	3.95 ± 0.07 ^{ab}	3.70 ± 0.14 ^{b}	3.60 ± 0.14 ^{ab}
P14	4.10 ± 0.14 ^{bc}	3.60 ± 0.14 ^{bc}	3.45 ± 0.07 ^{ab}	4.05 ± 0.21 ^{b}	3.55 ± 0.07 ^{b}	3.75 ± 0.07 ^{b}
P15	4.00 ± 0.14 ^{bc}	3.80 ± 0.28 ^{c}	3.75 ± 0.21 ^{bc}	4.15 ± 0.21 ^{b}	3.50 ± 0.00 ^{b}	3.55 ± 0.07 ^{ab}
P16	4.50 ± 0.00 ^{c}	4.25 ± 0.21 ^{cd}	4.20 ± 0.14 ^{bc}	4.15 ± 0.07 ^{b}	4.10 ± 0.14 ^{bc}	3.95 ± 0.07 ^{bc}
P17	3.80 ± 0.28 ^{bc}	3.75 ± 0.21 ^{c}	3.60 ± 0,14 ^{ab}	4.05 ± 0.21 ^{b}	3.45 ± 0.07 ^{b}	3.50 ± 0.14 ^{ab}
P18	4.20 ± 0.00 ^{bc}	4.05 ± 0.07 ^{cd}	3.90 ± 0.00 ^{bc}	4.05 ± 0.21 ^{b}	3.55 ± 0.21 ^{b}	3.55 ± 0.07 ^{ab}

*$^{a-d}$ Column means having a different letter or letters differ (P<0.05).
Means ± SD of duplicate samples.

Table 3.2. Contents of the aroma compounds in the different yoghurt combinations are produced with aroma cultures.

Yoghurt Samples	Acetaldehyde (mg/L)	Ethanol (mg/L)	Acetone (mg/L)	Diacetyl (mg/L)
Sample1	17.73 ± 0.33^{a}	3.30 ± 0.81^{b}	0.25 ± 0.00^{a}	0.48 ± 0.01^{c}
P2	17.93 ± 0.46^{a}	3.18 ± 0.64^{b}	0.38 ± 0.02^{c}	0
P3	17.48 ± 0.03^{a}	6.72 ± 0.16^{c}	0.27 ± 0.00^{a}	0.33 ± 0.01^{b}
P4	17.99 ± 0.39^{a}	6.49 ± 0.16^{c}	0.28 ± 0.01^{b}	0.37 ± 0.01^{b}
P5	34.05 ± 0.34^{d}	7.97 ± 0.00^{d}	0.41 ± 0.02^{c}	0
P7	23.47 ± 0.52^{c}	7.85 ± 0.16^{d}	0.45 ± 0.00^{d}	0.38 ± 0.07^{b}
P10	35.26 ± 0.67^{d}	3.42 ± 0.00^{b}	0.51 ± 0.03^{e}	0
P16	21.23 ± 0.02^{b}	1.56 ± 0.31^{a}	0.27 ± 0.00^{a}	0.30 ± 0.05^{b}

*$^{a-e}$ Column means having a different letter or letters differ (P<0.05).
Means ± SD of duplicate samples.

3.2. Chemical and Physical Analysis Results

pH, titratable acidity, syneresis, viscosity, aroma profile, lactic acid and lactose determination and proteolytic activity analyses were done for 2 samples during storage days. Sample 1 and sample 2 were compared for these analyses during storage days. Also pH and aroma characterization were done during fermentation hours and cooling hours. Total solid contents, total protein contents, total fat contents were determined at day one of storage.

3.2.1. The pH and Titratable Acidity Results

During yoghurt fermentation, pH of milk decreased from 6.59 to 4.60-4.70 for both samples. When the gel was formed, fermentation was ended at an incubation time of 3–4h. The pH changes during fermentation and post fermentation from 4 to 8h were shown in Figure 3.1 and Table 3.3. During fermentation, the same results were reported by O'Neil et al. (1979) and Fadela et al. (2009), who measured that, the pH values of milk declined during the fermentation hours from 6.7 to 4.34.

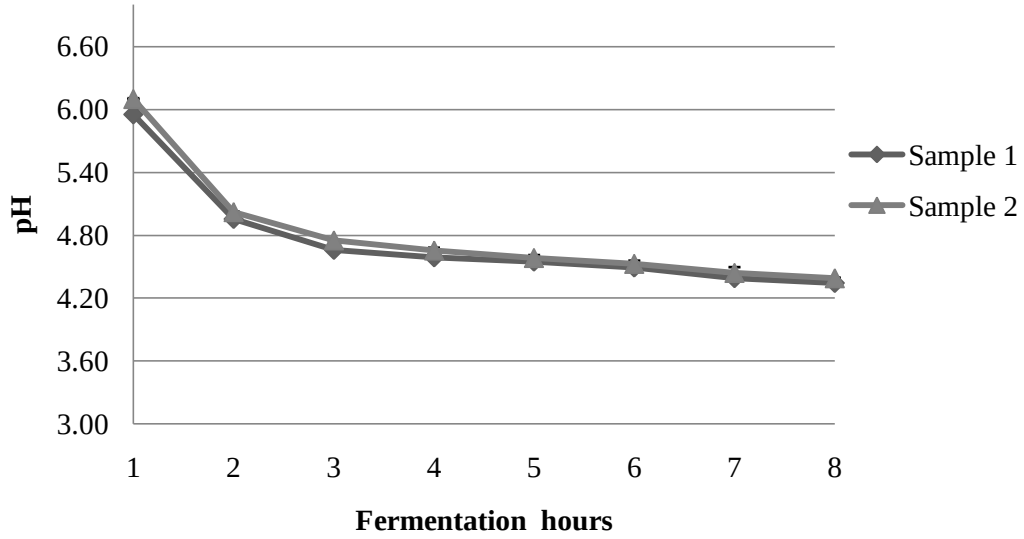

Figure 3.1. pH change in yoghurt samples during fermentation (3-4h, 42°C) and cooling hours (+4°C).

Table 3.3. pH changes during fermentation and cooling time (h).

Fermentation and cooling time (h)	Sample 1	Sample 2
1	$5.95 \pm 0.03^{c,A}$	$6.10 \pm 0.01^{c,B}$
2	$4.95 \pm 0.01^{b,A}$	$5.02 \pm 0.01^{b,B}$
3	$4.66 \pm 0.01^{a,A}$	$4.75 \pm 0.03^{b,B}$
4	$4.58 \pm 0.02^{a,A}$	$4.65 \pm 0.04^{b,A}$
5	$4.54 \pm 0.00^{a,A}$	$4.58 \pm 0.01^{b,B}$
6	$4.49 \pm 0.01^{a,A}$	$4.53 \pm 0.01^{b,B}$
7	$4.39 \pm 0.01^{a,A}$	$4.44 \pm 0.02^{a,B}$
8	$4.34 \pm 0.02^{a,A}$	$4.39 \pm 0.01^{a,B}$
Minimum	4.34	4.39
Maximum	5.95	6.10
Average	4.82	4.90

* $^{a-c}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)
* $^{A-B}$ Means in the same row with different superscript letters differ significantly ($P<0.05$)
Means ± SD of triplicate samples.

The pH values of samples during storage days were given in Table 3.4. The pH values were varied between 4.27 and 3.73 in both samples (see Figure 3.2). In agreement with those found in the literature and within the desirable range for a high quality product (Runge et al., 2003; Salvador & Fiszman, 2004).

The average of pH value for sample 1 and sample 2 were 3.98 and 4.02, respectively. The pH of sample 1 (control yoghurt) decreased from 4.60 to 4.27 at 24h of storage at 4 °C. The pH decrease after 24h storage for both samples varied between 4.27 and 3.73 for control sample 1 and 4.24 and 3.89 for sample 2 at the end of storage. Sample1 decreased slightly during cold storage days. Sample 2 observed a similar trend until day 14. However, the pH change was significantly different in sample 1 at day 21 than sample 2 ($P<0.05$). The pH results were significant and the results of high acid production and decreased pH. These results are in line with findings of Kondratenko et al. (1979). Also, use of aroma culture during storage days and fermentation had a significant effect on the pH values of yoghurt sample 2 ($P<0.05$).

Table 3.4. pH changes during storage days

Storage Days	Sample 1	Sample 2
1	$4.27 \pm 0.01^{c,A}$	$4.24 \pm 0.01^{c,A}$
7	$3.96 \pm 0.01^{b,A}$	$3.93 \pm 0.01^{b,A}$
14	$3.93 \pm 0.04^{b,A}$	$3.90 \pm 0.02^{b,A}$
21	$3.73 \pm 0.02^{a,A}$	$3.89 \pm 0.01^{b,B}$
Minimum	3.73	3.89
Maximum	4.27	4.24
Average	3.98	4.02

* $^{a–c}$ Means in the same column with different superscript letters differ significantly (P<0.05)

* $^{A-B}$ Means in the same row with different superscript letters not differ significantly (P>0.05)
Means ± SD of triplicate samples.

