CHEMICAL AND RHEOLOGICAL PROPERTIES OF YOGHURT PRODUCED BY LACTIC ACID CULTURES ISOLATED FROM TRADITIONAL TURKISH YOGHURT

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CHEMICAL AND RHEOLOGICAL PROPERTIES OF YOGHURT PRODUCED BY LACTIC ACID CULTURES ISOLATED FROM TRADITIONAL TURKISH YOGHURT

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

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Yoghurt is a fermented milk product which is produced by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. The production of yoghurt has started in Middle East and spread all over the world. The aim of this study is to select the culture combination which is appropriate to Turkish taste and have the best yoghurt characteristics by means of post-acidification and whey separation properties, texture of gel formation, exopolysaccharide and acetaldehyde content; and to observe the effect of freeze-drying of cultures on these yoghurt properties.

At the first part of this study, six *L.delbrueckii* spp. *bulgaricus* isolates and six *S.thermophilus* isolates were used with different combinations to produce 36 yoghurt samples. These isolates were selected among a strain collection which contains 111 *L.delbrueckii* spp. *bulgaricus* and 56 *S.thermophilus* isolates which were isolated from traditional Turkish yoghurt according to their acidification activity and acetaldehyde production properties. In addition, two commercial *S.thermophilus* isolates and one commercial *L.delbrueckii* spp. *bulgaricus* isolate were used to produce two commercial yoghurt samples. 38 yoghurt samples were examined in terms of pH and total titratable acidity changes during 21-day storage, syneresis and hardness. According to these three analyses, six yoghurt samples were chosen, which give the best results, for the determination of exopolysaccharide and acetaldehyde content. In addition, two yoghurt samples produced by commercial cultures and one sample, which gives average results in experiments, were also examined for these compounds to provide a good comparison.

In the second part of the study the amount of exopolysaccharide and acetaldehyde of nine yoghurt samples were determined. In addition, sensory analysis was conducted to see consumer perception. According to the results, one culture combination was obtained as the best combination which produces the appropriate yoghurt to Turkish taste with the closest chemical analysis results to the commercial samples.

In the last part, freeze drying process was examined if this has a significant effect on the selected LAB combination as well as yoghurt produced by using this.

Keywords: Yoghurt, starter culture, exopolysaccharide, acetaldehyde, texture analysis

ÖZ

GELENEKSEL TÜRK YOĞURTLARINDAN İZOLE EDİLEN LAKTİK KÜLTÜRLER TARAFINDAN YAPILAN YOĞURTLARIN KİMYASAL VE FİZYOLOJİK ÖZELLİKLERİ

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Yoğurt, *Streptococcus thermophilus* ve *Lactobacillus bulgaricus* tarafından üretilen fermente bir süt ürünüdür. Yoğurt üretimi Orta Doğu'da başlamış ve buradan tüm dünyaya yayılmıştır. Bu çalışmada, Türk damak tadına en uygun ve aynı zamanda post-acidifikasyon, serum ayrılması, yapısal özellikler, ekzopolisakkarit ve asetaldehit içeriği açısından en iyi özelliklere sahip yoğurdu üreten kültür kombinasyonunu seçmek ve liyofilizasyonun bu yoğurt kültürleri ve özellikleri üzerindeki etkisini görmek amaçlanmıştır.

Bu çalışmanın ilk bölümünde, altı *L.delbrueckii* spp. *bulgaricus* ve altı *S.thermophilus* izolatı kullanılarak çeşitli kombinasyonlar oluşturulmuş ve 36 yoğurt örneği üretilmiştir. Bu izolatlar, geleneksel Türk yoğurtlarından izole edilen 111 *L.delbrueckii* spp. *bulgaricus* ve 56 *S.thermophilus* izolatı arasından asidifikasyon aktiviteleri ve asetaldehit üretme özelliklerine göre seçilmiştir. Ek olarak, 2 tane ticari *S.thermophilus* ve 1 tane de *L.delbrueckii* spp. *bulgaricus* izolatı 2 tane ticari yoğurt örneği üretmek üzere kullanılmıştır. 38 yoğurt örneği 21 günlük depolama boyunca oluşan pH ve titrasyon asitliği değişikliği, serum ayrılması ve sertlik özellikleri yönünden incelenmiştir. Sonuç olarak, en iyi sonuçları veren altı yoğurt örneği ekzopolisakkarit ve asetaldehit miktarının belirlenmesi analizlerinde kullanılmak için seçilmiştir. Ek olarak, ticari kültürler kullanılarak üretilen iki yoğurt örneği ve testlerde ortalama sonuçlar veren bir örnek de karşılaştırma yapılabilmesi için bu maddeler yönünden incelenmiştir.

Çalışmanın ikinci bölümünde, dokuz yoğurt örneğinin ekzopolisakkarit miktarı ve asetaldehit miktarları belirlenmiştir. Ek olarak, tüketici beğenisini ölçmek için duyusal analiz yapılmıştır. Sonuçlara göre, Türk damak tadına en uygun ve kimyasal analizleri ticari yoğurt örneklerine en yakın olan bir kültür kombinasyonu liyofilizasyon işlemi için seçilmiştir.

Son bölümde, liyofilizasyon işleminin seçilen laktik asit bakteri kombinasyonunun ve bu kültürle üretilen yoğurdun üzerindeki etkisi incelenmiştir.

Anahtar Kelimeler: Yoğurt, starter kültür, ekzopolisakkarit, asetaldehit, yapısal analiz

To my family

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INTRODUCTION

1.1 Milk

Milk is one of the most valuable and natural food materials. It is a white opaque liquid produced by the mammary glands of mammals to feed the newly born before they are able to digest other types of food. According to evidence, animal milk has been used as a food material since around 5000 BC (McGee, 2004).

The basic components of milk are water, fat, lactose, protein, mineral substances, organic acids and vitamins. Milk is collected from different sources; namely, cow, buffalo, goat, yak, sheep, horse and camel so milk may have different compositions depending on the source and the compositions are given in Table 1.1. In addition, the source and content of milk affect the pH. Milk has a pH ranging from 6.3 to 6.9 (Kanwal, Ahmed, & Mirza, 2004).

Table 1.1 Composition of milk from different sources (Tamime & Robinson, 2007)

	Buffalo	Cow	Goat	Sheep
Total solid (%)	14.04	13.73	13.55	18.53
Fat (%)	5.25	4.56	4.73	8.96
Solid-Non-Fat (%)	8.79	9.17	8.92	9.71
Lactose (%)	3.92	4.03	4.66	3.57
Protein (%)	3.87	5.23	2.38	6.57
Total Nitrogen (%)	0.62	0.86	0.39	1.03
Non-Protein-Nitrogen (%)	0.004	0.004	0.001	0.005
Ash (%)	0.4	0.36	0.28	0.58
Water (%)	85.96	86.27	86.45	81.47

As all mammals, milk has an important role in human diet from the birth. It has an antimicrobial activity through some enzymes. Therefore, it is also a protective nutrition for all the newborns of mammals. One liter of milk supplies the daily requirements are given in Table 1.2 for the human being (Spreer, 1998).

Table 1.2 Daily requirements supplied from one liter of milk (Spreer, 1998)

Nutrients and Energy	Percent of daily requirement (%)
Calcium	100
Phosphorus	67
Vitamin A	30
Vitamin B_1	27
Vitamin B_2	66
Vitamin C	19
Protein	49
Iron	3
Energy	20

Milk is processed to produce lots of products. These processes are generally used to make shelf-life longer. Yoghurt, cheese, butter, milk powder, cream, kefir can be counted among the milk products.

1.2 Fermentation and Fermented Milk Products

Fermentation is a method used for thousands of years to provide longer shelf life to perishable foods and also different flavor. It is known that fermented foods have been made since Neolithic times. The first examples of fermented foods are wine, bread and cheese. In East Asian regions, yoghurt and other fermented milk products, traditional alcoholic beverages, vinegar and pickles followed these examples

(Shurtleff & Aoyagi, 2007). Fermentation is a chemical process in which enzymes break down organic substances into smaller compounds. In the result of fermentation, more digestible, more stable and more flavored foods are produced. Fermentation is carried out by molds, yeast or bacteria. During the growth of these microorganisms, fermented foods are produced incidentally.

Milk can be fermented by all these organisms. The products of these different processes can be classified as shown in the Figure 1.1 (Tamime & Robinson, 2007).

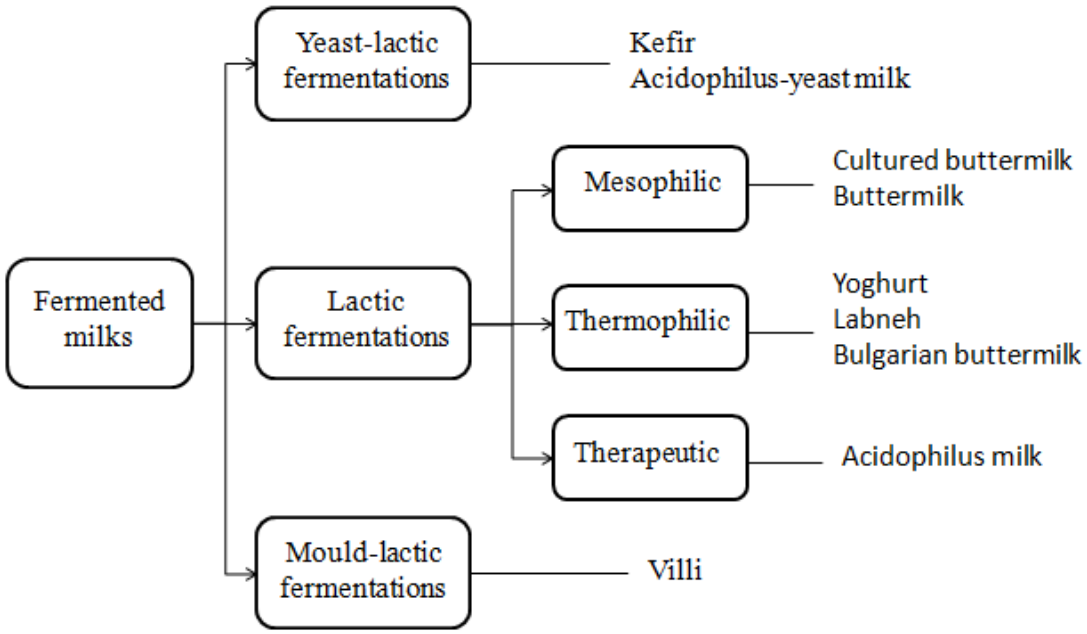


Figure 1.1 Classification of fermented milk (Tamime & Robinson, 2007)

1.3 Yoghurt

Yoghurt is possibly the oldest fermented milk product (Harper & Hall, 1981). It is obtained from lactic fermentation of milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus.* It is thought that the origin of yoghurt was Middle East (Tamime & Robinson, 2007).

Yoghurt has characteristics between cultured milk and semi-soft cheese (Webb & Whittier, 1970). Texture of yoghurt mostly depends on the strains of lactic acid culture and content of the milk.

Yoghurt, similar to milk, is an excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitamin B_{12}, folate, niacin, magnesium and zinc. Since lactose in milk is converted into lactic acid during fermentation, lactose intolerant people can consume yoghurt without any adverse effect. In addition, yoghurt consumption causes a small increase of stomach pH and this reduces the risk of the pathogen passage and the effects of low gastric juice secretion problem.

1.4 Economical Aspects

Traditional product demand decreased by improvement in cooling technology. However, worldwide yoghurt consumption statistics show significant increase between 1975-2000 (Akın, 2006). In Turkey, 45% of raw milk is processed in urban areas, 40% is processed in small size dairy houses without technological machinery

and only 25% is processed in modern factories according to regulations and hygienic conditions. In European Union, this ratio is about 90-95% (Akın, 2006).

Incentives given in recent years in Turkey help foundation of new dairy houses and modernization of existing facilities. As a result the number of dairy houses increased from 815 to 1035 between 2003 and 2008, according to Turkish Statistical Institute, detailed numbers of dairy houses are given in Table 1.3. In addition, Table 1.4 shows percent yearly increase statistics of dairy production in Turkey (Şahin, 2011).

Table 1.3 Number of dairy houses, annually (Şahin, 2011)

Year	2003	2004	2005	2006	2007	2008
Dairy products production	966	1112	1061	1250	1311	1262
Dairy houses and cheese production	815	920	887	1024	1098	1035
Ice cream production	151	192	174	226	213	227

Table 1.4 Yearly increase of dairy production (Şahin, 2011)

Year	2006 (%)	2007 (%)	2008 (%)	2009 (%)	2010 (%)	Average (%)
Dairy production	10.1	7.6	8.5	0.0	12.4	8.9

1.5 Yoghurt Manufacturing

In industry, yoghurt generally produced in two types, namely, set yoghurt and stirred yoghurt. Flow chart for each yoghurt production is given in Figure 1.2 (Walstra, Wouters, & Geurts, 2006).

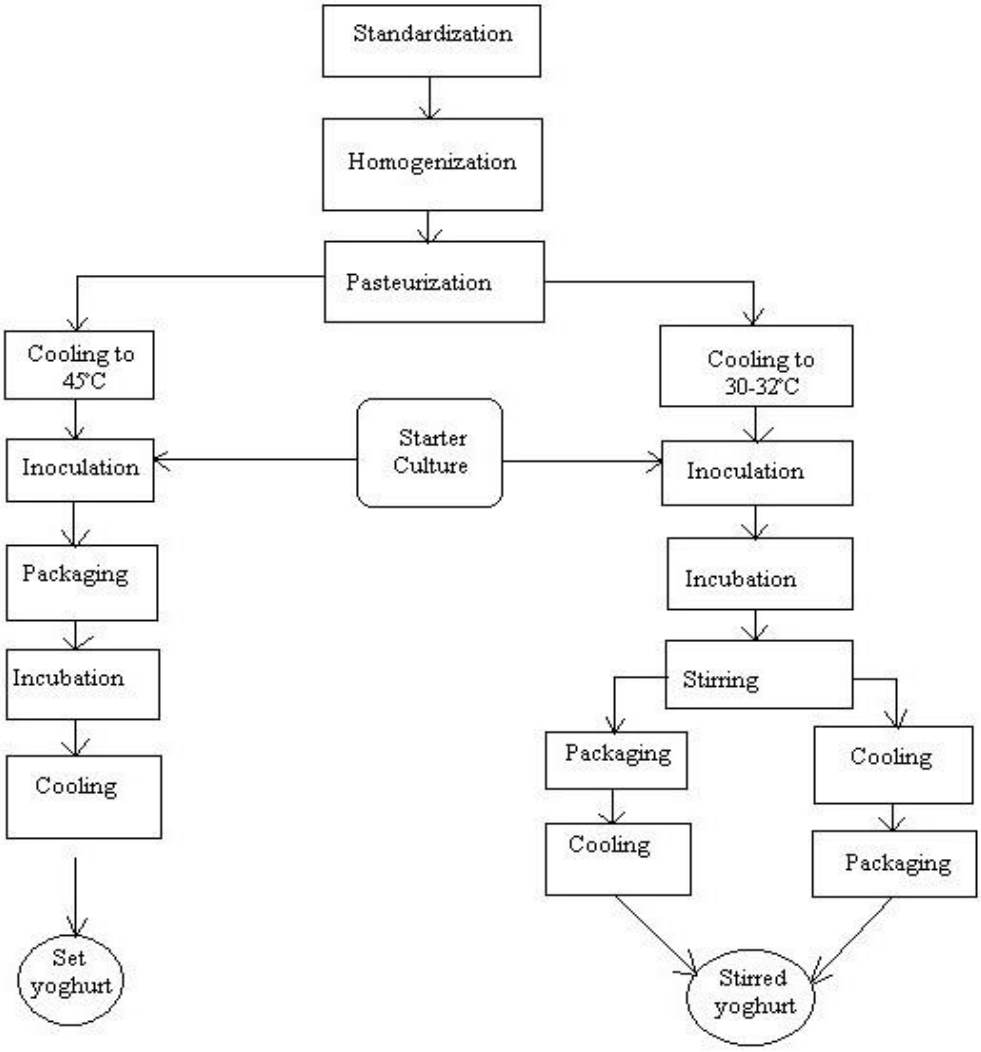


Figure 1.2 Flow chart of set yoghurt and stirred yoghurt production (Walstra, Wouters, & Geurts, 2006)

Set yoghurt is made of concentrated milk. It is firstly packaged and then incubated. After fermentation, a firm gel is obtained and maintained. However, stirred yoghurt is made generally non-concentrated milk and fermented in bulk. The formed gel after fermentation is stirred to get smooth and pourable yoghurt. In addition, between these two yoghurts there is a microbiological difference because there are only certain strains that can reach the correct consistency and thickness after stirring. The disadvantage of stirred yoghurt is that, at low temperature aroma compounds in yoghurt is produced less amount. Therefore, incubation temperature and time should be designed the same as set yoghurt (Walstra, Wouters, & Geurts, 2006).

According to Tamime and Robinson (2007) and Turkish Standard (TS 10935/ April 1993), yoghurt manufacturing can be described as follow. In addition to yoghurt manufacturing rules, Turkish Standard 10935 also determines the content of yoghurt. According to TS 10935, the use of any other materials than milk, milk powder, milk fat and starter culture during yoghurt production are forbidden.

Standardization: In industry, fat content and non-fat-solid content of milk are standardized for a good and standard quality yoghurt production. Minimum fat content for normal yoghurt is 3%, for low-fat yoghurt is 1.5% and for non-fat yoghurt is lower than 1.5%. In addition, non-fat-solid content should be minimum 12% in commercial yoghurt (TS 1330/February 1999). To maintain required fat content, cream is removed or added to the milk (Tamime & Robinson, 2007). Non-fat solid content can be adjusted in several ways. One of the most used methods is evaporation in which water is removed from milk under pressure so total solid content of milk increases. The other way is adding skim milk powder to the milk. Also, membrane

filtration methods or condensed milk addition are other ways to increase the non-fat-solid content (TS 10935/April 1993).

Homogenization: It is applied to prevent separation of fat from the milk, especially during fermentation (Tamime & Robinson, 2007). Also, it is a good process for mixing the milk content after standardization step. Homogenization causes some chemical changes in milk. First, due to the reduced fat globule size casein micelles adsorbs fat and suspended matter volume increases and this increases the viscosity. Then, due to increase in total fat surface area, lipolysis increases. Also, cream line formation is prevented because of the prevention of fat separation from milk. Additionally, homogenized milk is seen whiter because small fat globules reflect and scatter the light. However, foaming is a disadvantage of homogenization. During homogenization, phospholipids are transferred to the skimmed milk part and this cause foaming while pumping to the fermentation tanks (Walstra, Wouters, & Geurts, 2006).

Heat treatment: Heat treatment is applied to kill pathogenic organisms in raw milk. Different time temperature relations can be used according to raw material, product and process requirements. In yoghurt production, pasteurization is made at 80-85°C for 20-30 minutes or 90-95°C for 3-5 minutes (TS 10935/April 1993). This process is called high pasteurization and destroys all vegetative cells but bacterial spores remain. Whey proteins are denatured and most enzymes are inactivated except bacterial proteinases and lipases. According to researches, pasteurization improves gel formation. Since in heated milk casein micelles form a chain matrix and maintain a good distribution of protein all over the yoghurt, aqueous phase is stuck in this

matrix. Therefore, syneresis is not likely and coagulation is firmer than unheated milk (Tamime & Robinson, 2007).

Inoculation: After pasteurisation, milk is cooled to 42-45°C. Mixed culture, which contains *S.thermophilus* and *L.delbrueckii* subsp. *bulgaricus* with a ratio 1:1, is used for inoculation. Inoculation rate can vary from 0.5% to 4% (v/v).

Incubation: After inoculation, milk is hold at 42-45°C that is the optimum growth condition for *S.thermophilus* and *L.delbrueckii* subsp. *bulgaricus* mixed culture. Fermentation is terminated when pH reaches to 4.5-4.6. Fermentation time depends on inoculation rate and incubation temperature. There are two types of fermentation. First one is overnight incubation which is done at 30°C for 16-18 hours (Tamime & Robinson, 2007). Second fermentation is done at higher temperature and lower time (e.g. at 42°C for 4-5 hours). This type of fermentation is the commonly used one in industry because of short production time.

Cooling and storage: In industry multi-stage cooling system is used for yoghurt process (Tamime & Law, 2001) (White, 1995). Multi-stage cooling is described in four steps. First step is shock cooling which is cooling from 42°C to 30°C. In second stage, called dysgentical stage, product is cooled to 20°C. Third stage is lact-less phase in which product is cooled to 14.5°C. Finally, in holding phase, product is kept at 2-4°C until transportation and then consumption. However, according to Tamime and Law. (2001), in industrial use, the steps of multi-stage cooling are not separated sharply and some stages are combined.

1.6 Components of Yoghurt and Effects on Health

The constituents of food materials are used for the determination of their nutritional values. Since milk is a very valuable and complete food, yoghurt becomes nutritive food stuff. Although the composition of milk does not significantly change during fermentation, lots of components chemically change and it is considered that these changes give certain beneficial effects to yoghurt (Walstra, Wouters, & Geurts, 2006). Table 1.5 shows the components of full-fat milk and yoghurt and non-fat milk and yoghurt (Akın, 2006).