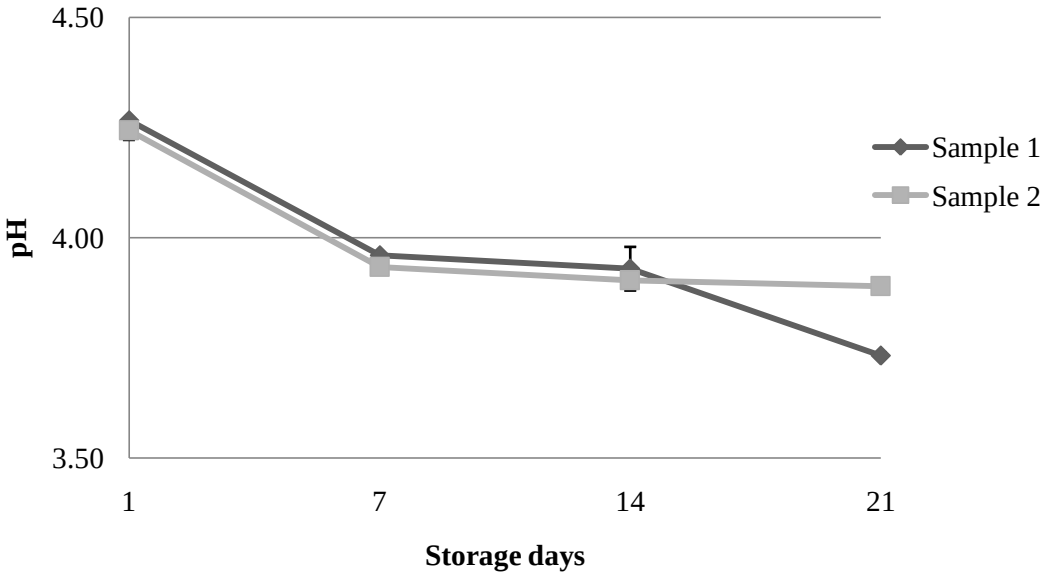

Figure 3.2. pH changes in yoghurt samples during storage days.

The pH decrease could be responsible for the variability of titratable acidity. In addition, acid production is directly related to lactose metabolism by yoghurt starter cultures and amino acids (Haj et al., 2007).

The titratable acidity results of yoghurt samples were given in Table 3.5. Titratable acidity results were given by lactic acid % for all samples during storage in

Figure 3.3. For sample 1, titratable acidity increased from 1.29% to 1.75% and also for sample 2 acidity increased from 1.27% to 1.58% in during storage days.

The titratable acidity values of samples varied between 1.27% and 1.75% and Sample 1 were not significantly difference from sample 2 for acidity value until 14th day ($P>0.05$). The average titratable acidity of yogurt sample 2 was lower than in control sample 1 ($P<0.05$) during storage days. Significant differences were observed in all samples during storage ($P<0.05$). No differences were observed among the all samples during storage ($P>0.05$). Results were investigated in proportional with pH results.

According to standards of yoghurt, titratable acidity results found in the acceptable limits (Codex Standard for Fermented Milks CODEX STAN 243-2006 FAO/WHO 2001, Turkish Food Codex; Fermente Sütler Tebliği, 2009)

Table 3.5. Titratable acidity (% lactic acid) changes during storage days

Storage days	Sample 1	Sample 2
1	$1.29 \pm 0.04^{a,A}$	$1.27 \pm 0.03^{a,A}$
7	$1.43 \pm 0.06^{bc,A}$	$1.36 \pm 0.04^{b,A}$
14	$1.49 \pm 0.03^{c,A}$	$1.56 \pm 0.03^{c,A}$
21	$1.75 \pm 0.04^{d,B}$	$1.58 \pm 0.01^{c,A}$
Minimum	1.29	1.27
Maximum	1.75	1.58
Average	1.49	1.44

* $^{a–c}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)

* $^{A-B}$ Means in the same row with different superscript letters not differ significantly ($P>0.05$)
Means ± SD of triplicate samples.

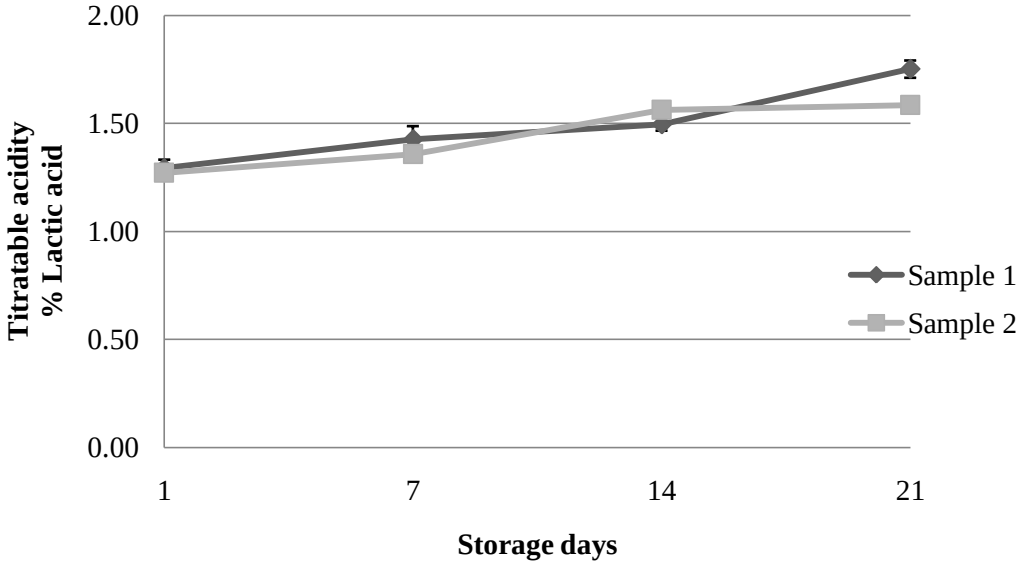

Figure 3.3. Titratable acidity in yoghurt samples during storage days**.**

3.2.2. Total Solids, Total Fat and Total Protein Contents Results

Total solids contents of yoghurt samples were varied between 20.20% and 20.50%. This variation occurred due to the total solids content of evaporated milk (17%) since no skim milk was added to increase the total solids content. Total solids of yoghurt are shown in Table 3.6. The milk solids content for yoghurt ranges from around 9% for skim milk yoghurt to more than 20% for certain types of concentrated yoghurt. Many commercial yoghurt products have milk solids contents of 14-15% (Tamime & Robinson, 1999). The total solids content of milk can be increased by concentration processes, such as, evaporation under vacuum, and membrane processing (i.e., reverse osmosis and ultra filtration) (Lee & Lucey, 2010).

Fat contents of sample 1 and sample 2 were varied between 4.46% and 4.06%. No additional cream was added during standardization of milk to increase its fat content. The maximum fat content is 15% according to codex standarts (Codex, 2008).

Protein contents of yoghurts were ranged from 2.39% to 2.45%.The minimum total protein content is 2.7% in milk and yoghurt products according to Turkish Food Codex; Fermente Sütler Tebliği (2009).

Table 3.6. Approximate composition of milk and yoghurt samples at the first day of storage.

Properties	Milk	Sample 1	Sample 2
Total solids (%)	17.47 ± 2.04^{a}	20.20 ± 0.62^{a}	20.50 ± 0.33^{a}
Fat (%)	4.43 ± 0.05^{b}	4.46 ± 0.15^{b}	4.06 ± 0.05^{a}
Protein (%)	2.39 ± 0.11^{a}	2.39 ± 0.03^{a}	2.45 ± 0.08^{a}

*a-b Raw means having a different letter not differ significantly (P>0.05).
Means ± SD of triplicate samples.

3.2.3. Syneresis Results

Syneresis changes of yoghurt samples were ranged between 38.33% and 53.83% at storage days were given in Table 3.7. Syneresis change was not significantly different among samples during storage (P>0.05). Although, syneresis was increased in sample 1 during storage, it is decreased in sample 2. The highest syneresis decrease was observed in sample 2 at day 21. Also, control sample 1 had higher syneresis values than aroma culture added yoghurt sample 2 at during storage days. This decrease of syneresis could be due to metabolic activity of starter cultures and decrease in pressure in the protein matrix which causes decrease of syneresis (Güler-Akın and Akın, 2007). Syneresis decreased just sample 2 at day 21, agreeing with the observations by Tamime et al. (1997), and İşleten and Karagül-Yüceer (2006). The most important negative effect in yoghurt is syneresis that can be defined as the expulsion of whey from the gel surface of yoghurts (Lucey, 2004). Also, Figure 3.4 shows the changes in syneresis during storage days.

Table 3.7. Syneresis changes during storage days

Storage days	Sample 1	Sample 2
1	$43.08 \pm 5.62^{ab,A}$	$41.11 \pm 3.57^{a,A}$
7	$43.83 \pm 3.74^{ab,A}$	$41.79 \pm 2.80^{a,A}$
14	$45.63 \pm 2.12^{ab,A}$	$39.61 \pm 3.00^{a,A}$
21	$53.83 \pm 1.71^{b,A}$	$38.33 \pm 5.45^{a,A}$
Minimum	43.08	38.34
Maximum	53.83	41.79
Average	46.59	40.21

* $^{a–b}$ Means in the same column with different superscript letters not differ significantly (P>0.05)
* $^{A-B}$ Means in the same row with different superscript letters not differ significantly (P>0.05)
Means ± SD of duplicate samples.