Table 1.5 Content of full-fat and non-fat milk and yoghurt (100 g) (Akın, 2006)

Components	Unit	Full-fat milk	Full-fat yoghurt	Non-fat milk	Non-fat yoghurt
Energy	Kcal	68	70	35	39
Protein	G	3.3	3.8	3.5	4.4
Fat	G	3.8	3.8	0.1	0.1
Carbohydrates	G	4.7	4.6	4.8	4.9
Lactic acid	G	0	0.8	0	1.0
Potassium	mg	157	157	150	187
Calcium	mg	120	120	123	143
Phosphorus	mg	92	92	97	109
Magnesium	mg	12	12	14	14
Sodium	mg	48	48	53	57
Iodine	mg	0.46	0.46	0.45	0.44
Chlorine	mg	102	102	100	121
Vitamin A	µg	30	29	-	0.8
Vitamin B_2	µg	180	180	170	180
Niacin	µg	90	90	90-100	92
Pantothenic acid	µg	350	350	280	360
Vitamin B6	µg	46	46	50	47
Biotin	µg	3.5	3.5	1.5	3.6
Folic acid	µg	0.29-6.88	1.0	-	0.5
Vitamin B12	µg	0.42	0.09	0.3	0.43
Vitamin C	mg	1.7	1.0	0.23-2	1-2.5

1.6.1 Total Solid Content

Total solid content expresses the amount of lactose, other carbohydrates, fat, proteins and minerals in milk. Minimum solids-not-fat (SNF) content depends on legal standards. Existing legal standards require 8.2 - 8.6 % SNF content (TS 1330, 1999). This minimum limit is applied to protect the consumers' rights. This is approximately same with the SNF content of milk. From producer point of view, total solid content of yoghurt has an important role on consistency and viscosity of yoghurt.

According to Tamime's research in 1977, increasing total solid content in yoghurt from 12 % to 20 % maintains the higher consistency and viscosity values. Since this increase is less between 16 % and 20 % solid content, generally up to 16 % solid content is used for yoghurt production (Tamime & Robinson, 2007). On the other hand, if total solid content is above 25 %, because of less amount of available water content, propagation of some starter cultures strains might reduce (Patel & Chakraborty, 1985). Optimum total solid content for yoghurt starter cultures, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* are 12 % and 14 %, respectively (Al-Dabbagh & Allan, 1989).

There are different ways to increase the total solid content of yoghurt milk and these can be listed as below (Akın, 2006):

- Boiling of milk
- Milk concentration by vacuum evaporation

- Membrane filtration (ultra-filtration, reverse-osmosis)
- Milk powder addition
- Whey concentrate or whey powder addition
- Whey protein concentrate or whey protein powder addition
- Casein addition
- Non-milk-protein addition
- Stabilizer or emulsifier addition

1.6.2 Lactose

In yoghurt, there are many types of monosaccharide and disaccharide but in trace amount. However, lactose content is very high even after fermentation since the main sugar in milk is lactose. Lactose content in milk, which is 4.8%, decreases to 2.5-2.6% in yoghurt. It cannot be allowed hydrolysis of all lactose in milk by lactic acid bacteria because as an end product of hydrolysis, lactic acid is produced and decreases the pH of yoghurt to the unacceptable levels (Walstra, Wouters, & Geurts, 2006). However, remaining lactose in yoghurt does not show the same discomfortability with the lactose in milk on people who are lactose-intolerant or lactose maldigestor.

Lactose intolerance can be described as the inability of human to metabolize lactose. At birth, babies can secrete β-galactosidase enzyme and break down lactose into glucose and galactose. Since milk consumption decreases after childhood, secretion of this enzyme gets slower or stops. Therefore some problems rise after consumption in milk, such as abdominal bloating, cramp and diarrhea. Although, yoghurt consists

high amount of lactose, it does not cause problems in lactose-intolerant. Different researches showed that after ingestion of yoghurt lactic acid bacteria continue to metabolize lactose (Gallagher, Molleson, & Caldwell, 1974). The researchers who have worked on surviving of lactic acid bacteria until small intestine, showed that the yoghurt curd protect strains from gastric digestion so lactic acid bacteria can survive and break down lactose until small intestine (Pochart, Dewit, Desjeux, & Bourlioux, 1989). In addition to that, in small intestine, lactic acid bacteria cells autolysis and lactase in the cell is released into small intestine and helps the digestion of lactose (Martini, Bollweg, Lewitt, & Savaiano, 1987).

1.6.3 Proteins

Milk is a valuable dietary source also from the point of view of proteins. Milk includes caseins (α-La), whey proteins (β-Lg) and essential amino acids (Tamime & Robinson, 2007). The difference between protein amounts of milk and yoghurt is caused by standardization because in this step milk is condensed to increase the total solid content. In addition to that, milk protein digestibility increases because of proteolytic activity of starter cultures during fermentation (Breslaw & Kleyn, 1973). Thus, daily protein requirement can be provided by yoghurt with fewer amounts than milk as, 200-250 ml yoghurt per / day (Altschul, 1965).

Proteolysis ability, rate and type of substrate strictly depend on the strains of starter cultures. For example, while enzymes of *L.delbrueckii* spp. *bulgaricus* show the proteolytic activities on casein molecules, enzymes of *S.thermophilus* work on the

intermediate products produced during casein hydrolysis (Poznanski, Lenoir, & Mocquot, 1965).

Proteolytic activity maintains some advantages to yoghurt. Firstly, some amounts of free amino acids, which are given in Table 1.6, formed during fermentation are used for growth of starter cultures (Tamime & Deeth, 1980). Secondly, as mentioned before, protein digestibility increases by means of protein degradation by enzymes (McLaughlan, Anderson, Widdowson, & Coombs, 1981). In addition, some people have an allergy against milk proteins and protein degradation caused by fermentation and heat treatment prior to fermentation may decrease these reactions (Akın, 2006).

Table 1.6 Free amino acid content of cow's milk and yoghurt (mg per 100 mL) (Tamime & Deeth, 1980)

Amino acid	Milk	Yoghurt
Alanine	0.16-0.64	1.17-3.80
Arginine	0.16-0.96	0.70-1.39
Aspartic acid	0.23-0.52	0.70-1.20
Glycine	0.30-0.53	0.28-0.45
Glutamic acid	1.48-3.90	4.80-7.06
Histidine	0.11	0.80-1.70
Isoleucine	0.06-0.15	0.15-0.40
Leucine	0.06-0.26	0.70-1.82
Lysine	0.22-0.94	0.80-1.11
Methionine	0.05	0.08-0.20
Phenylalanine	0.05-0.13	0.17-0.61
Proline	0.12	5.40-7.05
Serine	0.08-1.35	1.50-2.90
Threonine	0.05-0.26	0.24-0.70
Tryptophan	Tr*	0.2
Tyrosine	0.06-0.14	0.18-0.61
Valine	0.10-0.25	0.90-1.86
Total	3.29-10.31	18.77-33.06

* Tr: Trace

1.6.4 Lipids

Lipids are the most valuable energy source by providing 9 kcal / gram and required for a balanced diet. In addition to being an energy source, lipids are used in the body in two ways (Tamime & Robinson, 2007):

- storage fat; composed of saturated fatty acids and used as protector for vital organs
- structural fat; with proteins forms essential membranes in animal cells

A research conducted with 54 voluntaries shows that consumption of yoghurt in diet may help decrease serum cholesterol (Hepner, Fried, Jeor, Fusett, & Morin, 1979).

Milk fats are composed of more than 400 different fatty acids which are in the form of glycerides (Patton & Jense, 1974). These different types of fatty acids contain also the volatile ones which are shown in Table 1.7 (Yukuchi, Goto, & Okonogi, 1992). During fermentation and storage, volatile fatty acids in yoghurt show a significant increase. The level of this increase depends on the used strains, temperature of heat treatment, incubation temperature and time. Researches claim that *L.delbrueckii* spp. *bulgaricus* strains produce more volatile fatty acids than *S.thermophilus* strains during yoghurt fermentation (Tamime & Robinson, 2007). Although these volatile fatty acids are very small amounts in yoghurt to have nutritional value, these are very important role in organoleptic properties of yoghurt and so in consumer acceptance.

Table 1.7 Volatile fatty acids content of raw milk and full-fat yoghurt (Tamime & Robinson, 2007)

Fatty Acid	Raw Milk		Full-fat yoghurt	
	mg	%	Mg	%
Citric Acid	229.6	89.4	232.40	28.1
Lactic Acid	8.82	3.4	486.45	58.9
Succinic Acid	0	0	18.95	2.3
Fumaric Acid	1.10	0.4	8.41	1.0
Ketoglutaric Acid	0.74	0.3	0.87	0.1
Pyruvic Acid	0.09	0	2.38	0.3
Formic Acid	1.33	0.5	19.51	2.4
Acetic Acid	8.35	3.2	43.80	5.3
Propionic Acid	0.74	0.3	1.78	0.2
n-Butyric Acid	0.35	0.1	0.70	0.1
n-Valeric Acid	0.20	0.1	-	(0)
Caproic Acid	1.04	0.4	1.32	0.2
Caprylic Acid	2.88	1.1	6.63	0.8
Caprinoic Acid	1.72	0.7	2.58	0.3
Lauric Acid	-	-	-	-

1.6.5 Lactic Acid

S.thermophilus and *L.delbrueckii* spp. *bulgaricus* and *L.acidophilus* are the bacteria which can catabolise lactose into mainly lactic acid. This reaction can be simplified into following equation:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 4\ C_3H_6O_3$$

Lactose Water Lactic acid

Lactic acid is an important factor for yoghurt production. First of all, it reduces the pH level of milk to 4.6-4.7. During this decrease, lactic acid converts the colloidal calcium-phosphate bridges, which maintain the stability of casein micelles, to the soluble fractions. When these fractions start to diffuse out of the casein micelles, micelles coagulate and yoghurt gel formation occurs. About pH 5.0 the gel formation starts and gel firmness reaches the maximum at 4.6 - 4.7 (Tamime & Robinson, 2007). Gel firmness increases with high heat treatment and time in cold storage (Lee & Lucey, 2003). This formation is also called acid gelation. Loosening of gel network may be caused by low temperature heat treatment, high incubation temperature and low inoculation rate. Loosening can be described as whey syneresis on gel surface. Acid gelation can be shown as follows (Dyachenko, Chemistry of Milk, 1971):

Ca-caseinate-phosphate complex + lactic acid ⟶ casein complex
+
Ca-lactate
+
Ca-phosphate

Secondly, lactic acid is required for characteristic yoghurt taste. It gives acidic taste and helps contribution of aroma compound. Lactic acid can be found in different forms as L(+), D(-) or DL(±) which are called as isomers. Production of these isomers during fermentation depends on the strain of lactic acid bacteria (LAB) used. It is claimed that *S.thermophilus* is a L(+) lactic acid producer and *L.delbrueckii* spp. *bulgaricus* is a D(-) lactic acid producer (Tamime & Robinson, 2007).

L(+) and D(-) lactic acid content of yoghurt are generally 45-60% and 40-55%, respectively and the ratio of L(+):D(-) determines the quality of yoghurt with respect to flavor. For example, L(+):D(-) ratio should be small for a sharp and acidic yoghurt.

1.6.6 Vitamins and Minerals

Vitamins are used as co-factors in metabolic reactions. Fermented milk products can be thought as vitamin source like milk. However, vitamin contents of fermented milk products are variable because some LAB strains need especially vitamin B for growth and some can synthesis this by itself. Thus, yoghurt produced by different strains include vitamins in different compositions according to the strains (Akın, 2006).

Smid et al. (2001) , Lin and Young (2000a) (2000b) said that *L.delbrueckii* spp. *bulgaricus* uses riboflavin, folic acid and vitamin B_{12} in yoghurt for growth so causes reduction in amounts of these and some strains of *S.thermophilus* can be assumed as folic acid producer.

In addition, sensibility of vitamins against the processing conditions like heat treatment, incubation temperature, time and storage conditions makes the yoghurt content determination more difficult (Rao & Shahani, 1987). Approximate vitamin content of yoghurt and comparison of this with milk are given in Table 1.8 (Deeth & Tamime, 1981).

Table 1.8 Vitamin content of milk and yoghurt (in 100 g) (Deeth & Tamime, 1981)

Vitamin	Milk		Yoghurt	
	Whole	Skimmed	Full Fat	Low Fat
Retinol (µg)	52	1	28	8
Carotene (µg)	21	Tr*	21	5
Thiamin (B_1) (µg)	30	40	60	50
Riboflavin (B_2) (µg)	170	170	270	250
Pyridoxine (B_6) (µg)	60	60	100	90
Cyanocobalamine (B_{12}) (µg)	0.4	0.4	0.2	0.2
Vitamin C (mg)	1	1	1	1
Vitamin D (µg)	0.03	Tr*	0.04	0.01
Vitamin E (µg)	90	Tr*	50	10
Folic acid (µg)	6	5	18	17
Nicotinic acid (µg)	100	100	200	100
Pantothenic acid (µg)	350	320	500	450
Biotin (µg)	1.9	1.9	2.6	2.9
Choline (mg)	12.1	4.8	-	0.6

* Tr: Trace

Since minerals are resistant to processing conditions unlike vitamins, mineral content does not change during production of yoghurt from milk. Although there are lots of minerals in yoghurt, calcium and phosphorus are the most important ones for bones. Milk and fermented milk products are main sources of these elements. Also, yoghurt is not only the main calcium source for lactose-intolerant people but also its calcium

can be better absorbed (McKinley, 2005). Researches show that phosphorus, magnesium and zinc are also absorbed easily from yoghurt (Butriss, 1997). Although there are different data on calcium content and iron absorption relationship, most of the researches show that changes in the calcium content of diets have only a small influence on iron absorption (Lynch, 2000). However, calcium and iron may be taken at different times of the day as a precaution.

1.7 Yoghurt Starter Cultures

Lactic acid bacteria (LAB) are Gram (+), non-sporulating, catalase-negative, acid-tolerant, facultative anaerobic, fermentative bacteria (Mozzi, Raya, & Vignolo, 2010). LAB produce lactic acid from fermentation of carbohydrates and give some organoleptic, rheological and nutritive properties to the end product (Leroy & de Vuyst, 2004). In addition to use in fermentation process, they are commonly found in gastrointestinal and genitourinary tract of human and animals where they have important role on health such as immunomodulation, intestinal integrity and pathogen resistance (Vaughan, Heilig, Ben-Amor, & de Vos, 2005).

Dairy starter cultures are LAB added in milk to produce the selected fermented product. They can produce cheese, fermented milk, cream butter and yoghurt. They generally classified according to their optimum growth temperature, namely, mesophilic and thermophilic cultures. Mesophilic cultures have an optimum growth temperature about 30^0C. Thermophilic cultures have an optimum growth temperature about 42^0C. The most used thermophilic cultures are *Streptococcus thermophilus*, *Lactobacillus delbrueckii* and *Lactobacillus helveticus*. In yoghurt production,

Streptococcus thermophilus (*S.thermophilus*), *Lactobacillus delbrueckii* spp. *bulgaricus* (*L.delbrueckii* spp. *bulgaricus*) are used as starter cultures (Mozzi, Raya, & Vignolo, 2010).

These two cultures are used in combination for yoghurt production because there is a symbiotic relationship between them and this symbiotic relationship can be seen in Figure 1.3. In fact, in milk, peptides and amino acid amount is less than required amount to grow these two organisms. However, *L.delbrueckii* spp. *bulgaricus* releases amino acids like valine, glycine and histidine by the help of its proteolytic activity. Therefore, *L.delbrueckii* spp. *bulgaricus* forms a medium which is appropriate for the growth of *S.thermophilus*. Then, *S.thermophilus* promotes the growth of *L.delbrueckii* spp. *bulgaricus* by producing CO_2 and pyruate (Salminen, von Wright, & Ouwehand, 2004) (Tamime & Robinson, 2007).

During fermentation process, these two bacteria show different growth curve. At the beginning, *S.thermophilus* shows a fast growth by the help of *L.delbrueckii* spp. *bulgaricus* but its number decreases as it has low acid production ability and it dies faster in improving milk acidity. On the contrary, *L.delbrueckii* spp. *bulgaricus* shows an increase in number at high acidity level. Finally, as a result of these two bacteria's activity, pH of milk decreases from 6.3 – 6.5 to below 4.6. As a result of this pH decrease, protein molecules precipitate at isoelectric point that is called protein coagulation in other words, yoghurt. The activities of these bacteria not only conduct yoghurt formation but also affect the taste, aroma and texture of yoghurt by producing especially acetaldehyde, diacetone, acetone, acetic, capric and caprylic acids, volatile fatty acids and exopolysaccharides.

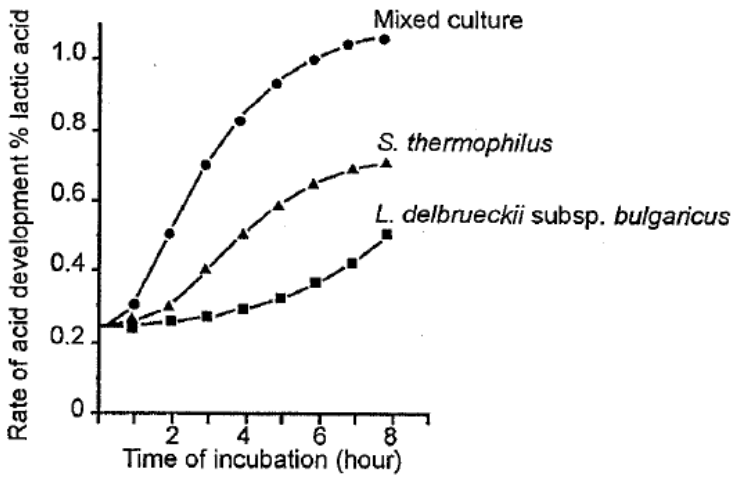


Figure 1.3 Single and mixed strain yoghurt cultures propagation at 40^0C in skimmed milk with 2% inoculation (Tamime & Robinson, 2007)

1.7.1 *Streptococcus thermophilus*

S.thermophilus is the only streptococcal species which is associated with food technology. It is a Gram (+) cocci which is shown in the Figure 1.4 (Doe Joint Genome Institute). Also, it is a non-motile and facultative anaerobe bacterium. *S.thermophilus* is a homofermentative bacterium and a member of alpha-hemolytic group of viridians. *S.thermophilus* does not produce endospores and does not have cytochrome, oxidase, and catalase enzymes. Although, in earlier times, *S.thermophilus* was considered as a subspecies of *Streptococcus salivarius* due to great DNA-DNA homology values, these are grouped as two different species

according their large phenotypic differences, like heat resistance and the ability to use limited number of carbohydrates (Salminen, von Wright, & Ouwehand, 2004).

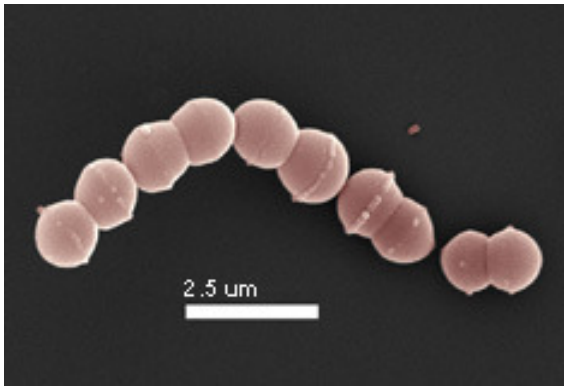


Figure 1.4 Electron micrograph of *Streptococcus thermophilus* by Robert Hutkins, University of Nebraska (Doe Joint Genome Institute)

1.7.2 *Lactobacillus delbrueckii* ssp. *bulgaricus*

The genus *Lactobacillus* is the largest genera in LAB. *L.delbrueckii* spp. *bulgaricus* was considered as a species until 1984 but now it is classified as a subspecies of *Lactobacillus delbrueckii. L.delbrueckii* spp. *bulgaricus* is a Gram (+), rod-shaped bacterium which is shown in the Figure 1.5 (Utah State University). It is facultative anaerobe, non-motile and non-sporeforming. In addition, it is classified as acidophilic bacterium because it requires low pH to grow. It can use only lactose among any kind of sugar and during fermentation of lactose produces acetaldehyde which is the main yoghurt aroma component.

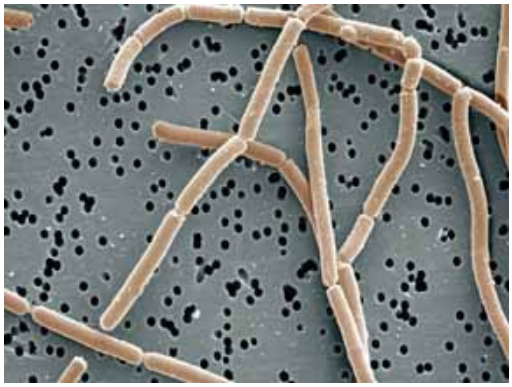


Figure 1.5 Electron micrograph of *Lactobacillus delbrueckii* spp. *bulgaricus* (Utah State University)

1.7.3 The Use of Starter Cultures in Dairy Industry

Starter cultures are the most important factors, which can affect the characteristics of final product, for fermented milk production. Thus, commercial manufacturers provide variety of starter culture mixtures with various characteristics by using some production techniques in different forms according to their usage and storage conditions.

The starter cultures are preserved in small quantities which are called stock cultures and they are used in dairy production plant according to their starter culture concentration. Figure 1.6 shows the different starter culture systems and their uses.