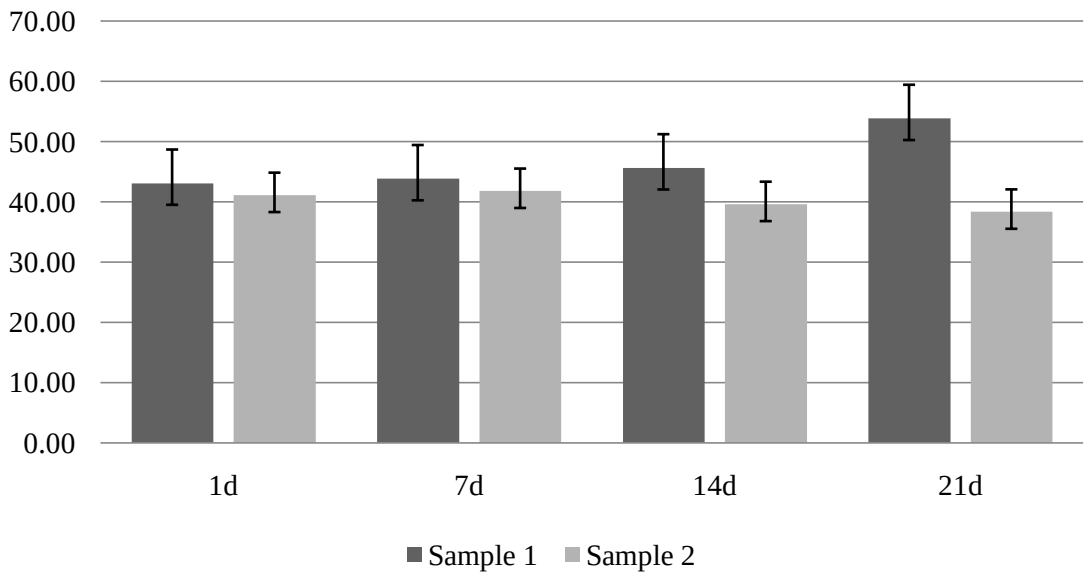

Figure 3.4. Syneresis change in yoghurt samples during storage days.

3.2.4. Apparent Viscosity Results

Two yoghurt sample equations were calculated related to apparent viscosity (Pa.s) and shear rate (1/sec) changes and R^2 of samples were determined 0.999 both of sample 1 and sample 2. Apparent viscosity results of yoghurt samples were given in Table 3.8.

In Figure 3.5, apparent viscosity (μ) changes were given at 300 s^{-1} during storage and it was stated as mPa.s. Apparent viscosity changes were significantly differences between sample 1 and sample 2 during storage ($P<0.05$). The apparent viscosity results of yoghurt samples ranged between 207.29 and 253.34 mPa.s during storage days. The highest apparent viscosity was observed for sample 2 which was 274.88 mPa.s at day 21. Viscosity values increased with storage time in sample 2, and the highest levels of viscosity were obtained in the yoghurts at day 21. Accelerating viscosity was also observed in plain yoghurt during storage by Abu-Jdayil and Mohameed (2002) and İşleten and Karagül-Yüceer (2006).

Table 3.8. Apparent viscosity changes during storage days (mPA.s).

Storage Days	Sample 1	Sample 2
1	$207.80 \pm 1.79^{a,A}$	$238.97 \pm 0.86^{bc,B}$
7	$207.29 \pm 1.37^{a,A}$	$240.55 \pm 0.70^{bc,B}$
14	$242.07 \pm 1.45^{c,A}$	$251.82 \pm 0.75^{d,B}$
21	$233.58 \pm 6.44^{b,A}$	$274.88 \pm 1.59^{e,B}$
Minimum	207.29	238.96
Maximum	242.07	274.88
Average	223.93	253.34

* $^{a-e}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)

* $^{A-B}$ Means in the same row with different superscript letters differ significantly ($P<0.05$)
Means ± SD of duplicate samples.

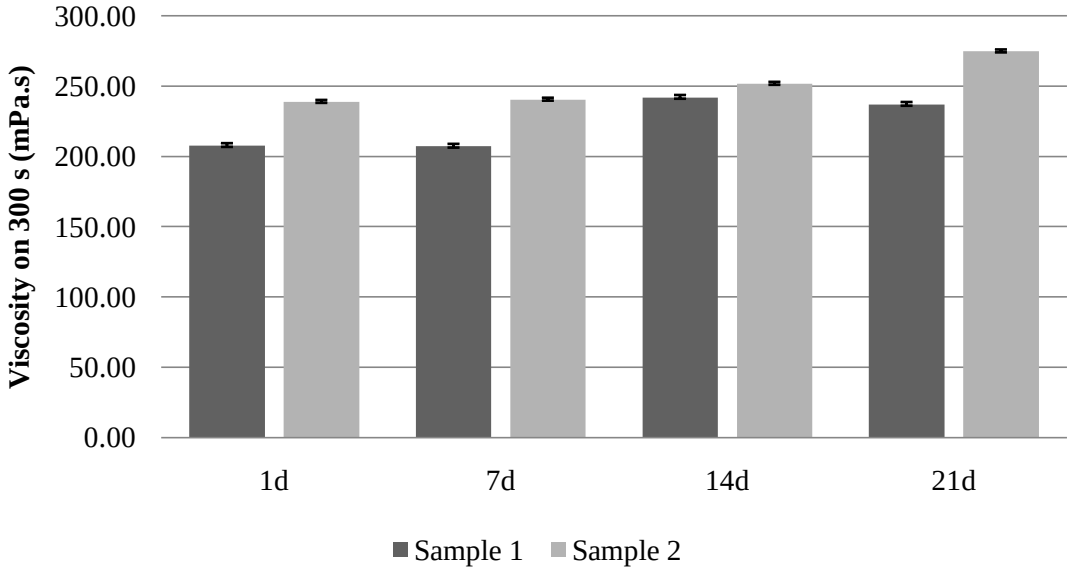

Figure 3.5. Apparent viscosity changes during storage days (mPA.s).

3.2.4. Aroma Profile Results

The amount of aroma compounds in yoghurt samples during fermentation and cooling time (h) are illustrated in Table 3.9 and also the amount of aroma compounds in yoghurt samples during storage days are illustrated in Table 3.10. Acetaldehyde, ethanol, acetone and diacetyl were found in samples during storage. During storage days, the acetaldehyde, ethanol, acetone and diacetyl contents of the yoghurt samples were varied between 17.01–31.41 mg/L, 1.12-4.93 mg/L, 0.32-0.63 mg/L, 0.24-0.84 mg/L, respectively.

Some researchers found that the most important flavor compounds are acetaldehyde (2.0 to 41.0 mg/kg), diacetyl (0.2 to 2.3 mg/kg), ethanol (0.2 to 9.9 mg/kg), acetone (1.8 to 3.4 mg/kg) (Kneifel et al. (1992); Rasic and Kurman (1978). According to the literature results, 2-butanone was not detected in any of yoghurt samples. This finding is also confirmed by Xanthopoulos et al. (2006).

Table 3.9. Changes amount of aroma compounds during fermentation and cooling time(h)

	Fermentaion and cooling time (h)	Sample 1	Sample 2
Acetaldehyde (mg/L)	**1**	$1.22 \pm 0.02^{a,A}$	$1.33 \pm 0.03^{a,A}$
	2	$10.91 \pm 0.04^{b,A}$	$9.13 \pm 0.28^{b,A}$
	3	$17.06 \pm 0.16^{cd,A}$	$15.48 \pm 1.16^{c,A}$
	4	$22.33 \pm 0.18^{ef,A}$	$23.51 \pm 0.82^{ef,A}$
	5	$18.04 \pm 0.21^{d,A}$	$22.45 \pm 0.26^{ef,B}$
	6	$17.77 \pm 3.53^{cd,A}$	$17.39 \pm 0.62^{cd,A}$
	7	$21.40 \pm 0.72^{e,B}$	$18.28 \pm 0.03^{d,A}$
	8	$24.75 \pm 0.21^{f,B}$	$19.92 \pm 0.18^{d,A}$
	Minimum	1.22	1.33
	Maximum	22.33	23.51
	Average	15.22	15.24
	Fermentaion and cooling time (h)	**Sample 1**	**Sample 2**
Ethanol (mg/L)	**1**	$0.47 \pm 0.11^{a,A}$	$0.59 \pm 0.16^{ab,AB}$
	2	$1.36 \pm 0.31^{ab,AB}$	$1.38 \pm 0.13^{ab,AB}$
	3	$1.49 \pm 0.27^{b,B}$	$2.33 \pm 0.56^{b,B}$
	4	1.20 ± 0.18^{abAB}	$1.84 \pm 0.58^{b,B}$
	5	$1.63 \pm 0.05^{b,B}$	$2.01 \pm 0.69^{b,B}$
	6	$1.31 \pm 0.53^{ab,AB}$	$2.32 \pm 0.26^{b,B}$
	7	$1.38 \pm 0.11^{ab,AB}$	$1.99 \pm 0.24^{b,B}$
	8	$1.41 \pm 0.06^{ab,AB}$	$1.99 \pm 0.24^{b,B}$
	Minimum	0.47	0.59
	Maximum	1.63	2.33
	Average	1.20	1.74
	Fermentaion and cooling time (h)	**Sample 1**	**Sample 2**
Acetone (mg/L)	**1**	$0.34 \pm 0.01^{a,A}$	$0.34 \pm 0.01^{a,A}$
	2	$0.43 \pm 0.01^{ab,A}$	$0.43 \pm 0.00^{ab,A}$
	3	$0.54 \pm 0.00^{b,A}$	$0.51 \pm 0.03^{b,A}$
	4	$0.51 \pm 0.03^{b,A}$	$0.49 \pm 0.00^{b,A}$
	5	$0.54 \pm 0.06^{b,A}$	$0.56 \pm 0.03^{b,A}$
	6	$0.46 \pm 0.10^{b,A}$	$0.58 \pm 0.06^{b,A}$
	7	$0.58 \pm 0.00^{c,A}$	$0.56 \pm 0.03^{b,A}$
	8	$0.59 \pm 0.00^{c,A}$	$0.56 \pm 0.03^{b,A}$
	Minimum	0.35	0.34
	Maximum	0.59	0.58
	Average	0.48	0.50
	Fermentaion and cooling time (h)	**Sample 1**	**Sample 2**
Diacetyl (mg/L)	**1**	$0.22 \pm 0.07^{a,A}$	$0.12 \pm 0.06^{a,A}$
	2	$0.78 \pm 0.14^{b,A}$	$1.00 \pm 0.06^{b,A}$
	3	$1.20 \pm 0.23^{b,A}$	$1.84 \pm 0.23^{cd,A}$
	4	$1.00 \pm 0.06^{b,A}$	$2.00 \pm 0.23^{d,B}$
	5	$0.92 \pm 0.06^{b,B}$	$0.58 \pm 0.01^{ab,A}$
	6	$0.62 \pm 0.04^{ab,A}$	$1.08 \pm 0.06^{b,B}$
	7	$0.69 \pm 0.15^{b,A}$	$1.92 \pm 0.11^{cd,B}$
	8	$0.70 \pm 0.14^{b,A}$	$1.48 \pm 0.51^{c,A}$
	Minimum	0.23	0.12
	Maximum	1.20	2.00
	Average	0.74	1.21

* $^{a–f}$ Means in the same column with different superscript letters differ significantly (P<0.05).

* $^{A-B}$ Means in the same row with different superscript letters differ significantly (P<0.05).
Means ± SD of duplicate samples.

Table 3.10. Amounts of the aroma compounds in the yoghurt samples during storage days.

	Storage days	Sample 1	Sample 2
Acetaldehyde (mg/L)	**1**	$20.30 \pm 0.27^{c,A}$	$31.41 \pm 0.31^{e,B}$
	7	$17.71 \pm 0.30^{b,A}$	$23.53 \pm 0.80^{d,B}$
	14	$17.01 \pm 0.72^{b,A}$	$17.69 \pm 0.35^{b,A}$
	21	$16.03 \pm 0.43^{a,A}$	$16.76 \pm 0.26^{ab,A}$
Ethanol (mg/L)	**1**	$1.12 \pm 0.14^{a,A}$	$1.17 \pm 0.10^{a,A}$
	7	$2.50 \pm 0.22^{c,B}$	$1.91 \pm 0.08^{b,A}$
	14	$3.64 \pm 0.22^{d,B}$	$2.35 \pm 0.13^{c,A}$
	21	$4.93 \pm 0.34^{e,B}$	$2.80 \pm 0.13^{c,A}$
Acetone (mg/L)	**1**	$0.42 \pm 0.03^{b,B}$	$0.32 \pm 0.01^{a,A}$
	7	$0.44 \pm 0.01b^{bc,A}$	$0.41 \pm 0.04^{b,A}$
	14	$0.47 \pm 0.04^{c,A}$	$0.46 \pm 0.04^{bc,A}$
	21	$0.63 \pm 0.02^{d,B}$	$0.45 \pm 0.03^{bc,A}$
Diacetyl (mg/L)	**1**	$0.24 \pm 0.02^{a,A}$	$0.84 \pm 0.08^{f,B}$
	7	$0.25 \pm 0.01^{a,A}$	$0.64 \pm 0.01^{e,B}$
	14	$0.30 \pm 0.01^{ab,A}$	$0.54 \pm 0.03^{d,B}$
	21	$0.41 \pm 0.07^{b,B}$	0

* $^{a–f}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)

* $^{A-B}$ Means in the same row with different superscript letters differ significantly ($P<0.05$)
Means ± SD of triplicate samples.

Yoghurt samples were produced under the same conditions, the amount of aroma compounds varied significantly different between sample 1 and sample 2 ($P<0.05$). Also, aroma compounds in sample 1 and sample 2 were significantly effected by storage days ($P<0.05$).

The results of acetaldehyde production during storage are presented in Figure 3.6. Acetaldehyde (fresh, fruity, pungent taste) is the one of the major aroma compound of yoghurt and is produced by starter cultures from lactose or due to the formation of acetyl coenzyme A (Chaves et al., 2002). Additionally, for desirable aroma in yoghurt

production, the amount of acetaldehyde should be among 23 and 41 mg/L of yoghurt (Tamime & Deeth, 1980).

The changes of acetaldehyde concentrations in sample 1 ranging from 16.03 to 20.30 mg/L and in sample 2 ranging from 16.76 to 31.41 mg/L during storage days. So, the yoghurt sample 2 (with aroma culture) had the highest mean of acetaldehyde amount at storage days than control yoghurt sample 1. But, acetaldehyde decreased in both samples during storage. Acetaldehyde production in yoghurt samples was decreased during storage days as reported by Güzel-Seydim et al. (2005). Also, Kneifel et al. (1992) found acetaldehyde to most typical yoghurt aroma; products with acetaldehyde levels <10 ppm were generally rated as "low" in flavor intensity.

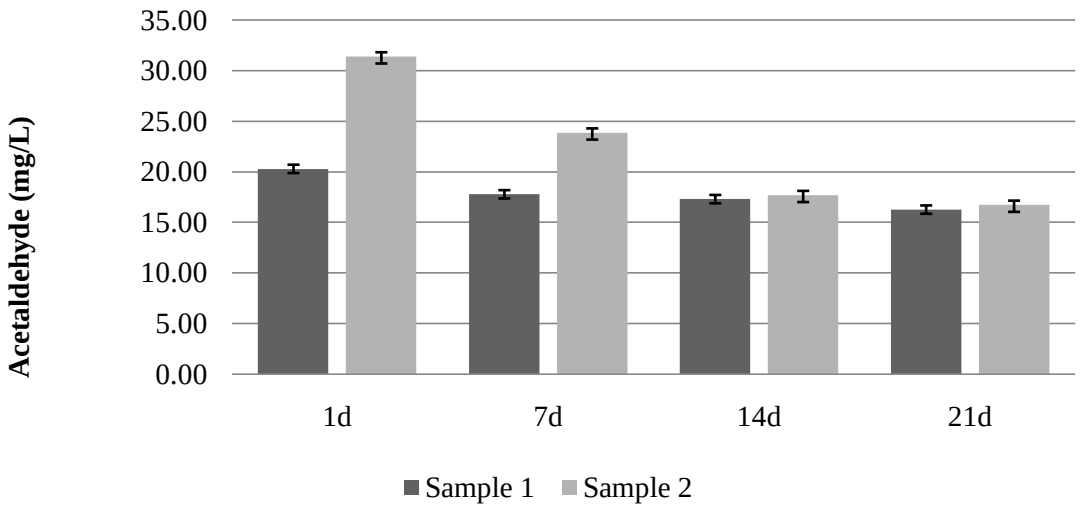

Figure 3.6. Changes of acetaldehyde concentration for yoghurt samples during storage days.

Higher amount of acetaldehyde in yoghurt with aroma culture should be due to the fast metabolic activity of aroma culture. Furthermore, when the pH values were lower, the concentration of acetaldehyde decreases through the acetaldehyde oxidized to acetate (Tamime & Robinson, 2001).

Acetaldehyde amount decreased in sample 1 and sample 2 but, ethanol amount increased during storage days. Varga (2006) and Özer et al. (2007) stated that acetaldehyde can be metabolized to ethanol by alcohol dehydrogenize of yoghurt starter

cultures, especially *S. thermophilus* strains. In addition, ethanol had the highest mean level (1.12-4.93 mg/L) in yoghurt control sample 1 compared yoghurt sample 2 with aroma culture and also, ethanol increased in all yoghurt samples (see Figure 3.7). The same results were showed by Güler (2007).