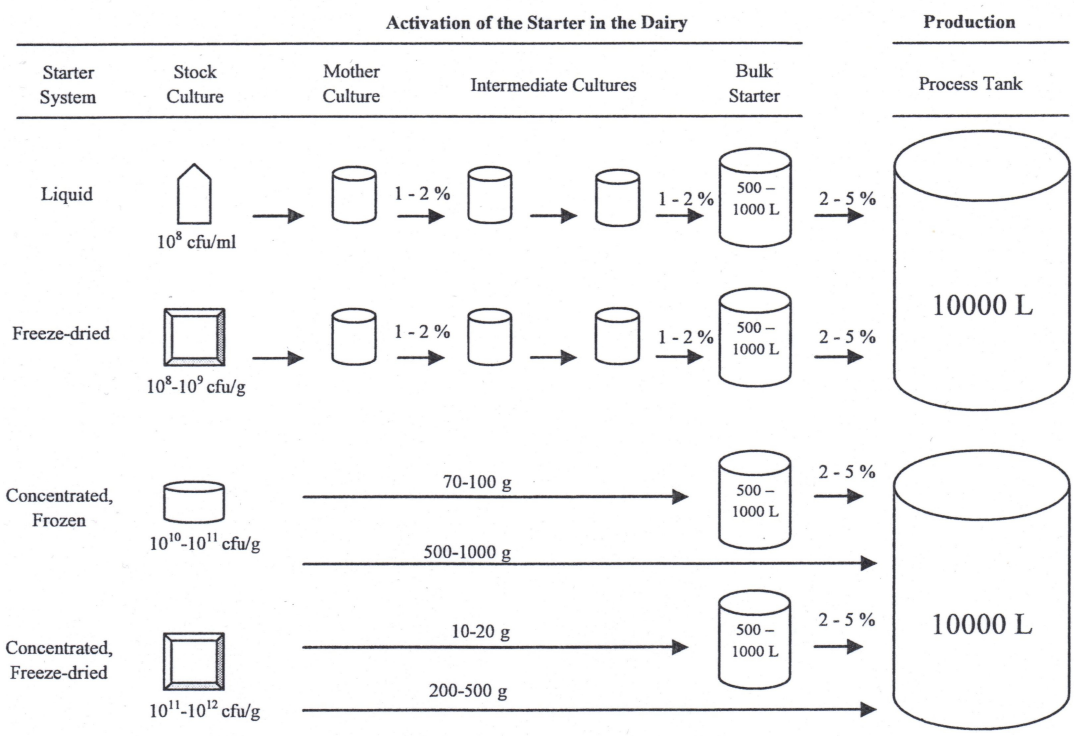


Figure 1.6 Use of starter culture systems (Mayra-Makinen & Birget, 2004)

Starter culture production is an increasing trend all over the world. First commercial starter culture was produced by Chr-Hansen laboratories. Starter cultures are offered to the market in liquid, dried ad lyophilized form. In recent years, the use of concentrated frozen and concentrated freeze-dried culture by dairy companies is increasing (Yaygın, 1988). There are several manufacturers of starter culture. Main starter culture producers in Turkey and in the world are listed in Table 1.9 (Gürakan & Altay, 2010). Christian Hansen and Danisco are the most important manufacturers. Chr. Hansen has made a decision to target only DVS, Danisco appears to be driven

by both the bulk starter and DVS markets but is applying its new technologies and strain development toward the DVS market. In Turkey, starter culture production is a new working area because academic researches and the relationship between industry and university is not enough for the development on this area. Thus, yoghurt production in Turkey depends on the starter culture production of foreign companies and these cultures may not be appropriate to Turkish taste. However, two companies had started to produce commercial starter culture, in recent years, in Turkey.

Table 1.9 Yoghurt starter culture producers and starter culture systems

Producer	Country	Starter System
Chr Hansen	Denmark	DVS[1]
Danisco	Denmark	DVS[2]
DSM	Netherlands	DVS – BS[3]
Sacco	Italy	DVS – BS[4]
CSL	Italy	DVS[5]
BioSource Flavors, Inc.	USA	Frozen, freeze-dried[6]
CSK Food Enrichment	Netherlands	DVS[7]
Maysa	Turkey	DVS – BS[8]
Intermak	Turkey	Liquid, frozen, freeze-dried[9]

DVS: direct-vat-set; BS: bulk starter culture(not concentrated) frozen and/or freeze-dried; Liquid, frozen, freeze-dried: not specified whether concentrated or unconcentrated (1: (Chr. Hansen, 2012); 2: (Danisco Inc., 2012); 3: (DSM, 2012); 4: (Clerici Sacco International, 2012); 5: (CSL, 2012); 6: (BioSource Flavors Inc., 2012); 7: (CSK Food Enrichment, 2012); 8: (Maysa Dairy&Food Ingredients, 2012); 9: (Intermak-Best Food, 2012))

1.7.3.1 Preservation Techniques of Starter Cultures

Starter cultures should be preserved to prevent any contamination or starter failure. Starter cultures can be preserved by using one of the techniques given below (Tamime & Robinson, 2007):

- Liquid starters
- Frozen starters
- Dried starters

Liquid starters can be produced in different growth media. In a sterile growth media is inoculated with 2 % starter culture and incubated at 42^0C for 3-4 h. Then, it can be stored at refrigeration temperature. Storage time can vary from one week to 12 months depending on the chemical components of growth media (Tamime & Robinson, 2007). A successive subculturing is a hard process while using this type of starters. Trained personnel are required in the laboratory because liquid starters can easily induce mutant strains.

Frozen starters are produced by inoculation of sterile milk with starters and frozen to - 20^0C or - 40^0C / - 80^0C or - 196^0C which are called freezing, deep freezing and ultra-low temperature freezing, respectively (Akın, 2006). Storage at low temperature may damage starter cultures; especially *L.delbrueckii* spp. *bulgaricus* is sensitive to freezing process. Glycerol is a cryogenic compound and is required for storage at - 20^0C. It is proved that when freezing temperature decreases, survival rate of

microorganism increases (Tsvetkov & Shishkova, 1982; Akın, 2006; Tamime & Robinson, 2007).

In dairy industry, dried starters are incrementally used. These can be done in 3 ways:

- Vacuum-drying
- Spray-drying
- Freeze-drying

Vacuum and spray drying are not used at this moment because they are old technology and survival rate is low with these methods. Thus, freeze-drying becomes the most common method. There are 3 advantages of freeze-dried cultures. Firstly, maintaining liquid cultures from freeze-dried cultures for inoculation is faster and easier. Secondly, shelf-life of cultures increases and finally, delivery of cultures can be done even by post. Until 1980s, freeze-dried cultures, also, were required propagation steps before fermentation. Amen and Cabau (1984), (1986) patented a method to produce active cultures. In this method, starters inoculated in a nutritive medium, generally milk with 16-25 % total solid content, with addition of neutralizing agent and then freeze-dried. Later, it was started to freeze-dry of concentrated cultures to be used in direct inoculation without any propagation steps (Tamime & Robinson, 2007). These cultures are called direct-to-vat set (DVS) or direct-to-vat inoculation (DVI) and there are lots of advantages for dairy manufacturers.

DVS may contain up to 10^{13} cfu/mL. DVS can be found in frozen or freeze-dried form. The cultures need no activation or other treatment prior to use and offer a number of advantages in terms of flexibility of use, consistent performance, possibility of using customized culture blends, and no investment in bulk starter equipment. Since these are not required propagation step, need for specialized hygiene precautions and trained personnel decreases. In addition, because these cultures are prepared outside of a dairy plant, bacteriophage contamination risk reduces (Carminati, Giraffa, Quiberoni, Binetti, Suarez, & Reinheimer, 2010).

1.7.3.2 Propagation of Starter Cultures

Industrial production of starter cultures is conducted by batch fermentations and this process can be divided some general steps as listed below (Mayra-Makinen & Birget, 2004):

- Preparation of inoculum
- Preparation of media
- Fermentation at constant pH
- Harvesting the culture
- Adding the cryoprotectant
- Freezing
- Freeze-drying
- Packaging and storage

There are some factors that affect fermentation and survival rate during freezing and drying. Growth medium is one of the most important ones (Altay Dede, 2010). Researches show that milk solids are necessary for synthesis of required enzymes during fermentations. In addition to skimmed milk, whey enriched by yeast extract can be used as growth medium. Also, addition of calcium into growth medium maintain higher survival rate for *L.delbrueckii* spp. *bulgaricus* (Tamime & Robinson, 2007).

pH level of growth medium and temperature during fermentation are other important factors. Yoghurt LAB are thermophilic cultures so fermentation temperature should be around 42^0C for high cell concentration. Medium pH should be stabilized at the optimum pH of strain by using neutralizer. Optimum pH level is determined as 5.4-5.6 for *L.delbrueckii* spp. *bulgaricus* and 6.5 for *S.thermophilus* (Mayra-Makinen & Birget, 2004) (Beal, Louvet, & Corrieu, 1989). Ammonium hydroxide is the most used neutralizer because in experiments, using ammonium hydroxide results higher yields of bacteria (Tamime & Robinson, 2007).

Most of LAB can be preserved by freezing and drying but *L.delbrueckii* spp. *bulgaricus* and *L.helveticus* are sensitive to these processes so harvesting time become important to prevent damaging of cells and loss of activity (Wright & Klaenhammer, 1983). If *L.delbrueckii* spp. *bulgaricus* and *S.lactis* supp. *cremoris* are harvested at the beginning of stationary phase during fermentation and *S.thermophilus* cells are harvested at the end of exponential phase, cells can be less damaged during freezing and drying (Akın, 2006). Concentration of cells can be done by using centrifugal separation at constant temperature between 5^0C and 15^0C

depending on strain (Porubcan & Sellars, 1979) (Salminen, von Wright, & Ouwehand, 2004).

Cryoprotective agents and freezing temperature are also important factors for survival rate. For high survival rate of culture during freeze-drying, freezing temperature should be between -20^{0}C and -30^{0}C and drying temperature should be between -10^{0}C and -30^{0}C (Akın, 2006). Mannitol can be used as a cryoprotective agent for freeze-drying of *S.thermophilus* and lactose and glycerol can be used for freeze-drying of *L.delbrueckii* spp. *bulgaricus* (Tamime & Robinson, 2007).

Preserved cultures show higher survival rate when they are stored at 5-10^{0}C than stored at room temperature (Nikolova, 1975). Also, preserved cultures are sensitive to oxygen so vacuum packaging recommended.

1.7.3.3 Phage Problems in Dairy Industry

Starter cultures are used in the production of fermented milk products. Final product characteristics are affected by not only the processing parameters, like, raw material quality, pasteurization temperature, etc. but also the starter culture properties. Thus, used starter cultures are selected according their productivity and bacteriophage resistant properties.

Although the use of commercial starter culture provides the standardization in product, it brings about the phage problem. Researches are focused on the source of the phage problem; whether phage contamination is originated from raw milk or the lysogenic strains (Acar Soykut & Tunail, 2009). Kaleli and Tunail (2001) and Kahraman (2006) were examined the rural and commercial strains and lysogeny was not encountered in these strains. On the other hand, because of the stability of phages at pasteurization conditions, it is thought that the source of phage contamination is raw milk.

To prevent the product loss due to phages, many precautions can be applied, for example, use of phage inhibitory medium, separation of culture preparation department from plant environment, improvement of sanitation procedures and the use of DVS cultures. However, these precautions can reduce phage propagation, cannot eliminate. Therefore, it is preferred the use of strains which are resistant to dominant phages in the environment. Starter culture producers help the manufacturers by offering rotation program but because of not having rural cultures in producers' culture collections, rotation program does not work to prevent the phage problem.

To overcome the phage problem and make the rotation program useful, dairy manufacturers should isolate the rural phages from their plants and determine the rural cultures which are resistant to these phages and use these resistant strains for the fermented milk production (Acar Soykut & Tunail, 2009).

1.8 Texture

1.8.1 Exopolysaccharides

Exopolysaccharides (EPS) are long-chain saccharides which are produced from sugar in milk or growth environment by many strains of LAB. These can be loosely attached to the cell wall and form a capsule structure which are capsular exopolysaccharides or can be secreted to environment which are ropy exopolysaccharides (Mayo, Aleksandrzak-Piekarczyk, Fernandez, Kowalczyk, Alvarez-Martin, & Bardowski, 2010).

EPS can be classified into two groups as homopolysaccharides and heteropolysaccharides. Homopolysaccharides consist of one type of monosaccharide like α-D-glucans, β-D-glucans, fructans, etc. and generally produced by *Leuconostoc mesenteroides* ssp. *mesenteroides*, *Streptococcus mutans, Streptococcus sobrinus* and *Streptococcus sangius* (Milci & Yaygın, 2005). Heteropolysaccharides which are composed of either linear or branched repeating units of different types of monosaccharide like D-glucose, D-galactose and D-rhamnose produced by *Lactococcus lactis* spp. *lactis Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* (Broadbent, McMahon, Oberg, & Welker, 2001).

Researches show that the amount of EPS production is affected by many factors such as incubation temperature, incubation time, growth medium, acidity of growth medium and type of strain (Tamime & Robinson, 2007; Akın, 2006). It was determined that the amount of EPS produced by *L.acidophilus* at 37-42^{0}C for 24

hours incubation is higher than that by the same strain at 30^0C (Mozzi, Oliver, De Giori, & De Valdez, 1995). Another important factor is pH level of the medium. Researches show that 6.5 pH is required for optimum EPS production by *L.delbrueckii* spp. *bulgaricus* (Duboc & Mollet, 2001). Amount of EPS synthesis was given by Cerning et al. (1990) as 80 mg / 100 ml when EPS-producer *L.delbrueckii* spp. *bulgaricus* and EPS-producer *S.thermophilus* were used.

Biosynthesis of EPS in LAB has four main steps starting with sugar transport into cytoplasm, synthesis of sugar-1P, polymerization of repeating unit precursors and lastly EPS transport outside the cell (Mayo, Aleksandrzak-Piekarczyk, Fernandez, Kowalczyk, Alvarez-Martin, & Bardowski, 2010). In Figure 1.7, EPS production by lactose metabolism of *S.thermophilus* is shown, where all four steps of EPS biosynthesis can be seen (Tamime & Robinson, 2007).

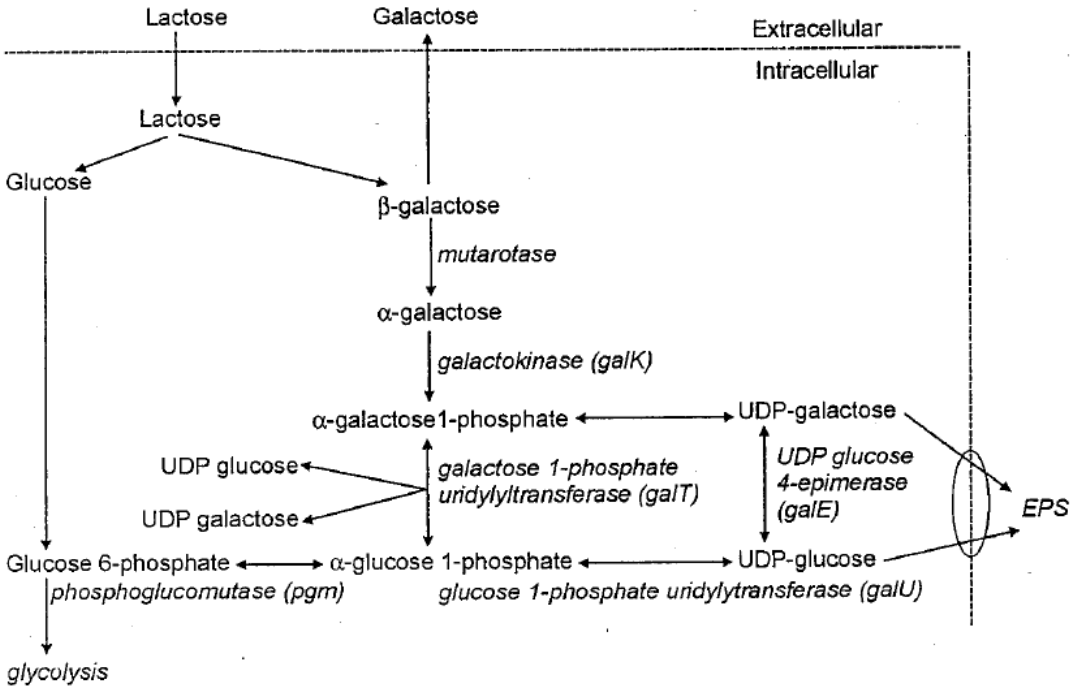


Figure 1.7 Lactose metabolism of *S. thermophilus* which results in production of EPS (Tamime & Robinson, 2007)

1.8.2 Effects of EPS on Rheological Properties of Yoghurt

Exopolysaccharides change the rheological properties of dairy products in a positive way due to their viscosity increasing, texture improving, water binding, stabilizing and emulsifying properties (Milci & Yaygın, 2005).

In yoghurt production, loose texture and serum separation are the main physical problems which can be solved by addition of some additives. Since consumers prefer

natural products and also additive use is forbidden by Turkish Standards which are TS 1330/February 2009 and TS 10935/ April 1993, EPS-producer starter cultures are used in fermentation of milk products (Ruas-Madiedo, Hugenholtz, & Zoon, 2002). In addition, exopolysaccharides have a positive impact on texture, stability, flavor and aroma of the final product.

Experiments show that there is not a significant correlation between viscosity of product and exopolysaccharide amount (Ruas-Madiedo, Hugenholtz, & Zoon, 2002). However, the amount of these molecules, their molecular weights, radius, chemical compositions and linkages strengths are very effective on the viscosity (Broadbent, McMahon, Oberg, & Welker, 2001). In recent years, it was found that yoghurt produced by EPS-producer starters have high viscosity values than yoghurt produced by non-EPS-producer starters (Hassan, Corredig, & Frank, 2001).

1.9 Aroma Compounds

Flavor compounds contribute the aroma to yoghurt and these flavor compounds can be examined into four main categories which is given below and also Figure 1.8 shows that the acetaldehyde and other aroma compounds production from pyruvate (Walstra, Wouters, & Geurts, 2006).

1. Non-volatile acids; lactic acid, pyruvic acid, oxalic acid and succinic acid
2. Volatile acids; formic acid, acetic acid, propionic acid and butyric acid
3. Carbonyl compounds; acetaldehyde, acetone, acetoin and diacetyl
4. Miscellaneous compounds; some amino acids or compounds formed due to thermal degradation of protein, fat and lactose (Tamime & Robinson, 2007).

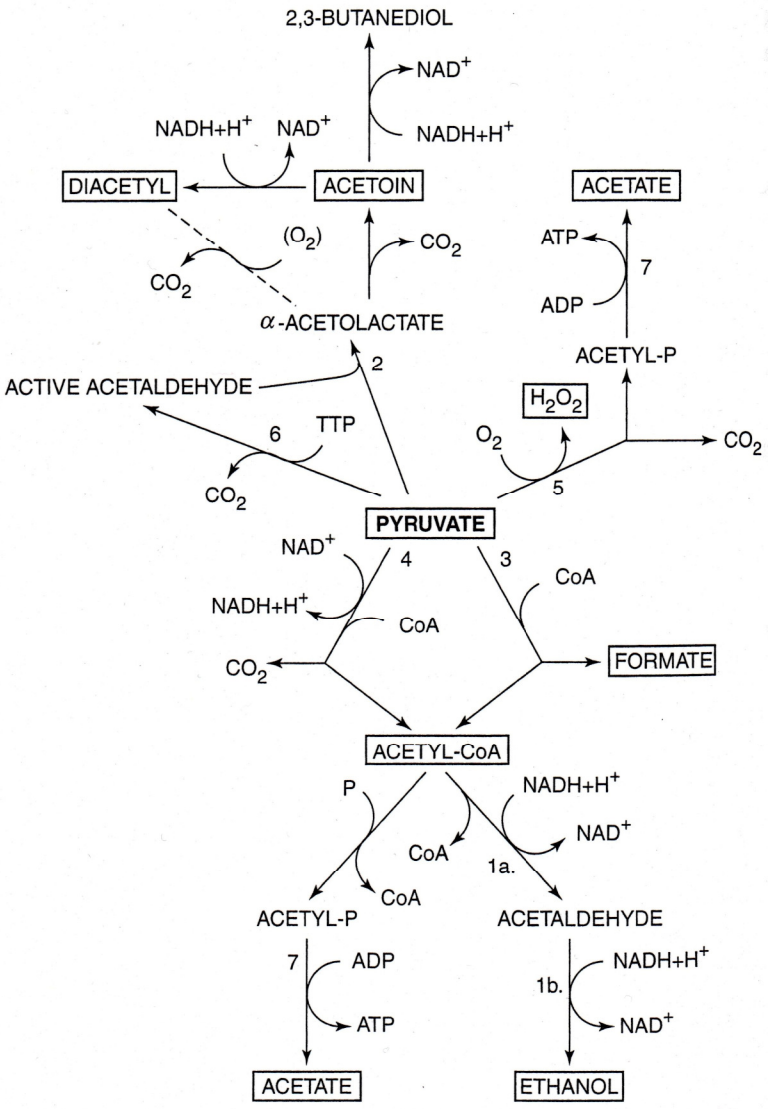


Figure 1.8 Different pathways of pyruvate. (1a) acetaldehyde dehydrogenase, (1b) alcohol dehydrogenase, (2) acetolactate synthase, (3) pyruvate formate lyase, (4) pyruvate dehydrogenase, (5) pyruvate oxidase, (6) pyruvate decarboxylase and (7) acetate kinase. Dashed arrow denotes a nonezymatic reaction (Walstra, Wouters, & Geurts, 2006; Axelsson, 2004).

Aroma compounds content of yoghurt depends on mainly two factors. One of them is the source of the milk which is used for yoghurt production. Table 1.10 shows that acetaldehyde, acetone and ethanol content of yoghurt produced by different mammalian milks. The other reason is the yoghurt starter cultures and their form of use. Yoghurt starter cultures are examined separately and in mixed form for their flavor compounds production capabilities. Table 1.11 shows the production of carbonyl compounds during fermentation process.

Table 1.10 Aroma compound content of yoghurt produced by using different mammalian milk (Tamime & Robinson, 2007)

Milk	Acetaldehyde	Acetone	Ethanol
Cow	4-26	3-25	19-365
Sheep	7-30	5-30	10-255
Goat	5-19	3-40	25-355
Buffalo	6-28	5-30	5-195

Table 1.11 Production of carbonyl compounds (µg/g) by yoghurt starter cultures (Tamime & Robinson, 2007)

Organism	Acetaldehyde	Acetone	Acetoin	Diacetyl
S.thermophilus	1.0-13.5	0.2-5.2	1.5-7.0	0.1-13.0
L.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

Researchers claim that the flavor and aroma of yoghurt are based on the non-volatile, volatile and carbonyl compounds content. However, acetaldehyde content is the most effecting factor of flavor because generally acetaldehyde presents in yoghurt much greater than other flavor compounds (Pette & Lolkema, 1950).

1.9.1 Flavor Formation in Yoghurt

Acetaldehyde is the major aroma compound in yoghurt and it is produced by lots of metabolic reactions which are shown in Figure 1.9.