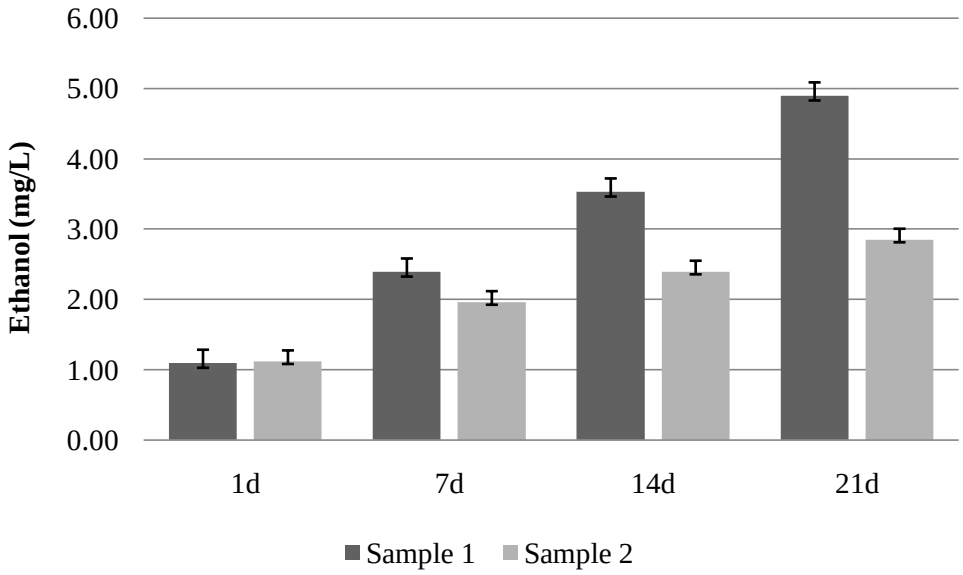

Figure 3.7. Changes of ethanol amount for yoghurt samples during storage days

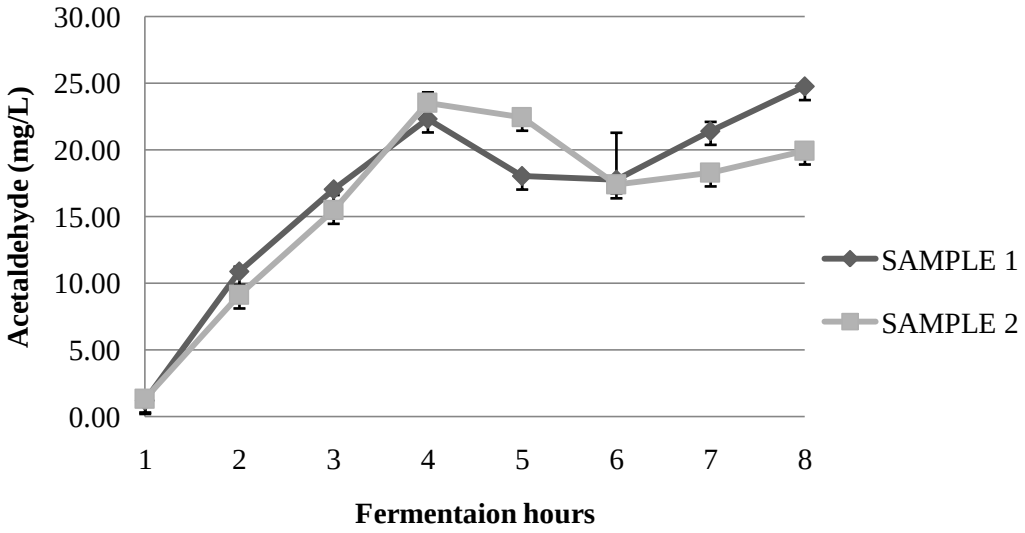

Figure 3.8. Changes of acetaldehyde amount for yoghurt samples during fermentation and cooling time (h)

The results of acetaldehyde production during the fermentation hours are presented in Figure 3.8. During fermentation, from the onset up to 4h of fermentation, acetaldehyde production increased at the same trend line in sample 1 and sample 2. After that point, acetaldehyde production decreased in sample 1 and sample 2, but in sample 1 was decreased higher than sample 2. In sample 1, the mean values of acetaldehyde varied from 1.22 to 22.33 mg/L during fermentation (1-4h) and also varied from 17.77 to 24.76 mg/L during cooling time (5-8h). In sample 2, the mean values of acetaldehyde varied from 1.33 to 23.51 mg/L during fermentation time (1-4h) and also varied from 17.39 to 22.45 mg/L during cooling time (5-8h).

Diacetyl (buttery, fatty) is another main aroma compound in yoghurt products. When acetaldehyde content is low, diacetyl is important for full aroma and taste of yoghurt (see Figure 3.9). The amount of diacetyl in yoghurt sample 2 with aroma culture had the highest value 0.84 ± 0.08 (mg/L) on 1st day of storage and then decreased by end of the storage (see Table 3.10). The decrease of diacetyl concentration in sample 2 by end of the storage could be hydrolyzed by microbial enzymes to form other compounds .Control yoghurt sample 1 had little diacetyl production (0.24-0.41 mg/L). However, in yoghurt sample 2 with aroma culture, the diacetyl concentration range was greater (0.54-0.84 mg/L). This result may be given explanation by the high ratio bacilli/cocci starter culture that is used at yoghurt production (Tamime & Robinson, 2007).

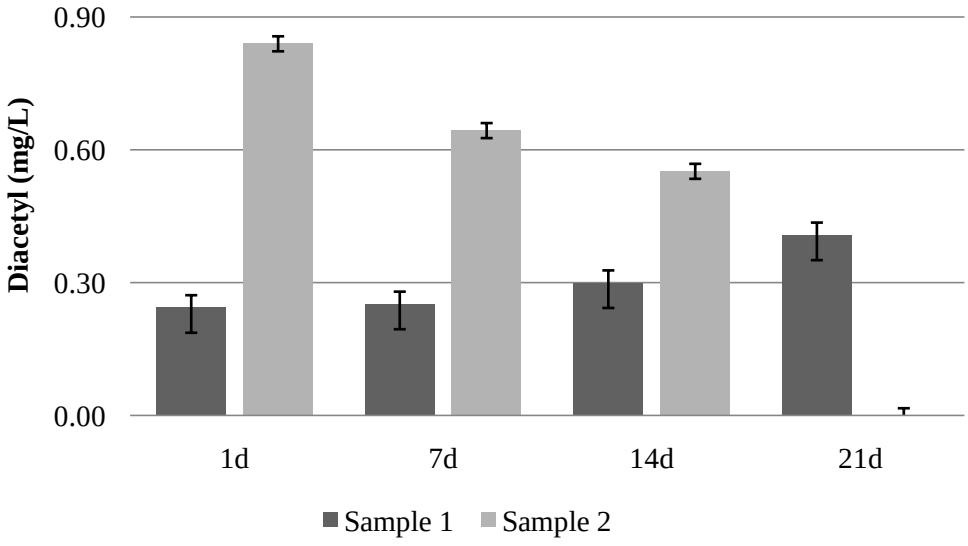

Figure 3.9. Changes of diacetyl amount for yoghurt samples during storage days.

Acetone has a sweet, fruity aroma and is known to influence the aroma and flavor qualities of yogurt. The changes of acetone concentrations in sample 1 ranging from 0.42 to 0.63 mg/L and in sample 2 ranging from 0.32 to 0.46 mg/L during storage days. So, the yoghurt sample 1 had the highest mean of acetone amount at day 21 than sample 2 (see Figure 3.10).

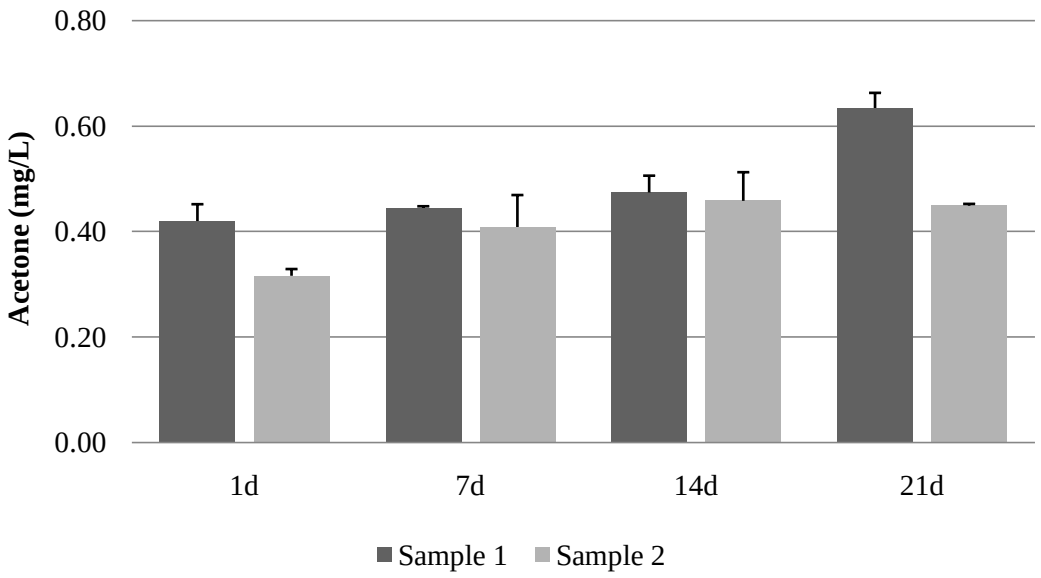

Figure 3.10. Changes of acetone amount for yoghurt samples during storage days.

3.2.6. Amount of Lactose and Lactic Acid Results

Lactic acid plays a specific role in the aroma and flavor properties of yoghurt. During yoghurt production, around 20-40% of lactose present in milk is transformed into lactic acid, and the content of lactic acid in yoghurt is around 0.9%. During storage, the decrease in lactose concentrations were generally accompanied by proportional increases in lactic acid concentration. Also, as decribed by Tamime and Robinson (2007), yoghurt after fermentation may contain between 4 and 5g of lactose per 100 mL yoghurt when milk is fortified to 14% non-fat solids.

Lactose and lactic acid amounts were determined during storage in all samples by HPLC. Retention times were determined for lactose on 8.055 minutes and for lactic acid on 12.968 minutes. Results were given in Table 3.11.

Table 3.11. Amounts of lactose and lactic acid in yoghurt samples during storage days.