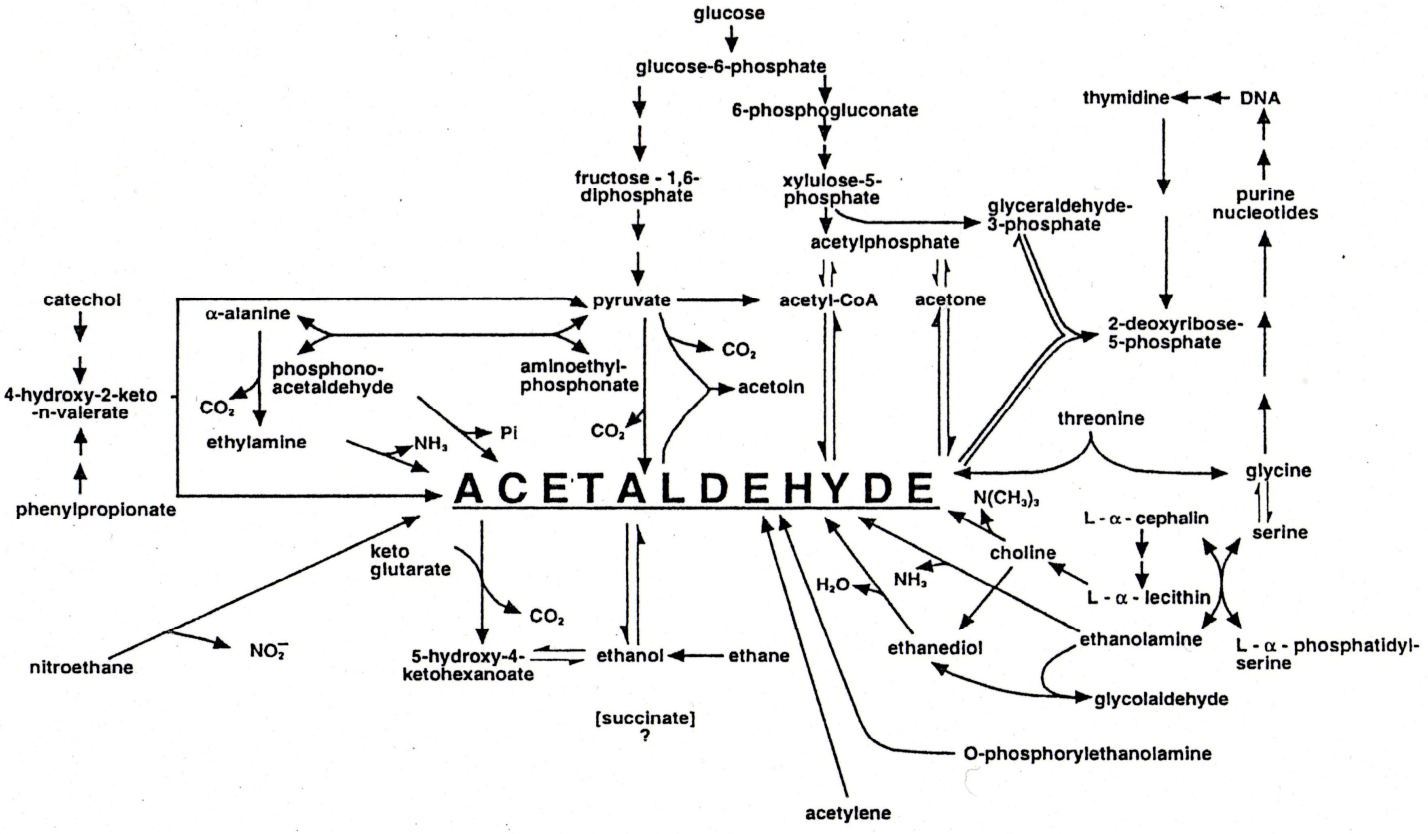


Figure 1.9 Acetaldehyde production pathways (Tamime & Robinson, 2007)

However, the high proportion of total acetaldehyde is produced by using mainly two metabolic pathways. Figure 1.10 explains briefly these two pathways.

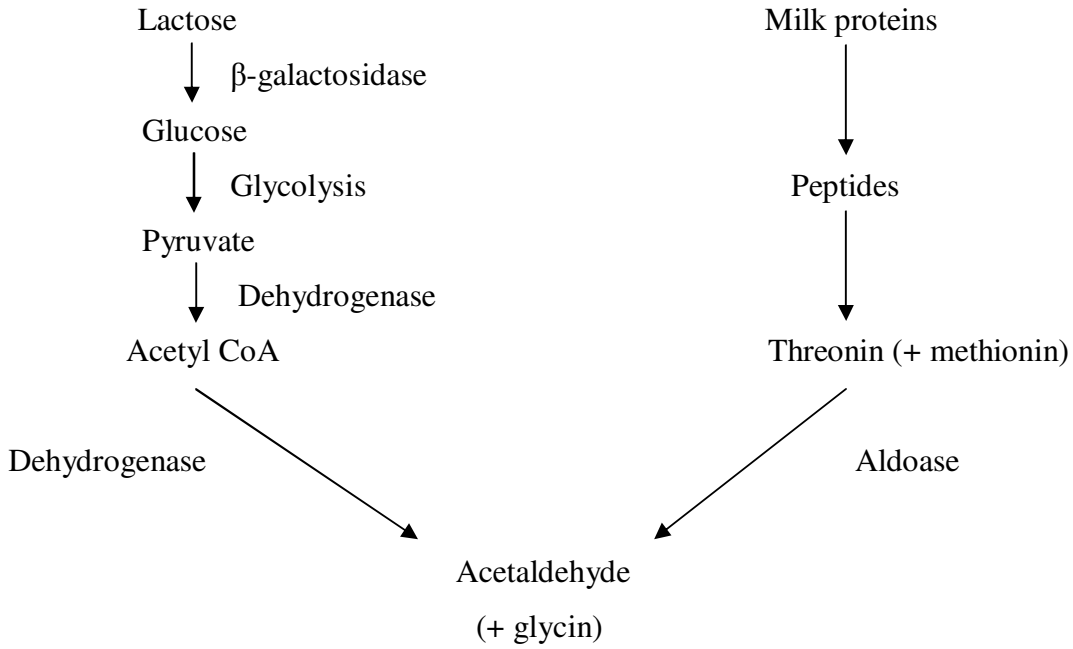
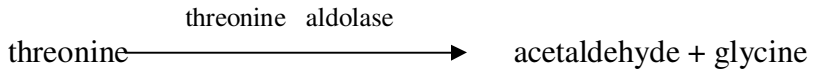


Figure 1.10 Two major pathways to produce acetaldehyde in yoghurt (Walstra, Wouters, & Geurts, 2006)

First one is Embden-Meyerhof-Parnas pathway, lactic acid bacteria that have no alcohol dehydrogenase enzyme because in the presence of this enzyme acetaldehyde produced from pyruvate can be broken down into ethanol (Walstra, Wouters, & Geurts, 2006). Second one is proteolysis that the production of acetaldehyde from

free amino acid, threonine with the activity of threonine aldolase enzyme by *L.delbrueckii* spp. *bulgaricus* and *S.thermophilus*. The chemical reaction is:



Walstra et al. (2006) claims that acetaldehyde amount produced by proteolysis is much higher than produced by carbohydrate metabolism. Organisms, *L.delbrueckii* spp. *bulgaricus* and *S.thermophilus*, both have threonine aldolase activity. Researches show that *S.thermophilus* threonine aldolase activity decreases by the temperature rise from 30^0C to 42^0C but the activity of this enzyme of *L.delbrueckii* spp. *bulgaricus* remains at the same level (Zourari, Accolas, & Desmazeaud, 1992). Therefore, it can be said that acetaldehyde production mainly depends on *L.delbrueckii* spp. *bulgaricus* strains because yoghurt fermentation temperature changes between 40 and 45^0C (Tamime & Robinson, 2007).

1.10 Aim of the Study

Yoghurt is a fermented milk product which is originated from Middle East but then, it has been started to be consumed all over the world with an increasing trend. Yoghurt is produced as a result of the symbiotic growth of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus*. Each strain of *L.delbrueckii* spp. *bulgaricus* and *S.thermophilus* has different capabilities for the production of lactic acid, carbon dioxide, diacetyl and acetaldehyde which give different flavor and texture characteristics to yoghurt. The amounts of these components determine the quality of yoghurt and consumer acceptance.

In this study, 6 *L.delbrueckii* ssp. *bulgaricus* and 6 *S.thermophilus* strains which were isolated from Turkish traditional yoghurts by Neslihan Altay Dede (2010) were used to produce yoghurt samples with different characteristics. Also, 2 commercial *S.thermophilus* strains and one *L.delbrueckii* ssp. *bulgaricus* strain was used for the production of two commercial yoghurt samples for comparison with the traditional ones. These strains were selected according to their acidification activity and acetaldehyde production properties. In order to determine the bacterial combination which produces the most appropriate yoghurt to Turkish taste, pH and titratable acidity change during storage, whey separation, exopolysaccharide and acetaldehyde content were determined. Also, texture and sensory analyses has been performed. The culture combination which have the best results in these analyses, was freeze dried and these freeze dried culture was used in yoghurt production. Another yoghurt sample was produced using commercial freeze-dried culture. Finally, these two samples were analyzed and compared with each other and the sample produced by conventional method to observe the effect of freeze drying on starter cultures and their yoghurt production properties.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

2.1.1 Lactic Acid Bacteria Strains

Eight *S.thermophilus* and seven *L.delbrueckii* spp. *bulgaricus* strains were used for yoghurt sample preparation. Two commercial *S.thermophilus* strains, one isolated from Danisco Yo-Mix 410 (M17 Dan-Yo-Mix410-1) and one isolated from Danisco TA 040 (M17 Dan TA040-1) were used as controls. One *L.delbrueckii* spp. *bulgaricus* strain isolated from Visby Visbyvac B1000 (MRS Visby-2) was also used as commercial control for yoghurt samples production. These cultures were selected among strains which were isolated from traditional and commercial cultures by Neslihan Altay Dede (2010). Strains were selected according to their acidification activity and acetaldehyde production properties. Acidification activity is calculated with the following equation and acidification activity is classified as in the Table 2.1 and Table 2.2.

$$\Delta pH = pH_{\text{at time zero}} - pH_{\text{at any time}}$$

Table 2.1 Classification of *S.thermophilus* strains according to acidification activity

ΔpH<1.3	1.3<ΔpH<1.4	1.4<ΔpH
Fair	Medium	Good

Table 2.2 Classification of *L.delbrueckii* spp. *bulgaricus* strains according to acidification activity

ΔpH<1.4	1.4<ΔpH<1.5	1.5<ΔpH
Fair	Medium	Good

According to Altay Dede (2010), acidification activity of *L.delbrueckii* spp. *bulgaricus* strains should be higher than 1.5 and acidification activity of *S.thermophilus* strains should be higher than 1.4 to be classified as good strain. In addition, acetaldehyde production properties of *L.delbrueckii* spp. *bulgaricus* strains should be as high as possible for a good flavor formation. All strains used in this study were listed in Table 2.3. Acidification activity and acetaldehyde production properties of used strains in this study were given in Appendix J.

Table 2.3 Lactic acid bacteria strains used for yoghurt sample preparation

S.thermophilus Strains	Source of Strains	*L.delbrueckii* spp. *bulgaricus* Strains	Source of Strains
M17 K1-14	METU_FDE Culture Collection*	MRS K1-43	METU_FDE Culture Collection*
M17 N2-3		MRS M2-16	
M17 N8-2		MRS M2-23	
M17 N5-7		MRS N6-2	
M17 N6-6		MRS N4-3	
M17 S1-3		MRS K2-1	
M17 Dan TA040-1	Danisco	MRS Visby-2	Visby
M17 Dan-Yo-Mix410-1			

* METU_FDE Culture Collection: Middle East Technical University Food Engineering Department culture collection contains *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus* strains which were isolated by Neslihan Altay Dede (2010).

2.1.2 Growth media and temperature

M17 broth (Merck) was used as a growth media of *S.thermophilus* strains after adjusting the pH of media to 6.8±0.1 at 25^{0}C which was originally 7.2±0.2 at 25^{0}C and sterilized at 121^{0}C for 15 minutes (pHmeter, Hanna instruments HI 221, EU). *L.delbrueckii* spp. *bulgaricus* strains were grown in MRS broth (Merck) with 5.7±0.2 pH value at 25^{0}C which was sterilized at 121^{0}C for 15 minutes. *S.thermophilus* and

L.delbrueckii spp. *bulgaricus* isolates were incubated at 42^{0}C for overnight unless otherwise noted.

2.2 Methods

2.2.1 Cultivation

During these experiment, cultures, stored at -80^{0}C with glycerol solution, were used. For activation of starter cultures, serial inoculations were conducted. The propagation step to produce the cultures used in yoghurt production was explained below:

1. Inoculate 10 mL of MRS or M17 broth (according to bacteria) by using the stock culture which is stored -80^{0}C with an inoculation rate 1% (CL HetoFrig -80, Heto, Denmark).
2. Incubate the broth at 42^{0}C overnight (Genlab INC/160, UK).
3. Inoculate a second 10 mL of MRS or M17 broth (according to bacteria) by using first broth and incubate at 42^{0}C overnight.
4. Inoculate 50 mL of MRS or M17 broth by using second broth and incubate at 42^{0}C overnight.
5. Take the broth in a falcon and centrifuge it to obtain pellet at 2000 rpm for 15 minutes (Thermo Electron IEC Centra CL2, US).
6. Remove the supernatant and add 10 mL of distilled sterile water to the pellet and centrifuge at 2000 rpm for 15 min.
7. Repeat step 6 until the pellet becomes clear.

8. Add 10 mL sterile distilled water and mix with vortex (Fisons Whirly-mixer, England).
9. Pipette 0.9 mL sterile distilled water in an eppendorf tube and add 0.1 mL of culture, use 1 mL of distilled water as a blank solution
10. Read absorbance at 600 nm wavelength (Specord 50, Analytikjena, Germany)
11. Adjust the optical density (OD) value to 2 at 600 nm by adding or removing sterile distilled water.

2.2.2 Yoghurt Production

Yoghurt samples were produced using standardized milk taken from Atatürk Orman Çiftliği Milk Factory. Standardized milk of AOÇ has 3±0.1% fat and 16±0.3% total solid content. Yoghurt sample numbers and *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus* strains used for the production of samples were listed in Table 2.4. Samples were produced for each culture combination by following the steps given below:

1. Inoculate 1000 mL of standardized milk at 42^0C with an inoculation rate of 4%. For inoculation, use the culture with adjusted OD.
2. Mix with magnetic stirrer for 10 minutes and pour sterile glass vessel as 100 mL (VELP Scientifica ARE, EU).
3. Incubate at 42^0C until pH values reach to 4.6 - 4.7.
4. After incubation, immediately store sample at 4^0C for 24 hours.

Table 2.4 Yoghurt sample numbers and *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus* strains used for production of yoghurt samples

Sample Number	*S.thermophilus* Strains Used in Sample	*L.delbrueckii* spp. *bulgaricus* Strains Used in Sample
1	M17 N8-2	MRS M2-16
2	M17 S1-3	MRS M2-23
3	M17 K1-14	MRS K1-43
4	M17 N8-2	MRS M2-23
5	M17 S1-3	MRS K1-43
6	M17 K1-14	MRS M2-16
7	M17 N8-2	MRS K1-43
8	M17 K1-14	MRS N4-3
9	M17 K1-14	MRS M2-23
10	M17 N8-2	MRS K2-1
11	M17 N5-7	MRS K2-1
12	M17 N8-2	MRS N4-3
13	M17 K1-14	MRS K2-1
14	M17 N2-3	MRS K2-1
15	M17 N6-6	MRS K2-1
16	M17 N5-7	MRS K1-43
17	M17 N6-6	MRS K1-43
18	M17 N2-3	MRS K1-43
19	M17 N5-7	MRS M2-16
20	M17 N6-6	MRS M2-16
21	M17 S1-3	MRS M2-16
22	M17 N2-3	MRS N4-3

Table 2.4 Yoghurt sample numbers and *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus* strains used for production of yoghurt samples (Cont'd)

Sample Number	*S.thermophilus* Strains Used in Sample	*L.delbrueckii* spp. *bulgaricus* Strains Used in Sample
23	M17 S1-3	MRS N4-3
24	M17 N6-6	MRS N4-3
25	M17 N5-7	MRS N4-3
26	M17 N2-3	MRS N6-2
27	M17 N5-7	MRS N6-2
28	M17 N2-3	MRS M2-23
29	M17 DanTa040-1	MRS Wisby2
30	M17 DanYoMix 410-1	MRS Wisby2
31	M17 N6-6	MRS N6-2
32	M17 N5-7	MRS M2-23
33	M17 N6-6	MRS M2-23
34	M17 N2-3	MRS M2-16
35	M17 K1-14	MRS N6-2
36	M17 N8-2	MRS N6-2
37	M17 S1-3	MRS N6-2
38	M17 S1-3	MRS K2-1

2.2.3 pH and Titratable Acidity Determination

pH and titratable acidity of yoghurt samples were measured at 1., 4., 7., 14. and 21. days of storage. Titratable acidity of yoghurt samples was determined according to Yoghurt Standard of Turkish Standards Institution (TS 1330/February 1999) and the procedure was given below. pH results and total titratable acidity results were given in Appendix B and Appendix C, respectively.

1. Weight 10 grams of yoghurt samples into a 100 mL erlenmeyer flask (Precisa BJ1000C, Switzerland).
2. Add 10 mL distilled water at 40^0C into the flask and mix with a glass bar until a smooth mixture reached.
3. Add 0.5 mL phenolphthalein solution.
4. Titrate with 0.1 N NaOH solution until 30 second-stable pink color maintained.
5. Calculate titratable acidity in yoghurt using the following equation.

$$A = \frac{V \times N \times 0{,}09}{m} \times 100$$

Where;

A: titratable acidity, wt % lactic acid

V: used 0.1 N NaOH solution during titration, mL

m: weight of sample used in titration, g

N: Normality of used NaOH solution in titration

2.2.4 Whey Separation

25 grams of yoghurt samples were weighted on a filter paper and stored at 4^0C for 2 hours to collect separated whey. Collected whey were measured in mL and divided by sample weight (Yılmaz, 2006) (Sezgin, Yıldırım, & Karagül, 1994). The results were given in Appendix D.

2.2.5 Texture Analysis

For texture analysis, three parallels, which were fermented from 100 mL of milk, were prepared for each sample. At first day of storage, the maximum forces were measured by using the cylindrical probe with a diameter 36 mm (Stable Micro Systems, TA.XT plus Texture Analyzer, UK). The hardness values of samples were given in Appendix D.

2.2.6 Selection of Yoghurt Samples for Chemical and Sensory Analysis

Nine yoghurt samples were selected for exopolysaccharides determination, acetaldehyde determination and sensory analysis. Yoghurt samples were analyzed according to their final pH, titratable acidity, whey separation and textural properties. By using these outcomes, samples which gave the best results for all the analysis, were chosen for further experiments. Samples, which had a pH score below 4.1 and had the highest seven titratable acidity score at the end of the storage time, were omitted even if these have high texture or whey separation scores. According to these selection criteria six yoghurt samples were selected. In addition, two yoghurt samples, which were made by using commercial cultures, and one yoghurt sample,

which was among the worst ones for each criteria, were analyzed for comparison. Yoghurt samples and culture mixtures were given in Table 2.5.

Table 2.5 Selected yoghurt samples for chemical and sensory analysis

Sample Number	*S.thermophilus* Strains Used in Sample	*L.delbrueckii* spp. *bulgaricus* Strains Used in Sample
6	M17 K1-14	MRS M2-16
9	M17 K1-14	MRS M2-23
11	M17 N5-7	MRS K2-1
15	M17 N6-6	MRS K2-1
24	M17 N6-6	MRS N4-3
25	M17 N5-7	MRS N4-3
27	M17 N5-7	MRS N6-2
29	M17 DanTa040-1	MRS Wisby2
30	M17 DanYoMix 410-1	MRS Wisby2

2.2.7 EPS Content Determination

The EPS assay, which was revised by Goh et al. (2005), was applied to determine the EPS amount in samples. The protocol was given below. Glucose standard curve at 485 nm and results were given in Appendix E.

1. Swirl to mix culture medium in bottle to ensure homogeneity.
2. Adjust the pH of the sample to pH 7 with 0.1N NaOH.
3. Add 100 µL of filter-sterilized Flavourenzyme (10% w/w) to 10 mL of sample.
4. Incubate the sample at 50^0C in a shaker for 4 hours (Infors HT, Aerotron, Switzerland).
5. Vortex the sample for approximately 15 seconds.
6. Pipette 2.9 mL of distilled water and 7 mL of chilled absolute ethanol in the falcon tube.
7. Pipette 100 µL of culture medium into the falcon tube.
8. Leave the sample overnight at 4^0C.
9. Centrifuge sample at 27.000g, 4^0C for 40 minutes. Ensure tubes are balanced within ±0.1g before centrifuging.
10. After centrifugation, carefully decant supernatant (pour away for pellet).
11. Invert the tubes on a piece of paper towel for approximately 10 minutes.
12. Pipette 3 mL of distilled water to re-suspend the pellet in falcon tube.
13. Pipette 7 mL of chilled 99.7% ethanol into falcon tube.
14. Repeat step 8-10.
15. Re-suspend the pellet in 1 mL of distilled water.
16. Transfer the sample to an eppendorf tube.
17. Prepare a blank sample using distilled water 1 mL.
18. Add 1 mL of 5% (w/v) phenol solution to the sample and mix using a vortex (15 seconds).
19. Add 5 mL of concentrated sulphuric acid directly to the sample.
20. Mix the sample thoroughly using a vortex.
21. Leave the sample to stand for 30 minutes.

22. Read absorbance at 485nm. Use the blank as the reference sample.
23. Obtain the amount of EPS from the glucose standard curve.
24. Amount of EPS is multiplied by 10 to account for the dilution factor.
25. Amount of EPS = EPS of the test sample – EPS of control sample

2.2.8 Acetaldehyde Content Determination

Acetaldehyde contents of samples were determined by Lees and Jago method (Lees & Jago, 1969). Procedure was given below (Yılmaz, 2006). Results were given in Appendix E.

1. Mix the sample to ensure homogeneity.
2. Weight 10 g of sample into a volumetric flask.
3. Add 30 mL of distilled water to the flask.
4. Distillate the mixture until gathering of 10 mL distillate.
5. Add 1 mL 0.25 M $NaHSO_3$.
6. Adjust the pH of the mixture to pH 9 with 0.1 N NaOH solution.
7. Cover the flask and leave for 15 minutes in a dark place.
8. Add 1 mL of 1% starch solution and titrate with 0.1 N iodine solution until reached a purple color.
9. Add 1 g $NaHCO_3$ and mix.
10. When the mixture becomes clear titrate with 0.005 N iodine solution until reached a purple color.
11. Amount of used 0.005 N iodine solution is used to determine the acetaldehyde amount using following equation.

$$A = \frac{44 \times V \times N}{m} \times 1000$$

Where;

A= Acetaldehyde amount, ppm

V= Used 0,005 N iodine solution during titration, mL

N= Normality of used iodine solution in titration

m = Sample weight, gram

2.2.9 Sensory Analysis

To find out the sensorial characteristics of yoghurt and consumer acceptance, sensory analysis was conducted. 9 different yoghurts were tried and scored by 11 participants. Samples were rated in terms of appearance, odor and flavor. Also, participants gave a score for overall acceptance of samples. A 5-degree scale was used for rating. Results were evaluated by using One-way ANOVA. Then, Tukey's test was used to see that whether a significant difference between samples. For all ANOVA applications confidence interval "CI" was taken as 95% (i.e; α=0.05). Calculations and results were given in Appendix G.

2.2.10 Freeze Dried Culture Preparation

The best combination of isolates of *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus* were determined by chemical and physical experiments and they were prepared as

freeze dried culture to see the effect of freeze drying of strains on yoghurt properties. Freeze drying procedure was given below:

1. Inoculate 10 mL of growth medium (M17 broth for *S.thermophilus* strain "M17 N6-6"; MRS broth for *L.delbrueckii* spp. *bulgaricus* strain "MRS N4-3") with a ratio of 1% by using stock cultures and incubate at 42^0C for 24 hours.
2. Repeat the step for once for each organism.
3. Inoculate 200 mL of medium and incubate at 42^0C for 14 hours.
4. Transfer the medium into falcon tubes and centrifuge at 5000 rpm for 45 minutes and remove supernatant.
5. Prepare suspension media with 11% non-fat milk solid and 15% sodium glycerophosphate.
6. Resuspend the pellet to 2% of original volume by using suspension media.
7. Transfer the cultures into an agar plate and spread it
8. Put the agar plates into the freeze-dryer and dried at -50^0C under 0.05 Torr vacuum for 18 hours (Heto FD8, Denmark).

CHAPTER III

RESULTS AND DISCUSSION

3.1 Experimental Design

This study was divided into three parts. First part includes the determination of physico-chemical and rheological properties. To observe the physico-chemical and rheological properties of yoghurt samples, change in pH and total titratable acidity during storage, whey separation and hardness values of yoghurt samples are determined (Fadela, Abderrahim, & Ahmed, 2009; Amatayakul, Halmos, Sherkat, & Shah, 2006).

Second part is the determination of chemical properties of yoghurt samples and consumer acceptance. Chemical properties of yoghurt can be determined by analyzing exopolysaccharide and acetaldehyde content of yoghurt samples (Amatayakul, Halmos, Sherkat, & Shah, 2006; Gündoğdu, Çakmakçı, & Dağdemir, 2009). Sensory analysis is conducted for the determination of consumer acceptance (Gündoğdu, Çakmakçı, & Dağdemir, 2009; Obi, Henshaw, & Atanda, 2010).

Third part is the determination of the effect of freeze-drying of lactic acid bacteria on yoghurt properties. In this study, 7 *L.delbrueckii* spp. *bulgaricus* strains and 8

S.thermophilus strains were used to produce yoghurt samples. These strains were selected from a collection which contains 111 *L.delbrueckii* spp. *bulgaricus* and 56 *S.thermophilus* isolates. These strains were isolated from traditional Turkish yoghurt and commercial cultures by Neslihan Altay Dede (2010). *L.delbrueckii* spp. *bulgaricus* strains were selected according to acidification activity and acetaldehyde production properties and *S.thermophilus* strains were selected according to acidification activity. The results of acidification activity and acetaldehyde production analyses were given in Appendix J.

3.1.1 Physico-Chemical and Rheological Properties Determination

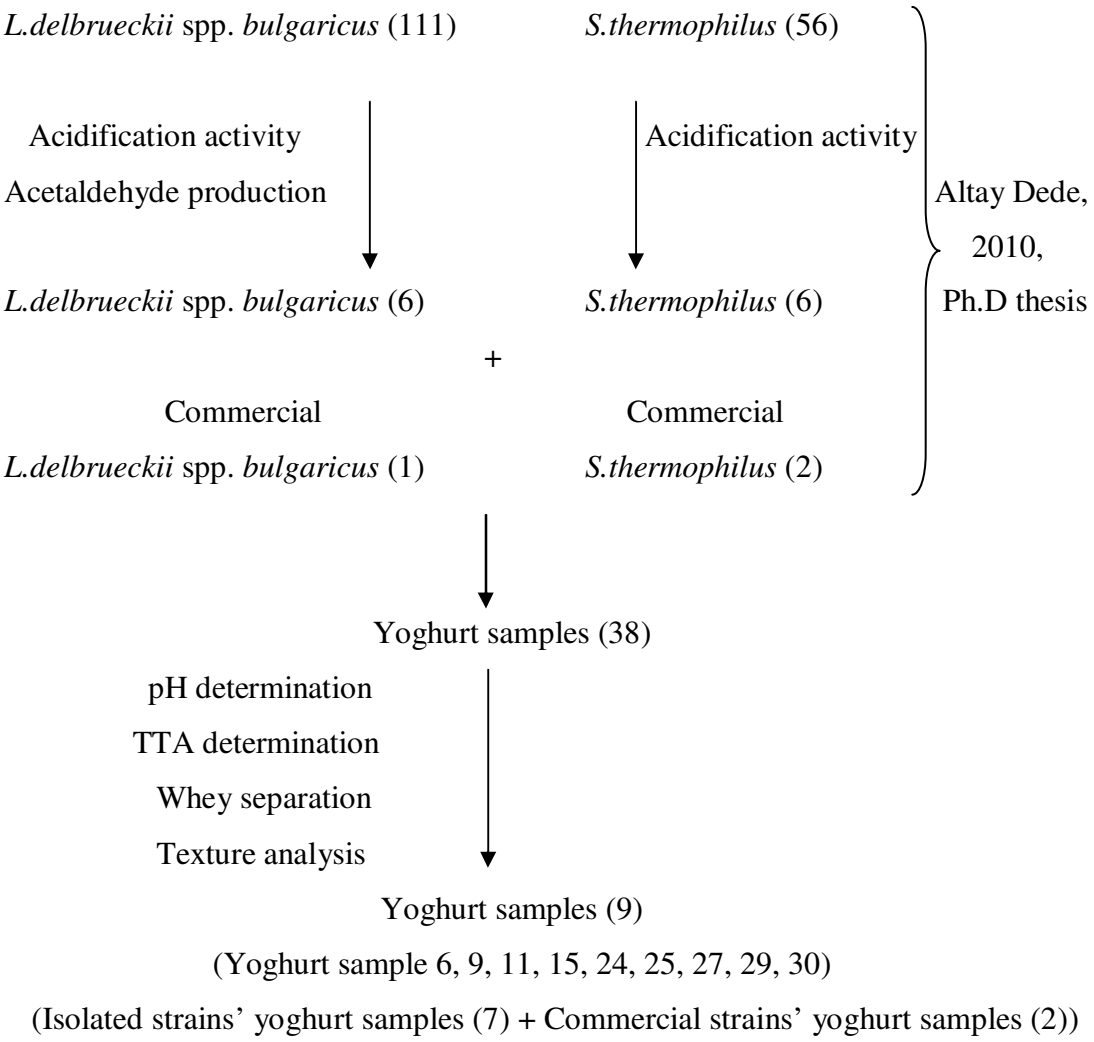


Figure 3.1 Determination of pH and titratable acidity, whey separation and textural properties of yoghurt samples and comparison with yoghurt samples produced by commercial strains. Numbers given in the parenthesis were the numbers of strains or numbers of yoghurt samples which were studied and selected.

Six *L.delbrueckii* spp. *bulgaricus* strains and six *S.thermophilus* strains were selected according to their acidification activity and acetaldehyde production characteristics which were determined by Neslihan Altay (Altay Dede, 2010). In addition, one *L.delbrueckii* spp. *bulgaricus* strain and two *S.thermophilus* strains, which were isolated from commercial mixed cultures, were selected for using in the production of 2 commercial yoghurt samples. 38 yoghurt samples were produced by using these 15 strains and pH and titratable acidity determination, whey separation and texture analysis were conducted. According to these results, 9 yoghurt samples were selected for further experiments. Two of these samples were the samples produced by commercial cultures and one of these was a sample which has average yoghurt characteristics, to provide a good comparison with commercials and average one.

3.1.2 Chemical Properties Determination

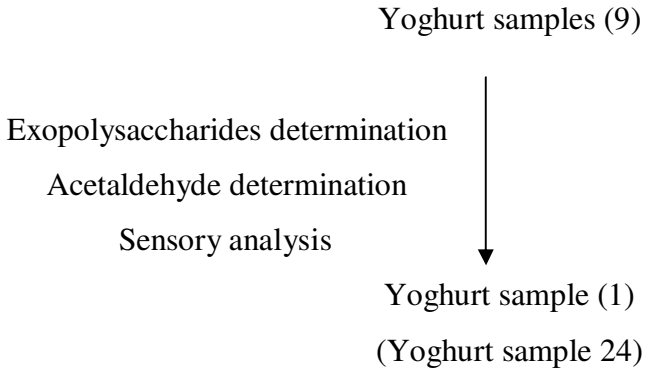


Figure 3.2 Determination of chemical properties and consumer acceptance of yoghurt samples and comparison with yoghurt samples produced by commercial strains.

Nine selected yoghurt samples were produced again and exopolysaccharides determination, acetaldehyde determination and sensory analysis were done to select the sample which have the best properties and which is accepted by the consumers. According to the results, strains used in the production of yoghurt sample 24 were selected for freeze-drying process because this sample had comparable results in all experiments with the samples which were produced by using commercial starter cultures.

3.1.3 Determination of the Effect of Freeze-Drying of Lactic Acid Bacteria on Yoghurt Properties

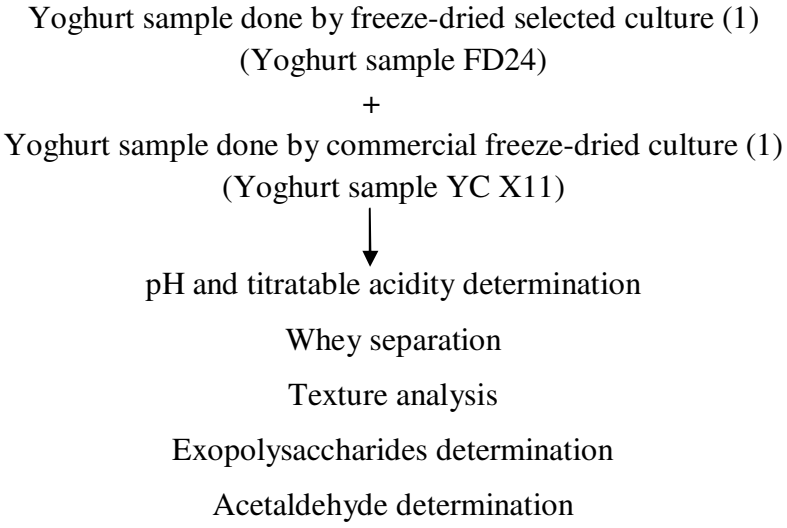


Figure 3.3 Determination of the effect of freeze-drying of LAB on yoghurt properties and comparison of these properties with a yoghurt sample produced by using a commercial freeze-dried culture.

Strains of selected yoghurt sample were freeze-dried to see the effect of freeze-drying process on the yoghurt. Yoghurt sample was produced by freeze-dried selected culture and yoghurt properties, which were pH and titratable acidity, whey separation, texture analysis, exopolysaccharide content and acetaldehyde amount were determined again to compare with yoghurt which was produced by a commercial freeze-dried culture. This commercial yoghurt was also studied with respect to these properties. To produce the commercial yoghurt sample, DVS YO-FLEX YC X11 culture of CHR-Hansen Company was used.

3.2 Results

All analyses results were given in Table 3.1. Thirty-eight yoghurt samples were analyzed for pH and total titratable acidity change during storage, whey separation and hardness. Yoghurt samples which gave the best results for each analysis were selected for chemical analyses.

As seen in Table 3.1, Yoghurt samples 8, 9, 11, 12, 31 and 33 gave the highest final pH value at the end of the storage time which means these yoghurt samples have longer shelf-life than the others. In addition to that acceptable yoghurt pH level is between 4.6-4.1 (Walstra, Wouters, & Geurts, 2006). According to this, yoghurt samples 2, 3, 4, 5, 26, 35, 36 and 37 were omitted.

Also, titratable acidity is an important parameter for shelf-life determination. According to the table, yoghurt samples 6, 7, 8, 9, 11, 12, 22 and 23 were the yoghurt samples with the lowest titratable acidity results and these were selected for further

chemical analyses. Yoghurt samples 13, 14, 26, 35, 36, 37 and 38 were omitted because they have the highest titratable acidity results.

Whey separation is an important quality parameter for consumers because when syneresis occurs in yoghurt it is thought that it is because of the low textural properties of yoghurt. According to whey separation analysis results, yoghurt samples 4, 6, 9, 15, 24, 36 and 37 have the lowest syneresis values and this may have a positive effect on consumer acceptance. Therefore, yoghurt samples 1, 2, 3, 7, 8, 21 and 23 were omitted due to the high syneresis value.

Finally, hardness of yoghurt samples were measured by using texture analyzer because texture of yoghurt is the second important factor which affects the consumer choice after taste of yoghurt. According to hardness values, yoghurt samples 4, 6, 11, 14, 15, 24, 25 and 29 were selected as the hardest yoghurt samples. Since texture is an important quality parameter, yoghurt samples 8, 12, 23, 31, 32 and 33, which have the lowest hardness values, were omitted.

As conclusion, after selecting and omitting of yoghurt samples for chemical analyses according to the results, 9 yoghurt samples, which are yoghurt samples 6, 9, 11, 15, 24 and 25, were selected for chemical analyses. To maintain a good comparison between traditional and commercial cultures' yoghurt, yoghurt samples 29 and 30 were also analyzed. In addition, to observe the difference between yoghurt samples which have good and poor physico-chemical and textural properties, yoghurt sample 27 which has average or poor results for each analysis was also analyzed in chemical analyses.

In chemical analyses part of the study, exopolysaccharide and acetaldehyde content determination were conducted. According to the acetaldehyde content analysis results, yoghurt samples 24 and 25 were the closest ones to yoghurt samples 29 and 30 which are the yoghurt samples produced by commercial strain combinations. In addition, in EPS content analysis, yoghurt sample 24 was found as the closest one to yoghurt samples 29 and 30.

In sensory analysis, 9 yoghurt samples were rated by 11 participants due to appearance, odor and flavor. According to overall scores, yoghurt sample 24 was found as the most preferred one among all 9 yoghurt samples.

In the last part of this study, strains of selected yoghurt sample (yoghurt sample 24) were freeze-dried separately and these freeze-dried cultures were used to produce yoghurt sample FD24. Yoghurt sample FD24 was analyzed to compare the results with yoghurt sample 24. Also, to compare yoghurt sample FD24 with a commercial yoghurt sample, DVS YO-FLEX YC X11 culture of CHR-Hansen Company was used.

Table 3.1 All analysis results for yoghurt samples

Sample Number	Final pH*	Final TTA* (% lactic acid)	Whey Separation (mL/g)	Hardness (N)	EPS Amount (g/mL)	Acetaldehyde Amount (ppm)	Sensory Analysis
1	4.17 ± 0.02	1.11 ± 0.03	0.362 ± 0.003	209.84 ± 51.49			
2	4.06 ± 0.02	1.18 ± 0.03	0.372 ± 0.002	241.59 ± 24.92			
3	4.08 ± 0.02	1.18 ± 0.01	0.354 ± 0.003	195.15 ± 16.33			
4	4.10 ± 0.02	1.12 ± 0.02	0.280 ± 0.003	270.65 ± 19.25			
5	4.11 ± 0.02	1.17 ± 0.05	0.333 ± 0.003	242.13 ± 6.92			
6**	4.20 ± 0.01	1.09 ± 0.03	0.205 ± 0.003	268.47 ± 72.85	$5.50 \times 10^{-5} \pm 4 \times 10^{-6}$	30.68 ± 0.32	4.0
7	4.21 ± 0.01	1.08 ± 0.03	0.377 ± 0.007	192.71 ± 9.20			
8	4.43 ± 0.35	1.10 ± 0.01	0.380 ± 0.003	151.55 ± 27.82			
9**	4.43 ± 0.02	1.00 ± 0.04	0.283 ± 0.002	225.05 ± 40.73	$3.68 \times 10^{-5} \pm 6 \times 10^{-6}$	29.13 ± 0.50	3.27
10	4.30 ± 0.02	1.15 ± 0.04	0.310 ± 0.004	173.91 ± 13.56			

Table 3.1 All analysis results for yoghurt samples (Cont'd)

Sample Number	Final pH	Final TTA* (% lactic acid)	Whey Separation (mL/g)	Hardness (N)	EPS Amount (g/mL)	Acetaldehyde Amount (ppm)	Sensory Analysis
11**	4.38 ± 0.06	1.05 ± 0.02	0.302 ± 0.002	272.97 ± 7.08	$3.51 \times 10^{-5} \pm 4 \times 10^{-6}$	28.74 ± 3.00	3.91
12	4.39 ± 0.03	1.04 ± 0.05	0.335 ± 0.003	132.95 ± 40.08			
13	4.25 ± 0.02	1.38 ± 0.07	0.327 ± 0.002	228.74 ± 10.12			
14	4.19 ± 0.01	1.29 ± 0.02	0.316 ± 0.001	255.09 ± 17.74			
15**	4.21 ± 0.02	1.27 ± 0.02	0.278 ± 0.002	265.56 ± 6.44	$5.20 \times 10^{-5} \pm 7 \times 10^{-6}$	31.87 ± 3.24	3.82
16	4.23 ± 0.03	1.27 ± 0.03	0.323 ± 0.001	220.69 ± 43.65			
17	4.26 ± 0.02	1.27 ± 0.04	0.306 ± 0.003	215.35 ± 60.74			
18	4.18 ± 0.02	1.23 ± 0.01	0.311 ± 0.002	209.09 ± 17.12			
19	4.31 ± 0.03	1.14 ± 0.01	0.351 ± 0.001	134.96 ± 2.93			
20	4.24 ± 0.03	1.14 ± 0.05	0.303 ± 0.003	174.11 ± 32.73			

Table 3.1 All analysis results for yoghurt samples (Cont'd)

Sample Number	Final pH	Final TTA* (% lactic acid)	Whey Separation (mL/g)	Hardness (N)	EPS Amount (g/mL)	Acetaldehyde Amount (ppm)	Sensory Analysis
21	4.17 ± 0.02	1.18 ± 0.04	0.433 ± 0.005	171.97 ± 52.07			
22	4.30 ± 0.02	1.06 ± 0.02	0.299 ± 0.005	239.37 ± 28.49			
23	4.30 ± 0.02	1.09 ± 0.01	0.373 ± 0.003	132.32 ± 37.36			
24**	4.31 ± 0.01	1.10 ± 0.01	0.278 ± 0.003	321.86 ± 4.33	$7.27 \times 10^{-5} \pm 4 \times 10^{-6}$	42.69 ± 8.12	4.18
25**	4.31 ± 0.02	1.10 ± 0.05	0.324 ± 0.002	286.58 ± 25.02	$3.51 \times 10^{-5} \pm 4 \times 10^{-6}$	39.29 ± 2.76	4.09
26	4.10 ± 0.01	1.30 ± 0.07	0.331 ± 0.001	237.20 ± 70.81			
27**	4.15 ± 0.01	1.20 ± 0.03	0.340 ± 0.002	168.68 ± 7.98	$3.47 \times 10^{-5} \pm 4 \times 10^{-6}$	24.52 ± 1.70	3.0
28	4.17 ± 0.03	1.17 ± 0.04	0.305 ± 0.004	194.72 ± 8.51			
29**	4.22 ± 0.01	1.17 ± 0.01	0.292 ± 0.002	305.00 ± 50.15	$8.35 \times 10^{-5} \pm 7 \times 10^{-6}$	41.97 ± 2.94	4.09
30**	4.27 ± 0.01	1.14 ± 0.02	0.292 ± 0.003	236.69 ± 31.15	$7.74 \times 10^{-5} \pm 9 \times 10^{-6}$	45.97 ± 3.25	3.91

Table 3.1 All analysis results for yoghurt samples (Cont'd)

Sample Number	Final pH	Final TTA* (% lactic acid)	Whey Separation (mL/g)	Hardness (N)	EPS Amount (g/mL)	Acetaldehyde Amount (ppm)	Sensory Analysis
31	4.40 ± 0.01	1.10 ± 0.02	0.331 ± 0.001	99.33 ± 22.97			
32	4.28 ± 0.01	1.16 ± 0.02	0.340 ± 0.002	119.83 ± 13.90			
33	4.39 ± 0.02	1.11 ± 0.03	0.305 ± 0.002	95.42 ± 14.20			
34	4.19 ± 0.01	1.22 ± 0.05	0.292 ± 0.004	186.06 ± 7.63			
35	4.09 ± 0.03	1.30 ± 0.01	0.293 ± 0.002	225.69 ± 21.00			
36	4.11 ± 0.02	1.30 ± 0.03	0.265 ± 0.004	233.91 ± 11.40			
37	4.02 ± 0.02	1.33 ± 0.02	0.241 ± 0.004	234.38 ± 31.00			
38	4.16 ± 0.02	1.29 ± 0.02	0.332 ± 0.001	178.71 ± 72.49			

***** Final pH and final TTA values are the values which were measured at the end of the 21-day storage

** These are the yoghurt samples which were selected for the chemical analyses part of the study.

3.3 Physico-Chemical Analyses

3.3.1 pH and Titratable Acidity Determination

Acidity is the most typical characteristic of yoghurt which is affecting consumer acceptance because that gives the taste to yoghurt and also determines the shelf-life of product. Since this characteristics have a crucial importance for both producer and consumer, determination of pH and titratable acidity was one of the most important aspects of this study. 38 yoghurt samples were studied during storage at 4^0C for 21 days and measurements were conducted according Chapter 2.2.2. According to Walstra et al. (2006), preferred yoghurt acidity for consumption is between 4.6 and 4.1. Therefore, these measurements were used to select the sample which has longer shelf-life. Final pH values of sample were given in Figure 3.4. As seen in the Figure 3.4, six yoghurt samples were below the pH of 4.1 and these were eliminated for the second part of the study which was the determination of chemical properties and consumer acceptance part.