	Storage days	Sample 1	Sample 2
Lactose (g/L)	1	$98.83 \pm 5.10^{c,A}$	$97.16 \pm 4.85^{c,A}$
	7	$69.17 \pm 0.14^{b,A}$	$94.57 \pm 9.32^{c,B}$
	14	$69.15 \pm 1.70^{b,A}$	$70.46 \pm 3.74^{b,A}$
	21	$53.76 \pm 0.01^{a,A}$	$68.84 \pm 1.82^{b,B}$
Lactic acid (g/L)	1	$12.61 \pm 0.78^{a,A}$	$12.65 \pm 0.44^{ab,A}$
	7	$13.06 \pm 1.29^{ab,A}$	$13.21 \pm 0.63^{ab,A}$
	14	$13.85 \pm 0.43^{ab,A}$	$13.48 \pm 1.12^{ab,A}$
	21	$14.81 \pm 1.07^{b,A}$	$15.30 \pm 0.90^{b,A}$

* $^{a–c}$ Means in the same column with different superscript letters differ significantly (P<0.05)
* $^{A-B}$ Means in the same row with different superscript letters differ significantly (P>0.05)
Means ± SD of triplicate samples.

No significant differences were observed among samples (P>0.05). Lactose and lactic acid changes during storage for sample 1 were given in Figure 3.11. Significant differences were observed during storage (P<0.05). Average lactose amount was determined 74.92 g/L. Minimum was determined 53.76 g/L on 21st day of storage and maximum was obtained 98.83 g/L on 1st day of storage. Lactose amount decreased during storage days. Lactic acid amount was determined between; 12.62 g/L to 14.81 g/L. Average amount of lactic acid was calculated 13.69 g/L. Lactic acid increased during storage.

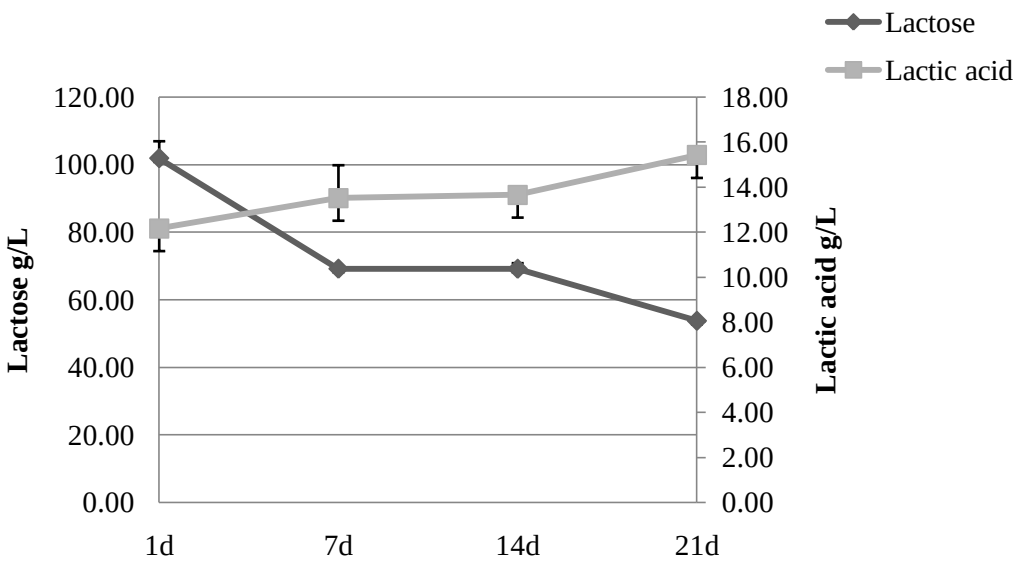

Figure 3.11. Lactose and lactic acid changes in sample 1 during storage.

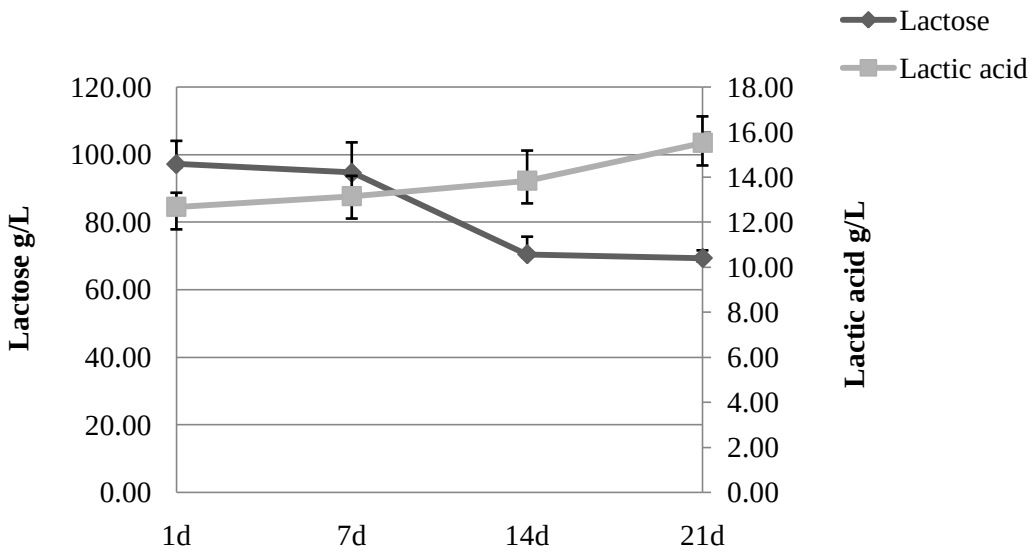

Figure 3.12. Lactose and lactic acid changes in sample 2 during storage.

Lactose and lactic acid changes during storage for sample 2 were given in Figure 3.12.Significant differences were observed in all samples during storage days ($P<0.05$). Average lactose amount was determined 82.88 g/L. Minimum was determined 68.84 g/L on 21st day of storage and maximum was obtained 97.16 g/L on 1st day of storage. Lactose amount decreased during storage days. Lactic acid amount was determined

between; 12.65 g/L to 15.30 g/L. Average amount of lactic acid was calculated 13.79 g/L. Lactic acid increased during storage.

3.2.7. Proteolytic Activity Results

Proteolytic activity of L. *bulgaricus*, *S. thermophilus and* aroma culture (P10) in yoghurts assessed during storage is shown in Fig 3.13. Generally, substantial proteolytic activity was detected in both yoghurt samples. The free amino acid content in both yoghurts was higher due to proteolytic activity of bacterias compared to (0h) milk as control (Donkor et al., 2007). Proteolysis is assessed by the release of free NH_3 groups by the OPA method, increased significantly ($P<0.05$) at a slower rate during storage for both yoghurt samples. However, proteolytic activity in sample 2 was significantly higher than that of control yoghurt at day 1 and the level of proteolytic activity remained substantially higher than the control yoghurt sample 1 during storage days. Results were observed between 0.213 to 0.340 units in sample 1 and between 0.365 to 0.567 units in sample 2.

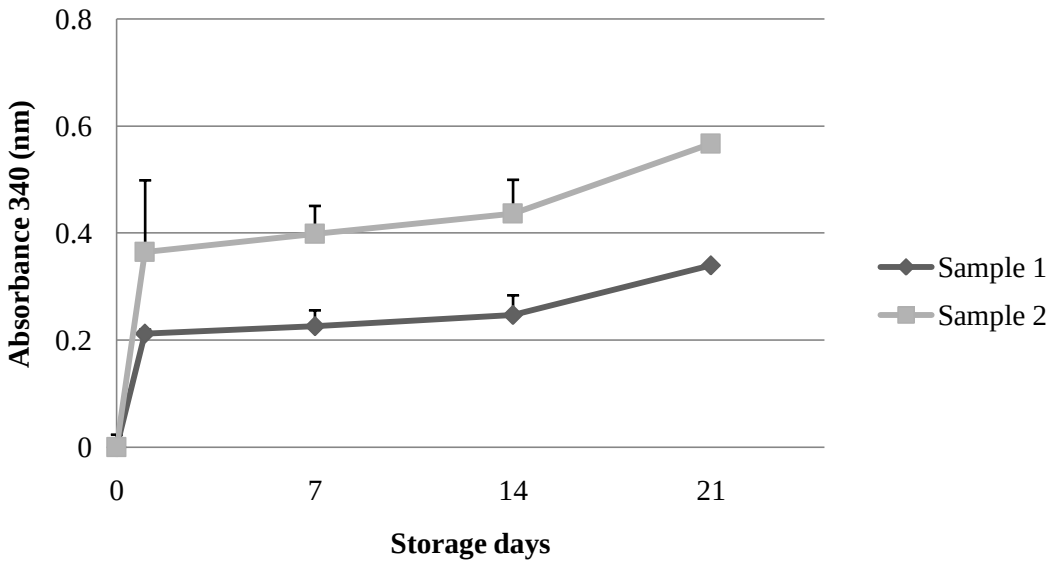

Figure 3.13. Proteolytic activity of control yoghurt sample 1, containing aroma culture yoghurt sample 2 during storage.

3.3. Lactic Acid Bacteria Counts

Cell growth of yoghurt samples during storage are shown in Table 3.12. Results were observed between 8.65 log cfu/mL to 11.57 log cfu/mL during storage. *S. thermophilus* counts were higher in sample 2 due to the stimulated growth of Streptococcus species and aroma culture. According to Figure 3.14 and Figure 3.15 both *L. bulgaricus* and *S. thermophilus* counts were decreased about 1 log cycle during storage. Similar results were reported by Donkor et al. (2007), Güler (2007). *L. bulgaricus* showed consistent decrease in cell concentration in both sample 1 and sample 2. Significant differences observed within samples during storage days ($P<0.05$). No significant differences were observed among samples during storage ($P>0.05$). Also, *St. thermophilus* showed consistent decrease in cell concentration in both sample 1 and sample 2. Significant differences were observed within all samples and among all samples during storage ($P<0.05$).