Total titratable acidity (TTA) of yoghurt is one of the quality control tests of yoghurt and should be appropriate to the limits stated in Turkish Codex as between 0.80-1.60 % lactic acid (TS 1330/February 1999). pH and TTA of yoghurt samples increase with the production of lactic acid which is related with the growth of lactic acid bacteria. Therefore, in this study, 7 yoghurt samples with the highest TTA values at the end of the storage period which was 21. day were omitted among the samples selected for second part of study even if there were in the acceptable limits with respect to final TTA. Total titratable acidity values of 38 samples were given in Figure 3.5.

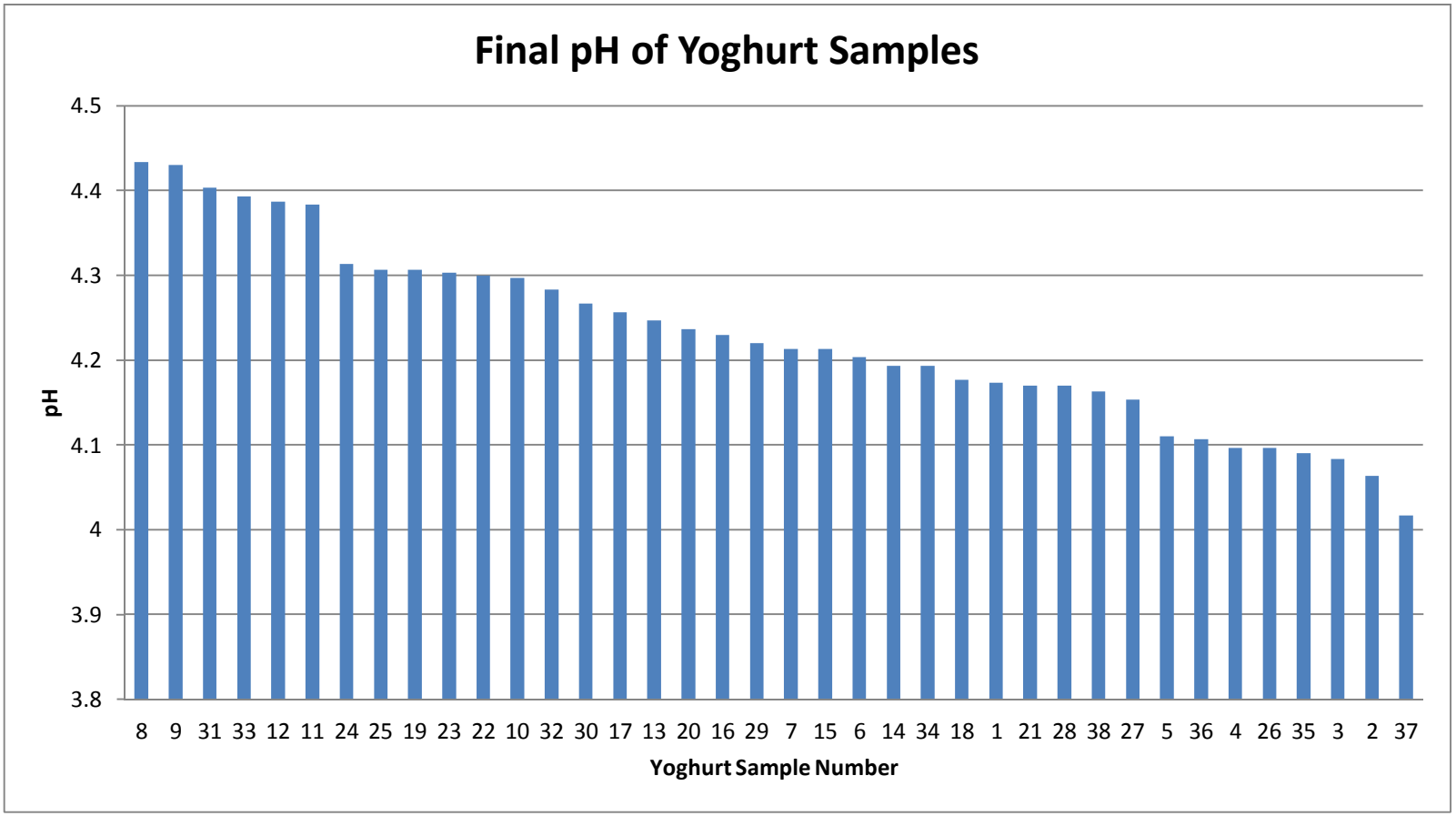


Figure 3.4 pH values of yoghurt samples at the end of the storage period which was 21. day

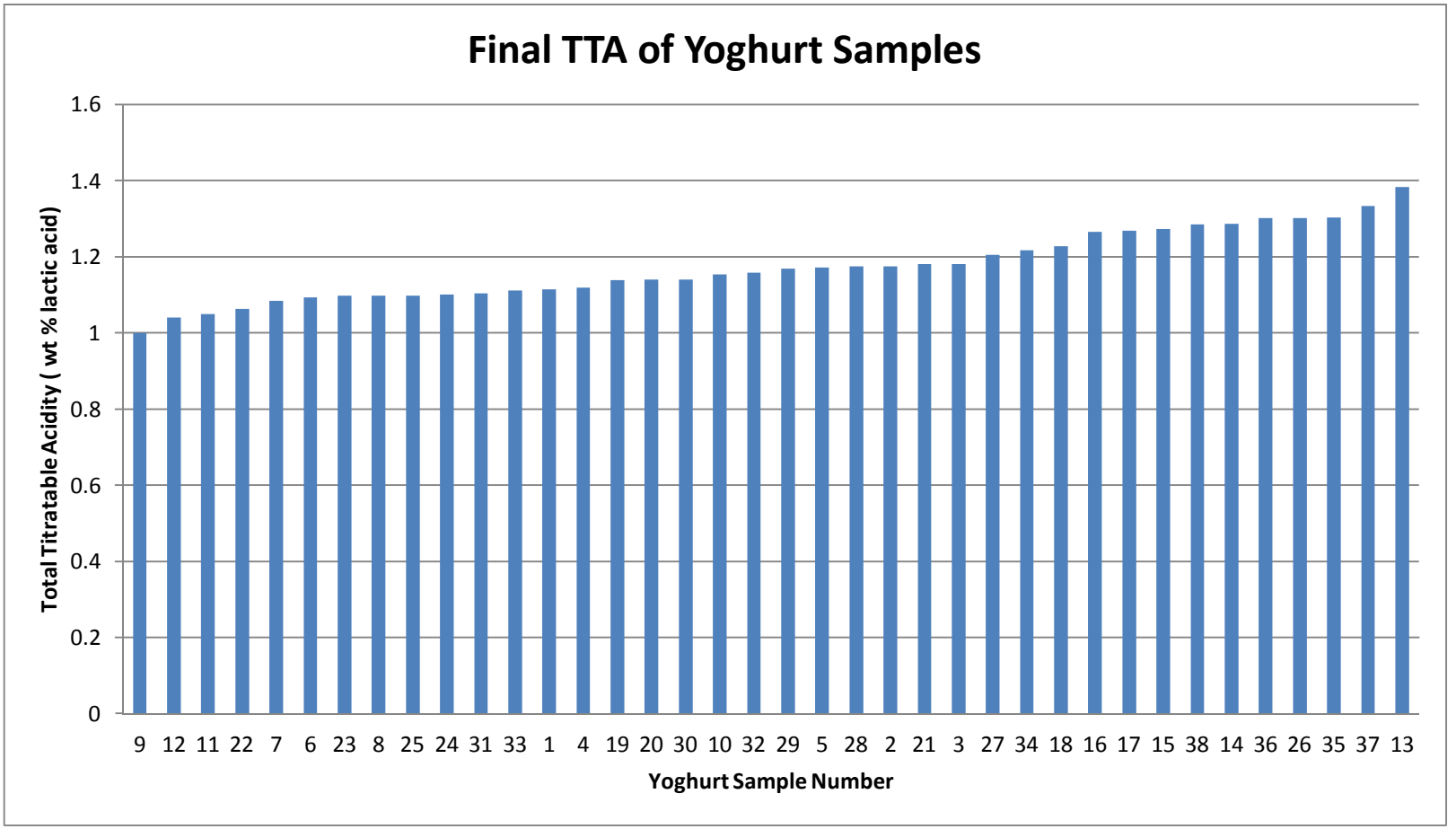


Figure 3.5 Total titratable acidity (TTA) values of yoghurt samples at the end of the storage period which was 21.day

Industrial starters have a small ratio of *L.delbrueckii* spp. *bulgaricus* compared to *S.thermophilus* and due to this ratio, enable the production of yoghurt with less potential of post-acidification (Donkora, 2006). *L.delbrueckii* spp. *bulgaricus* seems to be responsible for post-acidification of yoghurt. In addition, the results of this study showed that final acidity level of yoghurt depends on *L.delbrueckii* spp. *bulgaricus* strain in the culture. As seen in the Figure 3.6, final pH acidity levels of yoghurt samples are related with the used *L.delbrueckii* spp. *bulgaricus* strain. Figure 3.6 shows that final pH of yogurt samples produced by using 3 different *L.delbrueckii* spp. *bulgaricus* strains which are MRS N4-3, MRS K1-43 and MRS K2-1 and 3 different *S.thermophilus* strains which are M17 N8-2, M17 K1-14 and M17 S1-3. In the figure, group 1 shows the final pH of yoghurt samples produced by using *L.delbrueckii* spp. *bulgaricus* MRS N4-3 and 3 *S.thermophilus* strains and the average final pH of group 1 was found as 4.37±0.06. Group 2 shows the final pH of yoghurt samples produced by using *L.delbrueckii* spp. *bulgaricus* MRS K1-43 and 3 *S.thermophilus* strains and the average final pH of group 2 was found as 4.13±0.06. Finally, group 3 shows the final pH of yoghurt samples produced by using *L.delbrueckii* spp. *bulgaricus* MRS K2-1 and 3 *S.thermophilus* strains and the average final pH of group 3 was found as 4.24±0.07. Although final pH of yoghurt samples are affected by the *S.thermophilus* strains, final pH of yoghurt samples in the same group are very close to each other. However, the average final pH of groups are very different from each other. It can be concluded that *L.delbrueckii* spp. *bulgaricus* strains were much more effective on final pH of yoghurt samples. In addition to that graphic, two-way ANOVA test and Tukey test were conducted as statistical analysis. According to two-way ANOVA table both *L.delbrueckii* spp. *bulgaricus* and *S.thermophilus* strains significantly affect the final pH value of samples. However, Tukey test results showed that *L.delbrueckii* spp. *bulgaricus* strains are significantly different to each other. Therefore, it can be said that *L.delbrueckii* spp. *bulgaricus* is responsible from post-acidification of yoghurt. The results of statistical analysis were given in Appendix F.

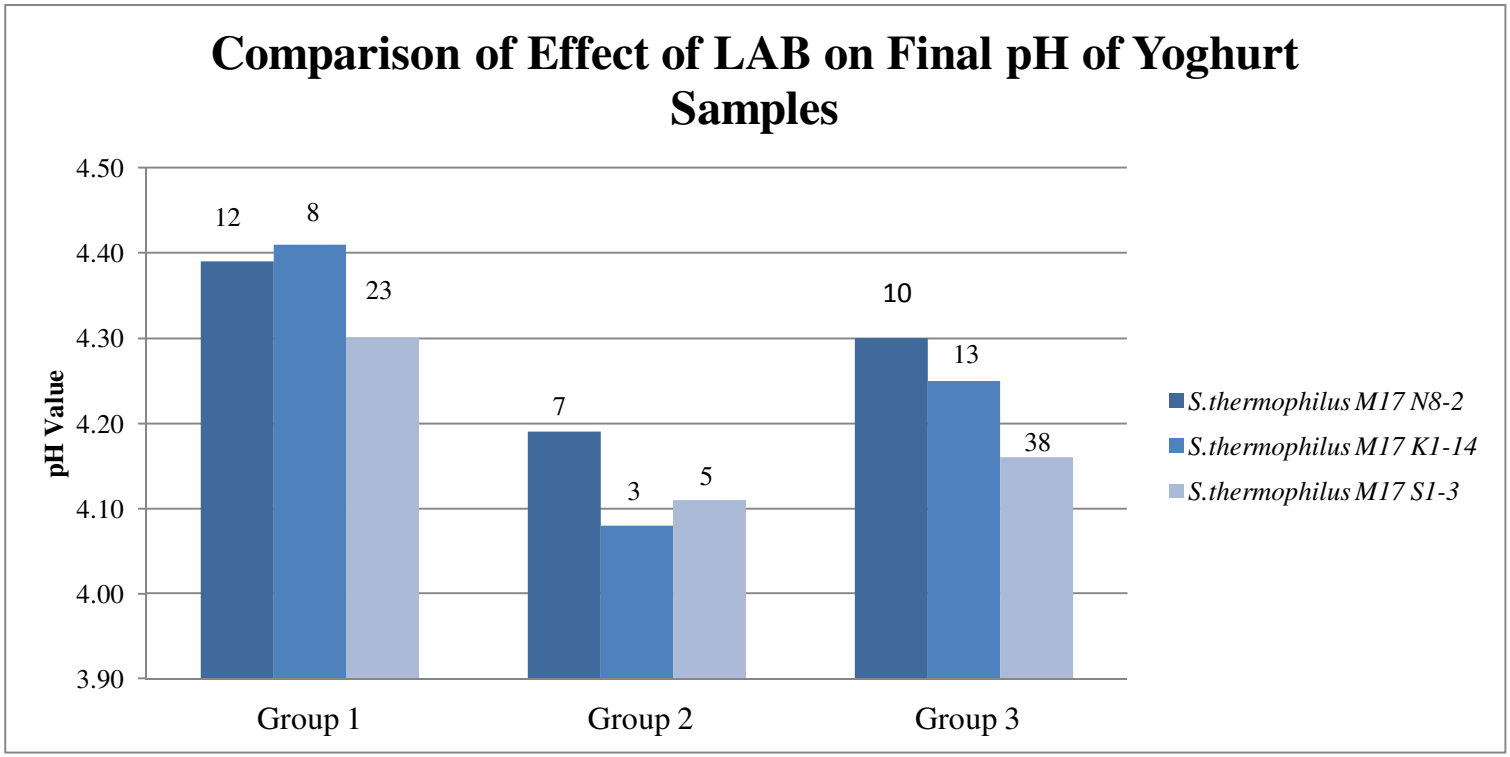


Figure 3.6 Effect of *L.delbrueckii* spp. *bulgaricus* and *S.thermophilus* strains on pH value. Group 1; yoghurt produced by using *L.delbrueckii* spp. *bulgaricus* strain "MRS N4-3", group 2; yoghurt produced by using *L.delbrueckii* spp. *bulgaricus* strain "MRS K1-43", group 3; yoghurt produced by using *L.delbrueckii* spp. *bulgaricus* strain "MRS K2-1". Yoghurt sample numbers are given at the top of the columns.

3.3.2 Texture Analysis and Whey Separation

Texture and whey syneresis of yoghurt are important parameters for consumer acceptance and these can be realized by consumers without any device. In this study, texture analyzer was used to measure the maximum force and whey syneresis was measured using the methods given at Chapter 2.2.4 (Yılmaz, 2006; Sezgin, Yıldırım, & Karagül, 1994). The maximum force of yoghurt samples is called the hardness of yoghurt. For the second part of the experiments, yoghurt samples, which have low whey syneresis value and high hardness value, were selected. Hardness values of samples were given in Figure 3.7 and whey syneresis results were given in Figure 3.8.

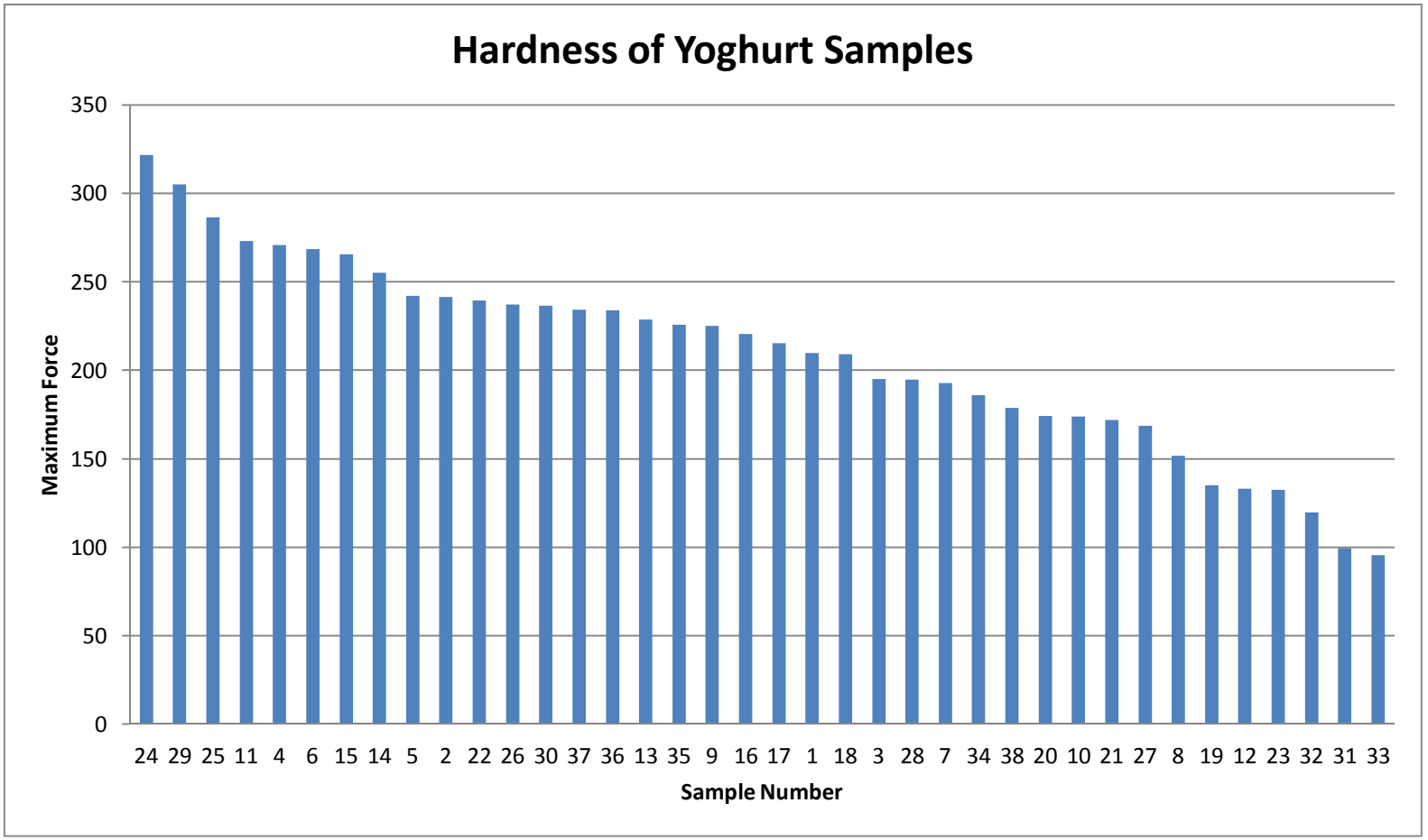


Figure 3.7 Peak forces of yoghurt samples which is called as hardness of yoghurt

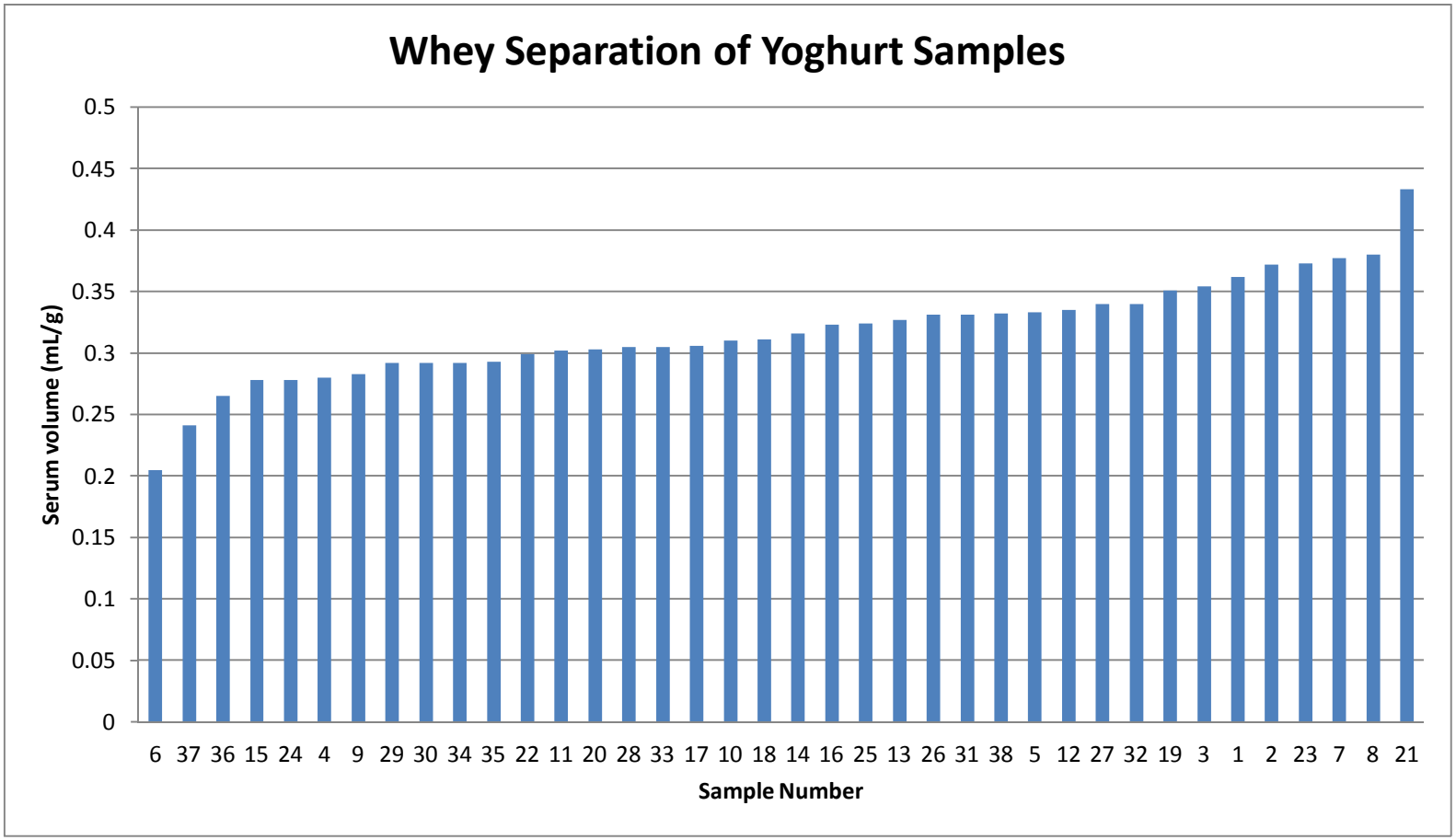


Figure 3.8 Separation of whey from yoghurt samples

3.4 Chemical Analyses

3.4.1 Exopolysaccharide Determination

Exopolysaccharide content of samples were determined according to Chapter 2.2.7 and Figure 3.9 shows the results.