Quantitative standards for yoghurt bacteria differ are generally accepted that the yogurt should contain 10^7 cfu of viable bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) per mL of yoghurt (Tamine & Death, 2007; Salvador & Fiszman, 2004).

Table 3.12. Cell growth of yoghurt samples during storage days

	Storage days	Sample 1	Sample 2
S. *thermophilus* (log cfu/mL)	**1**	$11.42 \pm 0.01^{c,A}$	$11.57 \pm 0.03^{c,A}$
	7	$11.20 \pm 0.01^{bc,B}$	$10.79 \pm 0.02^{bc,A}$
	14	$9.88 \pm 0.03^{ab,A}$	$10.50 \pm 0.04^{b,B}$
	21	$9.68 \pm 0.03^{b,A}$	$10.18 \pm 0.73^{ab,A}$
L. *bulgaricus* (log cfu/mL)	**1**	$11.01 \pm 0.02^{g,B}$	$10.83 \pm 0.07^{f,A}$
	7	$10.88 \pm 0.09^{fg,A}$	$10.67 \pm 0.00^{e,A}$
	14	$9.85 \pm 0.01^{c,A}$	$10.05 \pm 0.04^{d,A}$
	21	$8.65 \pm 0.04^{a,A}$	$8.94 \pm 0.05^{b,A}$

* $^{a-g}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)

* $^{A-B}$ Means in the same row with different superscript letters not differ significantly ($P>0.05$)
Means ± SD of duplicate samples.

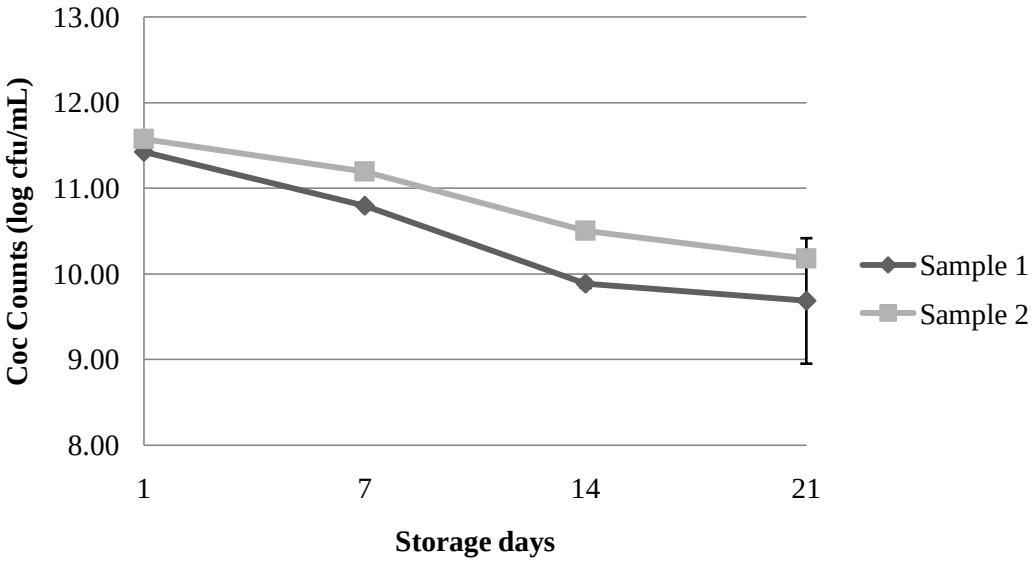

Figure 3.14. *S. thermophilus* enumeration changes in yoghurt samples during storage days. (*L. bulgaricus* + *S. thermophilus* + aroma culture = sample 2, *S. thermophilus* + *L. bulgaricus* = sample 1)

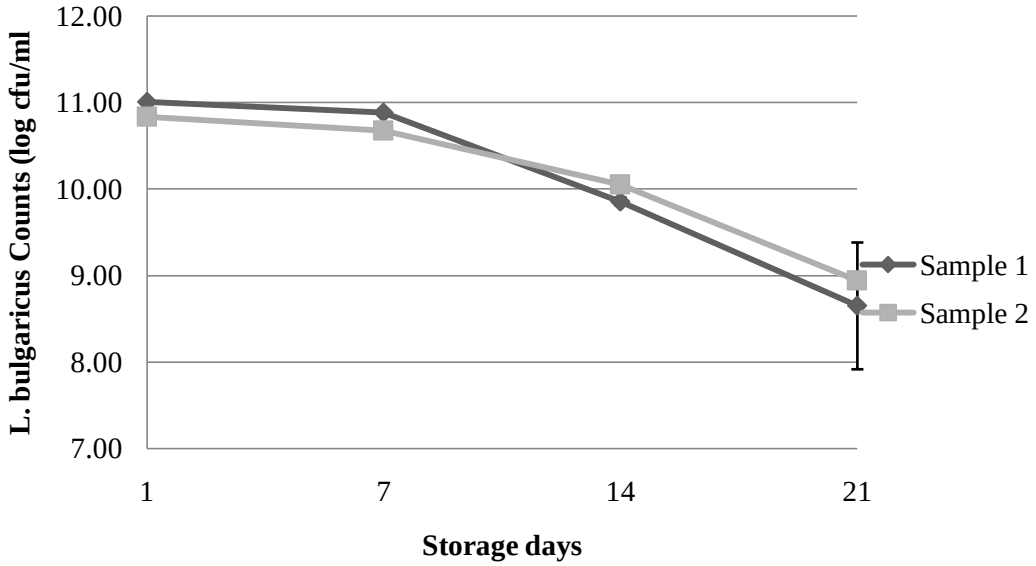

Figure 3.15. *Lactobacillus* ssp. enumeration changes in yoghurt samples during storage days.

3.4. Sensory Profile Results

Panelists scored sample 1 to 5 according to personal liking in sensory profile analyses. Sensory scores of yoghurt samples during storage days are presented in Table

3.13. According the results, samples sensory profile points were determined between; 4.75 to 3.47. Yoghurt sample 2 with aroma culture had the highest flavor and overall acceptability scores.

Appearance profiles of samples were compared. Average scores in appearance for sample 1; 4.22 and for sample 2; 4.30 were observed. No significant differences were determined among samples ($P>0.05$). But, significant differences were observed during storage days ($P<0.05$).

Average scores in odor were determined for sample 1; 4.16 and sample 2; 4.32. Sample 1 and sample 2 had scored close each other during storage. Among samples were significantly differences during storage ($P<0.05$). No significant differences were determined among samples ($P>0.05$).

No significant differences were determined among samples during storage days ($P>0.05$). Average scores of flavor were determined for sample 1; 3.92 and for sample 2; 4.26. Sample 1 and sample 2 had scored close each until to 14^{th} day. Additionally, yoghurt sample 2 with aroma culture had highest flavor score at 1^{st} day. This result supported aroma profile analysis.

Significant differences were obtained within the all samples during storage days ($P<0.05$). Average scores in overall acceptability were determined for sample 1; 4.02 and for sample 2; 4.48. Overall acceptability scores showed similarity with flavor scores. Sample 1 and sample 2 had scored close each other during storage ($P>0.05$). Only sample 1 more decreased on 14^{th} day of storage. These results showed that flavor was the most important factor in overall acceptability.

Moreover, samples from different brands of plain yoghurts were collected at a reigonal market. Sample 1 and Sample 2 were compared to 4 commercial yoghurt brands according to acetaldehyde concentration in Figure 3.16. Acetaldehyde amount was determined higher 33 mg/L approximately in sample 2 than the local market brands. Among the commercial yoghurts the highest acetaldehyde was obtained as 17.7 mg/L in Brand 2.

Also, flavor and overall sensory scores were compared for collected from market The highest flavor and overall sensory scores were in Brand 2 and Brand 3 among the commercial yoghurts. Sample 2 have highest sensory scores (see Table 3.13).

Table 3.13. Sensory scores for yoghurt samples during storage

Attributes	Storage days	Sample 1	Sample 2
Appearance	1	$4.45 \pm 0{,}07^{b,A}$	$4.10 \pm 0{,}28^{ab,A}$
	7	$4.30 \pm 0{,}14^{ab,A}$	$4.10 \pm 0{,}14^{ab,A}$
	14	$3.94 \pm 0{,}07^{a,A}$	$4.60 \pm 0.00^{b,B}$
Conistency on spoon	1	$4.20 \pm 0.14^{ab,A}$	$3.75 \pm 0.21^{a,B}$
	7	$4.25 \pm 0.07^{ab,A}$	$4.25 \pm 0.07^{ab,A}$
	14	$4.17 \pm 0.38^{ab,A}$	$4.60 \pm 0.14^{b,B}$
Conistency on mouth	1	$4.15 \pm 0.21^{b,A}$	$3.55 \pm 0.07^{a,A}$
	7	$4.35 \pm 0.07^{bc,A}$	$4.45 \pm 0.21^{bc,A}$
	14	$3.47 \pm 0.03^{a,A}$	$4.75 \pm 0.21^{c,A}$
Odor	1	$4.45 \pm 0.35^{a,A}$	$4.25 \pm 0.70^{a,A}$
	7	$4.00 \pm 0.14^{a,A}$	$4.70 \pm 0.28^{a,A}$
	14	$3.96 \pm 0.21^{a,A}$	$4.60 \pm 0.28^{a,B}$
Flavor	1	$4.15 \pm 0.49^{ab,A}$	$4.55 \pm 0.07^{b,B}$
	7	$4.20 \pm 0.28^{ab,A}$	$4.60 \pm 0.14^{b,B}$
	14	$3.52 \pm 0.10^{a,A}$	$4.00 \pm 0.42^{ab,A}$
Overall A.	1	$4.20 \pm 0.28^{ab,A}$	$4.65 \pm 0.07^{b,B}$
	7	$4.20 \pm 0.14^{ab,A}$	$4.40 \pm 0.00^{ab,A}$
	14	$3.74 \pm 0.05^{a,A}$	$4.35 \pm 0.35^{ab,A}$

* $^{a-c}$ Means in the same column (in each attribute) with different superscript letters differ significantly ($P<0.05$)

* $^{A-B}$ Means in the same row with different superscript letters not differ significantly ($P>0.05$)
Means ± SD of duplicate samples.