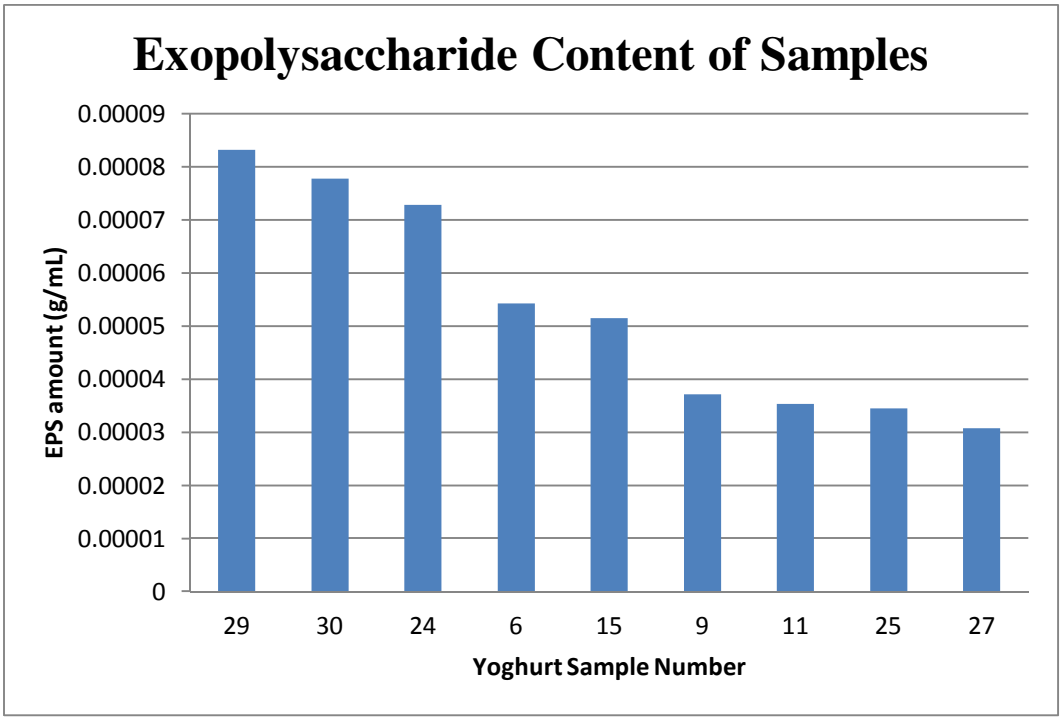


Figure 3.9 Exopolysaccharide content of yoghurt samples

In literature, there is a controversy about the effect of exopolysaccharide content of yoghurt on hardness value of yoghurt. In some researches, it is shown that there is no significant effect of EPS content on the hardness of yoghurt (Marshall & Rawson, 1999; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). However, some researchers claim that viscosity and texture of yoghurt is positively affected by the EPS content (Folkenberg, Dejmek, Skriver, Guldager, & Ipsen, 2006) (Duboc & Mollet, 2001). In this study, hardness and EPS content of yoghurt samples were measured and statistical analysis was conducted. According to one-way ANOVA results given in Appendix H, EPS level of yoghurt samples are not significantly effective on hardness of yoghurt ($p>0.05$). In Figure 3.10, hardness and EPS content of yoghurt sample were given and it can be seen that even though EPS amount is low in yoghurt sample, hardness value can be high. Yoghurt sample 11 and 25 has high hardness value but as seen in Figure 3.10, although their EPS amounts are relatively low.

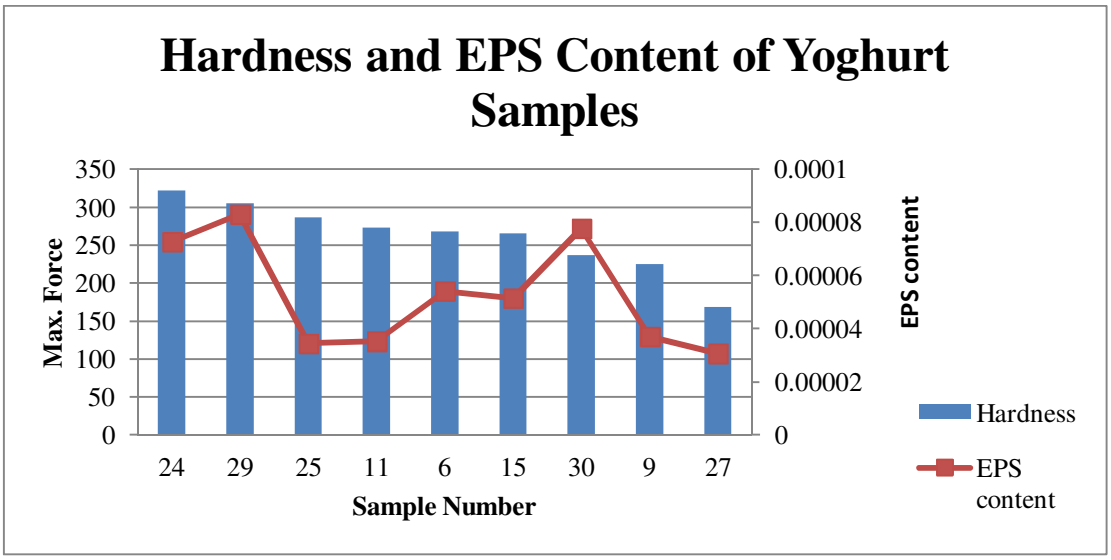


Figure 3.10 Comparison of hardness and EPS content of yoghurt

3.4.2 Acetaldehyde Determination

Acetaldehyde is the major aroma compound which gives the characteristic flavor of yoghurt and importance of acetaldehyde content on flavor characteristics in fermented milk was clearly demonstrated (Law, 1981) (Bottazzi & Vescovo, 1969) (Lees & Jago, 1969) (Schmidt, Davidson, & Bates, 1983). Another study on acetaldehyde content of yoghurt samples produced by Turkish yoghurt isolates was conducted by Çelik, E. S. (2007). Acetaldehyde contents of 20 yoghurt samples in Çelik's study were changing between 13.442±2.69 and 25.444±0.59 mg/L. In this study, acetaldehyde contents of yoghurt samples were measured and calculated according to Chapter 2.2.8. Acetaldehyde amounts in yoghurt samples produced by traditional cultures were varying between 24.25±1.63 and 41.93±3.02 ppm as shown in the Figure 3.11. Acetaldehyde contents of yoghurt samples produced by commercial strains were determined as 41.83±2.93 and 45.40±2.47 ppm. The highest acetaldehyde content was found in one of the commercial culture yoghurt but the second one was found sample number 24 which was produced by using traditional cultures.

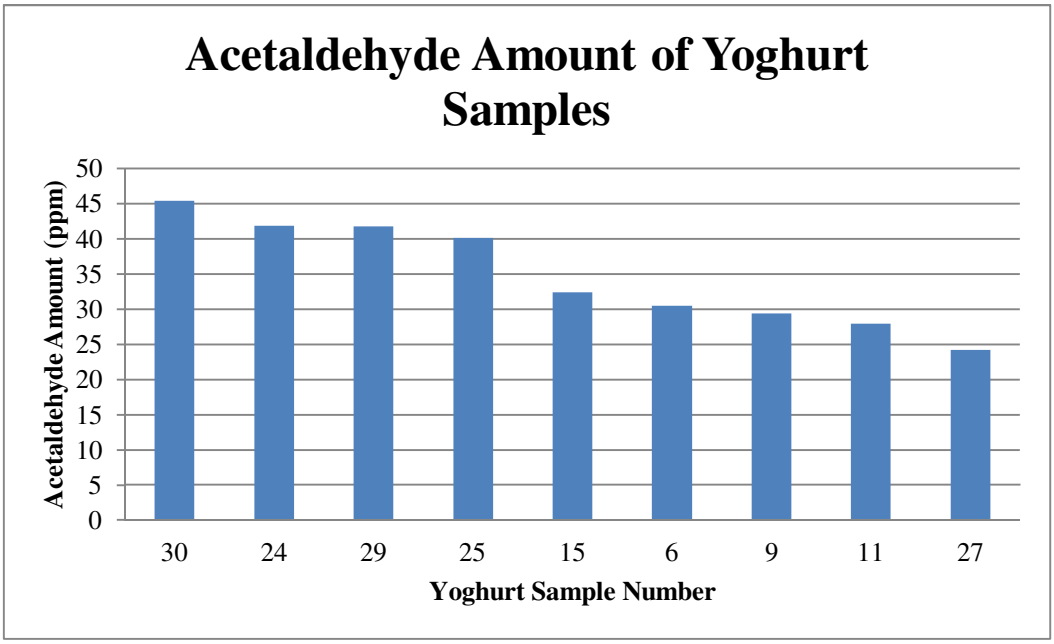


Figure 3.11 Acetaldehyde amount of yoghurt samples

3.4.3 Sensory Analysis

The most important parameter in all food industry is the consumer acceptance. Therefore, sensory analysis was carried out in this study to select the most accepted yoghurt sample for freeze-drying process. 9 different yoghurts were tried and scored by 11 participants. Yoghurt samples were rated in terms of appearance, odor and flavor. According to the results, overall acceptance of samples were significantly affected by odor and flavor ($p<0.05$). Figure 3.12 shows the overall scores of the yoghurt samples and as seen in the figure the highest score was given to the sample 24 which was produced by using traditional cultures. Sensory analysis questionnaire and statistical analysis results of yoghurt samples were given in Appendix G.

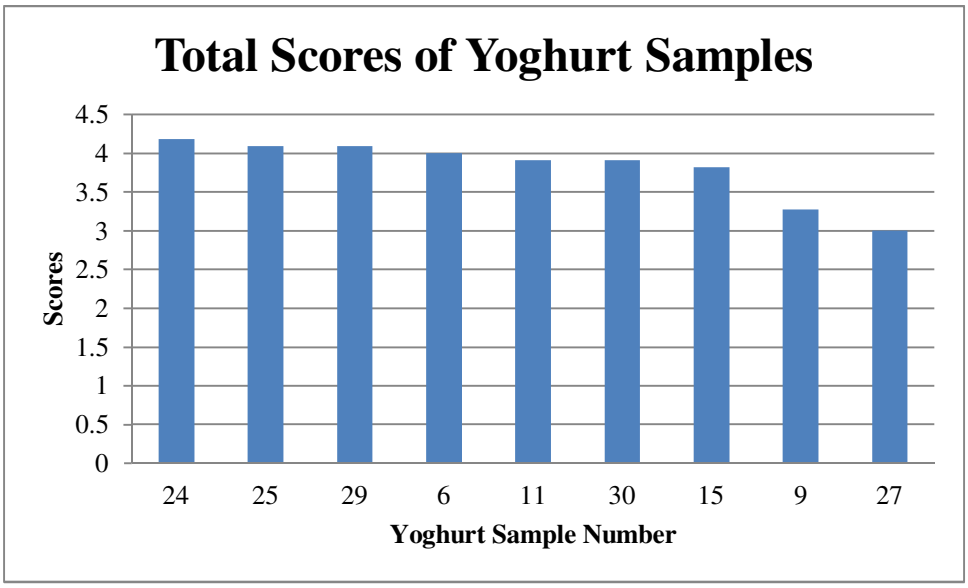


Figure 3.12 Total scores of yoghurt samples

3.5 Effects of Freeze-Drying on Yoghurt Properties

In order to observe the effect of using freeze-dried starter cultures on yoghurt properties, selected culture combination was freeze-dried and yoghurt samples were produced by using this freeze-dried culture. pH and TTA change during storage, whey syneresis, EPS and acetaldehyde content of these yoghurt samples were determined and then compared with that of yoghurt samples prepared using commercial freeze-dried culture and non-freze-dried culture. The results of physico-chemical and chemical analyses were given in Table 3.2 and Table 3.3.

Table 3.2 Physico-chemical properties of yoghurt samples produced by freeze-dried cultures

Yoghurt Sample	Final pH	Final TTA (% lactic acid)	Whey Separation (mL/g)	Hardness (N)
FD24	4.28 ± 0.02	1.11 ± 0.01	0.236 ± 0.002	334.94 ± 3.42
YCX11	4.25 ± 0.02	1.15 ± 0.01	0.277 ± 0.001	218.94 ± 3.90
24	4.31 ± 0.01	1.10 ± 0.01	0.278 ± 0.003	321.86 ± 4.33

Table 3.3 Chemical properties of yoghurt samples produced by freeze-dried cultures

Yoghurt Sample	EPS Amount (g/mL)	Acetaldehyde Amount (ppm)
FD24	$6.72 \times 10^{-5} \pm 7 \times 10^{-6}$	44.99 ± 3.73
YCX11	$5.06 \times 10^{-5} \pm 2 \times 10^{-6}$	48.40 ± 0.65
24	$7.27 \times 10^{-5} \pm 4 \times 10^{-6}$	42.69 ± 8.12

To produce the commercial yoghurt sample for comparison, DVS YO-FLEX YC X11 culture of CHR-Hansen Company was used. This culture is offered to the market as a structure culture which means that hardness of yoghurt produced by this culture is high.

An important difference was recognized during production of yoghurt between the incubation time of yoghurt samples produced by freeze-dried and conventional cultures. Although the rate of inoculation was same, it took longer time to decrease the desired pH. Actually, it can be seen in literature that freeze-drying may cause some decrease in activity of starter cultures (Bölükbaşı, 1985). However, higher incubation time may lead some positive aspects on flavor formation and textural properties (Tamime & Robinson, 2007).

Shelf-life, whey separation, hardness and acetaldehyde content are important quality parameters from the consumer point of view. Longer shelf-life is determined by the consumer according the acidity level and mouth-feel of yoghurt during storage. Also, whey of yoghurt is the unused part of yoghurt by consumers so low whey separation values is a desired yoghurt property.

An important difference recognized during production of yoghurt samples. The incubation time of yoghurt sample 24 and yoghurt sample FD24 are very different from each other and the incubation time of yoghurt sample FD24 was higher. In literature, activity loss of starters may cause the longer incubation time during production (Tamime & Robinson, 2007). However, it can be said that with the increasing bacteria number in yoghurt during fermentation, this activity loss lose its importance during storage because as seen in the Figure 3.13 and Figure 3.14, final pH and final TTA results were not affected by freeze-drying process. It is claimed that yoghurt is firmer at lower pH and longer incubation time means it takes longer time to reach a certain pH and so certain firmness. Since the gel formation takes longer time, gel structure become firmer. In Figure 3.15, it can be seen that whey

syneresis values are much lower in the samples produced by freeze-dried cultures rather than by conventional ones depending on the firm gel structure.

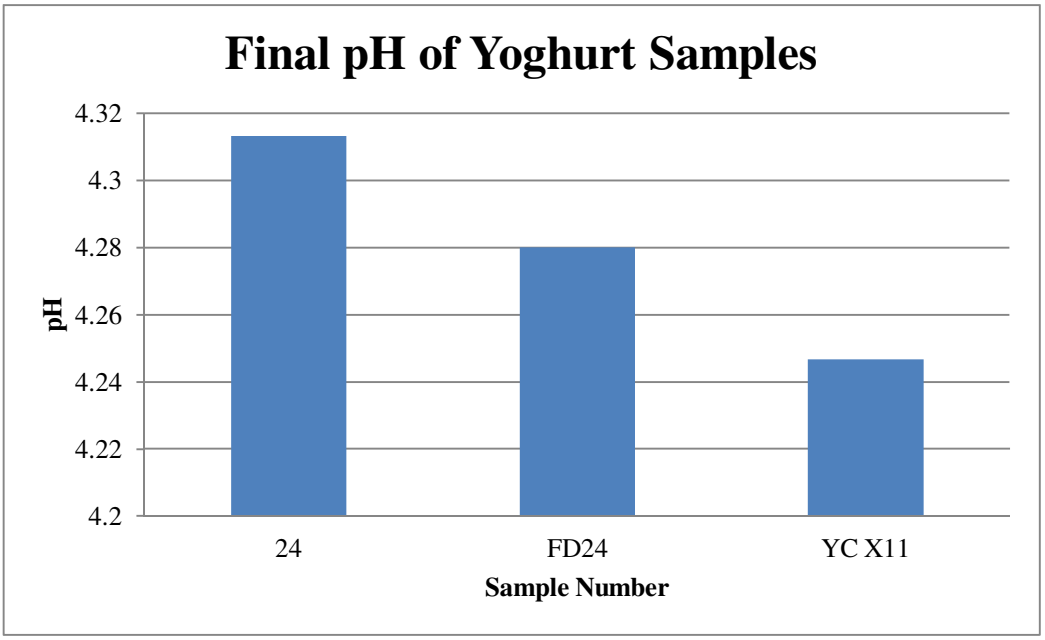


Figure 3.13 pH values of yoghurt samples at the end of the 21-day storage (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

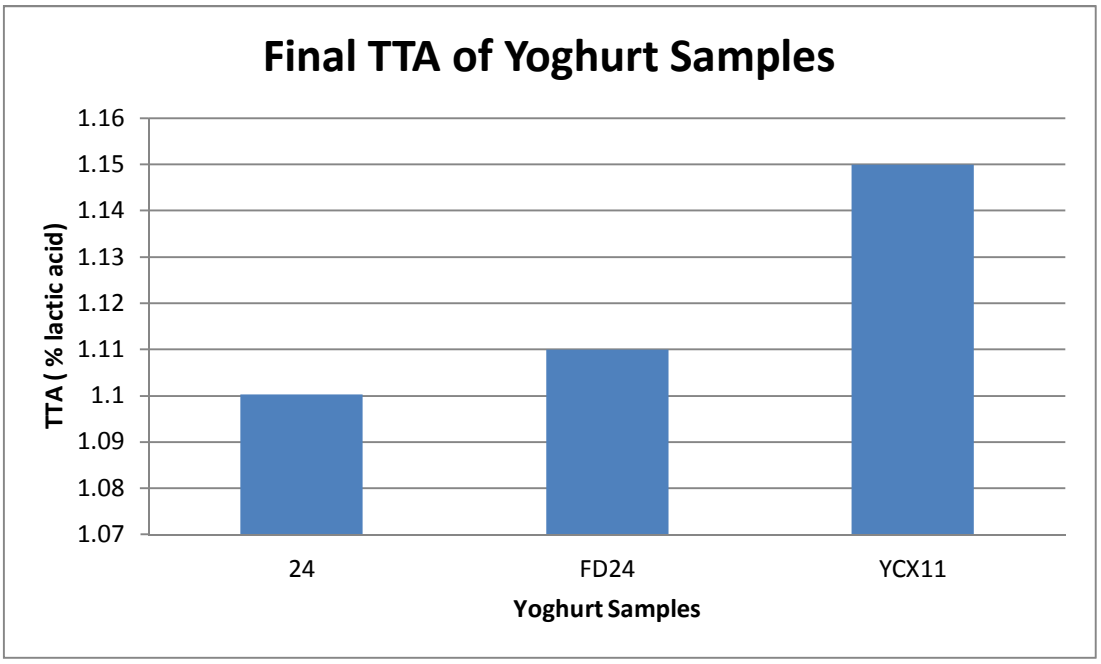


Figure 3.14 Total titratable acidity results of yoghurt samples at the end of the 21-day storage (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

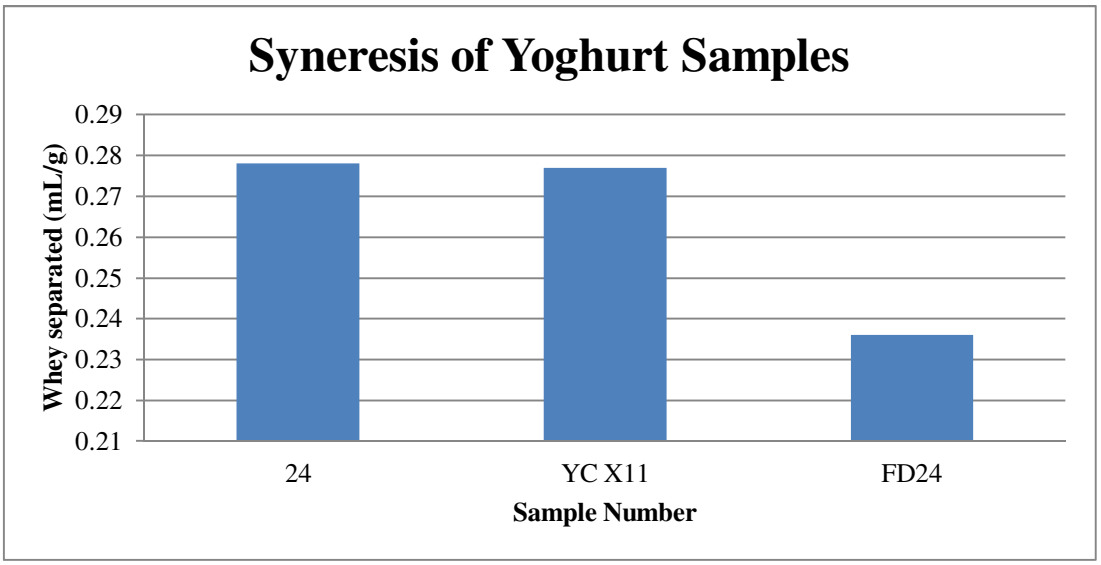


Figure 3.15 Syneresis results of yoghurt samples (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

In Figure 3.16, it can be seen that hardness of yoghurt samples produced by freeze-dried cultures rather than conventional method have higher hardness value. Although DVS YO-FLEX YC X11 culture is classified as a texture culture, the hardness value of this sample was measured lower than the traditional culture samples, both produced by conventional and freeze-dried ones. Exopolysaccharide content of yoghurt samples were determined and it was seen that freeze-drying process decreases the EPS production rate in yoghurt. Freeze-drying process may cause the loss of plasmid (Bouzar, Cerning, & Desmazeaud, 1997). Therefore, EPS production rate, depending on the enzymes which are encoded in plasmids, may decrease in freeze-dried yoghurt samples. Figure 3.17 shows EPS content of yoghurt samples.