Table 3.14. Sensory scores for commercial yoghurts, sample 1 and sample 2.

Yoghurt samples	Apearance	Consistency on spoon	Consistency on mouth	Odor	Flavor	Overall A.
Brand 1	4.10 ± 0.14^{ab}	4.20 ± 0.42^{a}	3.65 ± 0.21^{ab}	3.60 ± 0.14^{a}	2.85 ± 0.49^{a}	3.15 ± 0.21^{a}
Brand 2	3.85 ± 0.21^{a}	3.80 ± 0.28^{a}	3.95 ± 0.21^{ab}	4.25 ± 0.35^{ab}	4.30 ± 0.00^{b}	4.25 ± 0.07^{bc}
Brand 3	4.35 ± 0.07^{ab}	4.40 ± 0.00^{a}	4.05 ± 0.21^{ab}	4.20 ± 0.14^{ab}	3.70 ± 0.28^{ab}	$3,95 \pm 0,07^{b}$
Brand 4	4.55 ± 0.07^{b}	4.45 ± 0.07^{a}	4.15 ± 0.07^{b}	4.05 ± 0.07^{ab}	2.85 ± 0.21^{a}	3.20 ± 0.28^{a}
Sample 1	4.45 ± 0.07^{b}	4.20 ± 0.14^{a}	4.15 ± 0.21^{b}	4.45 ± 0.35^{b}	4.15 ± 0.21^{b}	4.20 ± 0.00^{bc}
Sample 2	4.10 ± 0.28^{ab}	3.75 ± 0.21^{a}	3.55 ± 0.07^{a}	4.25 ± 0.07^{ab}	4.55 ± 0.07^{b}	4.65 ± 0.07^{c}

* $^{a-c}$ Means in the same column with different superscript letters differ significantly ($P<0.05$)

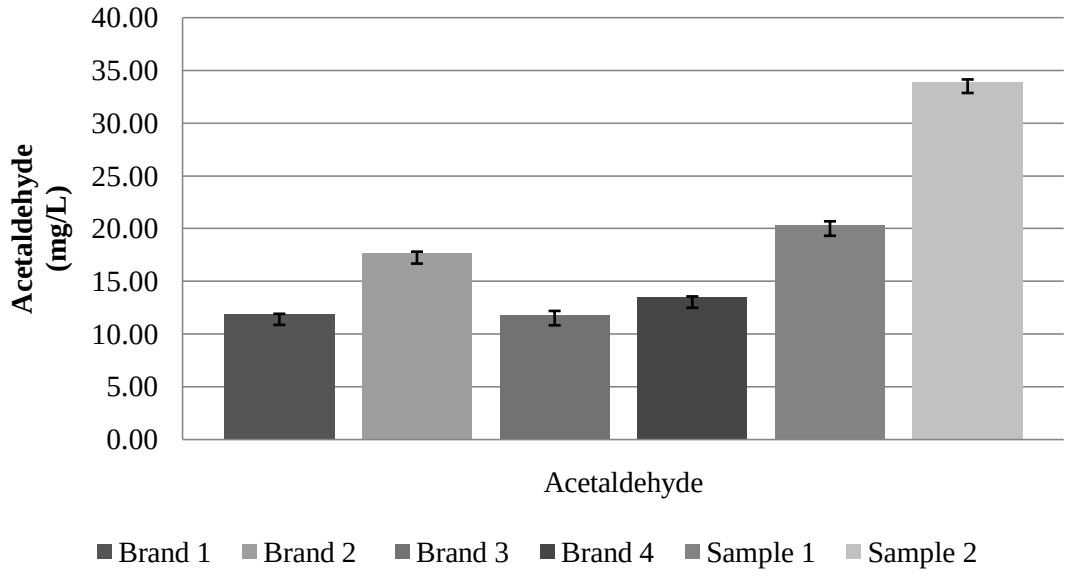

Figure 3.16. Acetaldehyde amounts in yoghurts acquired from a local market, sample 1 and sample 2.

CHAPTER 4

CONCLUSION

Investigation the chemical, physical, microbiological and organoleptic changes of the developed yoghurt products sample 1 and sample 2 during storage days were the objectives of this study.

A new yoghurt product sample 2 was produced according to screening results and chemical, physical, microbiological and organoleptic characteristics were determined in developed product during storage. Also, sample 2 was compared with control yoghurt sample 1 during fermentation hours and storage days.

There is an industrial need to produce traditional yoghurt with traditional taste and flavor. This can be only achieved by yoghurt starter cultures. Within this frame, the objective is to screen the artisanal yoghurt starter cultures for their aroma profiles and to explore the cultures having most desirable aroma for industrial production.

Average physicochemical characteristic for sample 1 were found as 3.98 pH, 1.49% titratable acidity, 20.20% total solids content, 2.39% total protein content, 4.46% total fat content, 46.59% syneresis, 223.93% mPa.s apparent viscosity were detected.

Volatile compounds of kefir samples were analyzed with GC-FID. Acetaldehyde, ethanol, acetone and diacetyl were determined during storage. All volatile compounds data were analyzed by amount mg/L. During storage days, the acetaldehyde, ethanol, acetone and diacetyl contents of the yoghurt samples were varied between 17.01–31.41 mg/L, 1.12-4.93 mg/L, 0.32-0.63 mg/L, 0.24-0.84 mg/L, respectively.

Acetaldehyde amount decreased in sample 1 and 2 and ethanol amount increased during storage days. The higher amount of acetaldehyde in yoghurt with aroma culture should be due to the fast metabolic activity of aroma culture. Furthermore, when the pH values were lower, the concentration of acetaldehyde increases through the acetaldehyde oxidized to acetate.

Ethanol amount of sample1 is higher than sample2. This result is not good for dairy industry and shelf life of yoghurt product.

The decrease of diacetyl concentration in sample 2 by end of the storage could be hydrolyzed by microbial enzymes to form other compounds .Control yoghurt sample 1

had little diacetyl production (0.24-0.41 mg/L). However, in yoghurt sample 2 with aroma culture, the diacetyl concentration range was greater (0.54-0.84 mg/L). This result may be given explanation by the high ratio bacilli/cocci starter culture that is used at yoghurt production

According to sensory results new yoghurt with aroma culture is suitable for consumer preference. Average scores of flavor were determined for sample 1; 3.92 and for sample 2; 4.26. Additionally, yoghurt sample 2 with aroma culture had highest flavor score at 1st day. This result supported aroma profile analysis. Average scores in overall acceptability were determined for sample 1; 4.02 and for sample 2; 4.48. Overall acceptability scores showed similarity with flavor scores. These results showed that flavor was the most important factor in overall acceptability.

In this study, addition of aroma cultures to the starter preparations had an effect on highest acetaldehyde production. The traditional yoghurt with aroma culture contained more acetaldehyde than well known commercial yoghurts in the region. Samples from different brands of plain yoghurts were collected at a reigonal market. Also, our samples sample 1 and sample 2 were compared to 4 commercial yogurts according to acetaldehyde concentration. The acetaldehyde production, flavor and overall sensory analysis were compared for collected from market.

Future investigations on the topic may include the following suggestions:

1. Artisanal yoghurt can be produced in the industrial scale.
2. Development of optimal aroma profiles experiments can be done using various scale up in industrial area during incubation hours and storage days.

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APPENDIX A

CHEMICAL AND MEDIA

Table A.1. Chemical Used

No	Chemical	Code
1	MRS Broth	Merck1.10661
2	M17 Broth	Merck 1.15029
3	Bacteriological Pepton	Oxoid LP037
4	Peptone water	Merck 1.07228.0500
5	NaOH	Riedel-de Haen 06203
6	Phenolftalein	Merck 1.07233.0100
7	Yeast extract	Merck A 1.03753
8	Agar	AppliChem A0949
9	Sulfuric acid	Merck 1.00729.2500
10	Filter paper(Whatman No: 42)	ISOLab
11	n-Amyl alcohol (for synthesis)	Merck 8.07500
12	HCl	Reidel-de Haen 07102
13	n-Amyl Alcohol	Merck 8.07500.1000
14	Sulfuric acid 95-97%	Fluka
15	Lactic acid	Sigma L1750
16	D(+) Glucose	AppliChem A3666
17	D(+) Lactose	Sigma L3750
18	Isopropanol	AppliChem A3928
19	Ethanol	Merck 100986
20	Diacetyl	Merck 8035280100
21	Glycerol	AppliChem A2926
22	Anaerogen	Oxoid AN0025A
23	Sodium phosphate monobasic dihydrate	Sigma 71505
24	Sodium hydroxide	Panreac 141687
25	Acetaldehyde	Merck 8450010100
26	Acetoin	Merck 8206640100