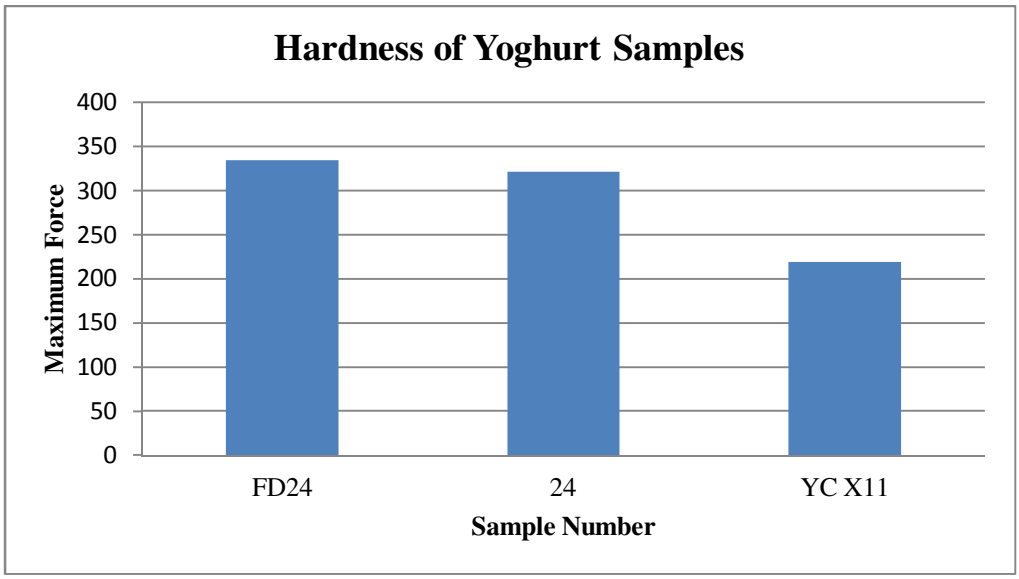


Figure 3.16 Hardness of yogurt samples and effect of freeze-drying on textural properties (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

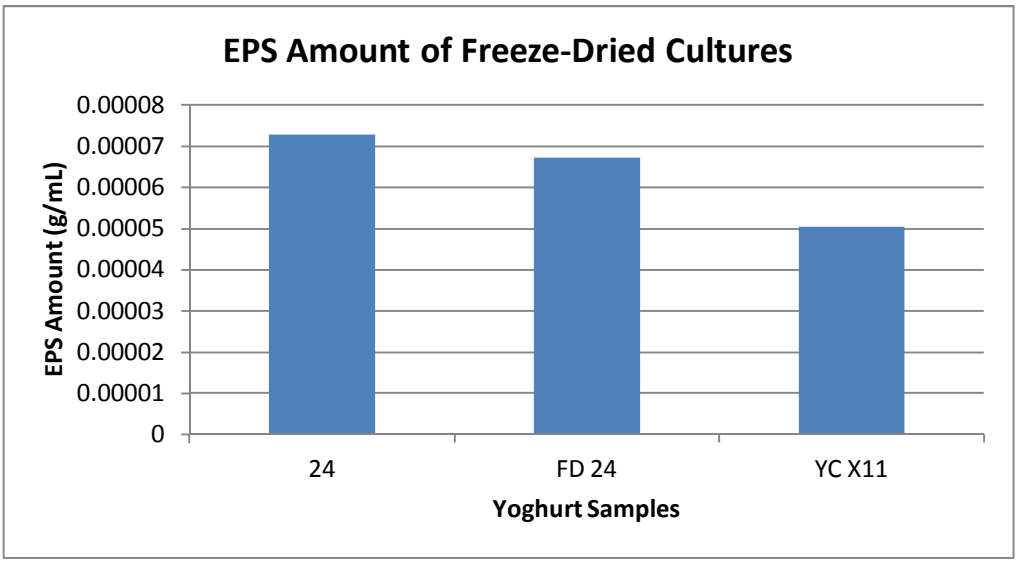


Figure 3.17 EPS amount of yoghurt samples and the effect of freeze-drying on EPS production of selected starter cultures (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

Acetaldehyde content of traditional freeze-dried culture sample was determined higher than the samples produced by conventional method as seen in the Figure 3.18 and it is thought that longer incubation time may provide this increase in acetaldehyde amount (Tamime & Robinson, 2007). Since DVS YO-FLEX YC X11 culture was used as a commercial reference, it was an expected result that this commercial sample has better results in the analyses.

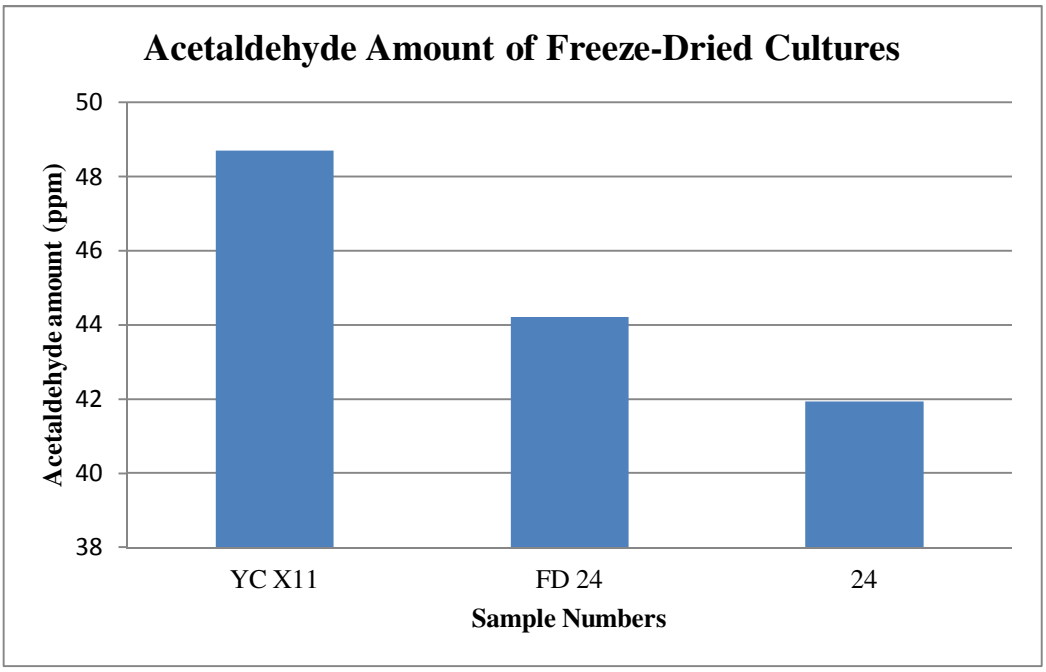


Figure 3.18 Acetaldehyde amount in yoghurt samples and the effect of freeze-drying on the acetaldehyde production of starter cultures (FD24: freeze-dried form of culture 24; YC X11: freeze-dried culture from Chr-Hansen Company; 24: non-freeze-dried culture)

CHAPTER IV

CONCLUSION

Yoghurt is a dairy product which is consumed all over the world with an increasing trend. It is produced as a result of the activity of *S.thermophilus* and *L.delbrueckii* spp. *bulgaricus*. In this study, 7 *L.delrueckii* spp. *bulgaricus* isolates, one of these isolated from commercial culture mixtures, and 8 *S.thermophilus* isolates, two of these isolated from commercial culture mixtures, were used to determine the yoghurt properties produced by these cultures. These cultures were isolated by Neslihan Altay Dede (2010) and selected as the strains having the best technological properties, namely, acidification activity and acetaldehyde production. Therefore, these strains were used in 38 different combination and 38 yoghurt samples were produced and examined in mainly three steps.

First step was the determination of pH and total titratable acidity (TTA) during 21-day storage, syneresis determination and texture analysis of 38 yoghurt samples. pH and TTA measurements are important to determine the shelf life of samples. Also, whey separation and textural properties are important by the consumer point of view to see the quality of yoghurt. At the end of the first step 9 yoghurt samples were chosen in total, two of these produced by commercial strains and one of these as the yoghurt sample, poor in quality.

In second step, 9 yoghurt samples were examined with respect to exopolysaccharide content, acetaldehyde content and consumer perception. At the end of the second step one yoghurt sample which had the best results in experiments and took the best scores from panelists was chosen for the third step experiments.

Finally in last step, the strains of culture mixture which produce the best yoghurt sample were freeze-dried. Two yoghurt samples were produced, one produced by using freeze-dried form of selected mixture and one produced by using commercial freeze-dried culture. All experiments were repeated for these two samples to see difference between the technological properties of these samples and also these results were compared with the data of selected mixture data before freeze-dried. Properties of the yoghurt sample made by freeze-dried culture was very close to that of yoghurt sample made by non-freeze-dried one so it can be said that freeze-drying process was successful and freeze-drying conditions were appropriate for the isolates.

After the third step, it can be said that one culture mixture was selected which has the ability of producing yoghurt as well as compete with the commercial cultures.

At the end of all experiments, it can be seen that the selected culture mixture has the ability to produce high quality yoghurt which is comparable with the commercial freeze-dried cultures.

CHAPTER V

RECOMMENDATION

Further studies can be carried on different freeze-drying conditions for the selected strains to minimize the adverse effects of freeze-drying process on strains because freeze-drying conditions may change from strain to strain. Bulk production of freeze-dried culture can be carried on. Freeze-drying of strains in high amount decrease the death rate during drying process, i.e., working with high amounts may increase the yield of culture production. Also, pilot-plant scale production can be done to see the problems during working with high amounts of cultures and yoghurt.

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APPENDIX A

CHEMICAL USED IN EXPERIMENTS

Table A.1 Chemicals used in experiments

M17 Broth	Merck 1.15029
MRS Broth	Merck 1.10661
Sodium hydroxide, NaOH	Merck 1.06498
Iodine	Merck 1.04761
Potassium Iodide	Merck 1.05051
Starch	Sigma S4126
Phenol crystalline	AppliChem A1594
Sulphuric acid, H_2SO_4	Merck 1.00713
Sodium bisulphate, $NaHSO_3$	Riedel-de Haen 13437
Sodium bicarbonate, $NaHCO_3$	Sigma S5761
Ethanol	Merck 1.00983
Flavourenzyme	Sigma P6110

APPENDIX B

pH OF YOGHURT SAMPLES DURING 21-DAY STORAGE

Table B.1 pH change of yoghurt samples during 21-day storage

Sample Number	pH				
	Day 1	Day 4	Day 7	Day 14	Day 21
1	4.48 4.41 4.49	4.27 4.24 4.26	4.18 4.20 4.23	4.18 4.20 4.21	4.16 4.17 4.19
2	4.43 4.38 4.40	4.32 4.30 4.30	4.25 4.22 4.23	4.16 4.11 4.11	4.08 4.04 4.07
3	4.51 4.50 4.54	4.43 4.44 4.44	4.29 4.29 4.30	4.15 4.13 4.13	4.08 4.07 4.10
4	4.54 4.52 4.54	4.46 4.45 4.41	4.34 4.34 4.30	4.19 4.17 4.16	4.11 4.08 4.10
5	4.43 4.41 4.48	4.22 4.21 4.26	4.17 4.20 4.22	4.13 4.19 4.18	4.09 4.11 4.13
6	4.54 4.58 4.52	4.33 4.31 4.25	4.26 4.31 4.24	4.22 4.24 4.24	4.20 4.21 4.20
7	4.43 4.44 4.49	4.27 4.28 4.31	4.25 4.24 4.27	4.22 4.22 4.26	4.22 4.20 4.22
8	4.64 4.68 4.65	4.49 4.51 4.52	4.45 4.50 4.48	4.44 4.48 4.42	4.43 4.47 4.40

Table B.1 pH change of yoghurt samples during 21-day storage (Cont'd)

Sample Number	pH				
	Day 1	Day 4	Day 7	Day 14	Day 21
9	4.69 4.61 4.67	4.50 4.47 4.49	4.49 4.46 4.47	4.46 4.46 4.44	4.45 4.42 4.42
10	4.59 4.52 4.56	4.43 4.41 4.46	4.39 4.37 4.38	4.32 4.34 4.33	4.28 4.30 4.31
11	4.57 4.63 4.62	4.41 4.49 4.45	4.39 4.46 4.42	4.34 4.41 4.39	4.32 4.44 4.39
12	4.63 4.60 4.60	4.54 4.50 4.52	4.47 4.44 4.45	4.44 4.39 4.40	4.42 4.37 4.37
13	4.40 4.43 4.42	4.38 4.41 4.37	4.33 4.37 4.34	4.26 4.29 4.26	4.24 4.27 4.23
14	4.39 4.37 4.36	4.37 4.35 4.35	4.33 4.31 4.32	4.22 4.22 4.20	4.19 4.20 4.19
15	4.45 4.40 4.42	4.38 4.36 4.36	4.36 4.32 4.33	4.28 4.24 4.27	4.19 4.22 4.23
16	4.33 4.39 4.37	4.29 4.35 4.31	4.29 4.31 4.27	4.23 4.27 4.25	4.21 4.26 4.22
17	4.44 4.40 4.43	4.35 4.35 4.32	4.34 4.35 4.30	4.27 4.28 4.26	4.27 4.26 4.24
18	4.30 4.34 4.35	4.26 4.27 4.27	4.25 4.26 4.25	4.20 4.20 4.18	4.19 4.18 4.16
19	4.45 4.51 4.49	4.40 4.37 4.41	4.35 4.35 4.36	4.30 4.32 4.34	4.28 4.31 4.33

Table B.1 pH change of yoghurt samples during 21-day storage (Cont'd)

Sample Number	pH				
	Day 1	Day 4	Day 7	Day 14	Day 21
20	4.50 4.46 4.47	4.37 4.32 4.35	4.34 4.27 4.30	4.33 4.25 4.29	4.27 4.21 4.23
21	4.40 4.39 4.40	4.32 4.32 4.31	4.29 4.29 4.30	4.21 4.24 4.23	4.15 4.17 4.19
22	4.43 4.47 4.43	4.39 4.40 4.40	4.36 4.36 4.35	4.33 4.31 4.30	4.31 4.31 4.28
23	4.46 4.42 4.45	4.40 4.36 4.39	4.39 4.34 4.37	4.35 4.31 4.32	4.33 4.29 4.29
24	4.47 4.44 4.46	4.42 4.41 4.41	4.37 4.36 4.35	4.34 4.35 4.34	4.31 4.32 4.31
25	4.47 4.41 4.45	4.42 4.37 4.39	4.40 4.34 4.35	4.35 4.31 4.31	4.33 4.29 4.30
26	4.46 4.44 4.47	4.33 4.27 4.29	4.24 4.17 4.21	4.15 4.16 4.15	4.10 4.09 4.10
27	4.51 4.51 4.55	4.37 4.38 4.41	4.21 4.20 4.25	4.17 4.18 4.17	4.15 4.16 4.15
28	4.53 4.52 4.50	4.43 4.38 4.40	4.25 4.21 4.21	4.21 4.19 4.18	4.20 4.15 4.16
29	4.47 4.52 4.53	4.41 4.44 4.43	4.35 4.36 4.33	4.26 4.25 4.26	4.22 4.23 4.21
30	4.54 4.57 4.55	4.45 4.47 4.49	4.41 4.40 4.42	4.36 4.37 4.36	4.28 4.26 4.26

Table B.1 pH change of yoghurt samples during 21-day storage (Cont'd)

Sample Number	pH				
	Day 1	Day 4	Day 7	Day 14	Day 21
31	4.53 4.55 4.55	4.49 4.50 4.48	4.45 4.48 4.45	4.42 4.44 4.43	4.40 4.41 4.40
32	4.45 4.46 4.50	4.40 4.40 4.43	4.38 4.34 4.37	4.35 4.32 4.33	4.29 4.28 4.28
33	4.52 4.56 4.55	4.48 4.50 4.51	4.44 4.42 4.48	4.40 4.41 4.43	4.41 4.38 4.39
34	4.47 4.48 4.42	4.43 4.43 4.39	4.34 4.33 4.33	4.25 4.25 4.28	4.20 4.18 4.20
35	4.53 4.47 4.52	4.42 4.40 4.40	4.27 4.25 4.27	4.22 4.18 4.17	4.08 4.12 4.07
36	4.55 4.51 4.51	4.42 4.43 4.40	4.28 4.25 4.26	4.22 4.21 4.18	4.13 4.10 4.09
37	4.45 4.40 4.37	4.30 4.27 4.28	4.18 4.17 4.17	4.09 4.13 4.12	4.02 4.00 4.03
38	4.40 4.44 4.44	4.33 4.32 4.35	4.24 4.26 4.29	4.22 4.20 4.24	4.18 4.16 4.15
FD24	4.52 4.54 4.54	4.45 4.47 4.42	4.35 4.35 4.39	4.30 4.30 4.33	4.27 4.27 4.30
YCX11	4.49 4.46 4.50	4.42 4.40 4.39	4.35 4.36 4.33	4.31 4.29 4.30	4.25 4.24 4.25

APPENDIX C

TITRATABLE ACIDITY OF YOGHURT SAMPLES DURING 21-DAY STORAGE

Table C.1 Titratable acidity of yoghurt samples during 21-day storage

Sample Number	Titratable Acidity (% lactic acid)				
	Day 1	Day 4	Day 7	Day 14	Day 21
1	0.992 0.989 0.987	1.057 1.060 1.059	1.071 1.070 1.070	1.082 1.079 1.080	1.114 1.119 1.112
2	1.001 1.007 1.006	1.036 1.035 1.035	1.068 1.066 1.068	1.118 1.115 1.119	1.175 1.181 1.180
3	0.980 0.976 0.980	1.012 1.010 1.014	1.048 1.053 1.050	1.111 1.113 1.110	1.181 1.182 1.180
4	0.967 0.971 0.967	0.996 0.996 0.998	1.021 1.029 1.022	1.096 1.103 1.097	1.120 1.124 1.122
5	1.008 1.007 1.005	1.089 1.093 1.086	1.135 1.138 1.130	1.149 1.147 1.147	1.172 1.180 1.172
6	0.946 0.943 0.946	1.040 1.038 1.043	1.078 1.081 1.079	1.081 1.084 1.083	1.094 1.090 1.095

Table C.1 Titratable acidity of yoghurt samples during 21-day storage (Cont'd)

Sample Number	Titratable Acidity (% lactic acid)				
	Day 1	Day 4	Day 7	Day 14	Day 21
7	0.997 1.005 0.999	1.036 1.043 1.038	1.053 1.059 1.053	1.073 1.076 1.075	1.085 1.088 1.082
8	0.858 0.863 0.860	0.885 0.890 0.887	0.951 0.948 0.955	1.003 1.006 1.000	1.098 1.096 1.099
9	0.796 0.799 0.796	0.903 0.903 0.906	0.944 0.949 0.941	0.955 0.950 0.958	1.000 1.008 1.004
10	0.874 0.874 0.877	0.893 0.891 0.892	0.940 0.941 0.939	1.053 1.058 1.053	1.154 1.148 1.154
11	0.824 0.822 0.825	0.843 0.849 0.847	0.938 0.942 0.941	0.980 0.981 0.981	1.049 1.052 1.053
12	0.871 0.869 0.870	0.893 0.897 0.896	0.923 0.920 0.921	1.022 1.022 1.020	1.041 1.050 1.044
13	0.978 0.984 0.979	1.051 1.055 1.052	1.122 1.124 1.124	1.190 1.187 1.188	1.383 1.392 1.379
14	0.906 0.901 0.902	1.051 1.043 1.047	1.092 1.096 1.095	1.106 1.115 1.108	1.287 1.290 1.288
15	0.963 0.968 0.961	1.067 1.071 1.065	1.091 1.090 1.090	1.132 1.134 1.136	1.273 1.272 1.276

Table C.1 Titratable acidity of yoghurt samples during 21-day storage (Cont'd)

Sample Number	Titratable Acidity (% lactic acid)				
	Day 1	Day 4	Day 7	Day 14	Day 21
16	0.947 0.940 0.948	1.085 1.091 1.084	1.125 1.135 1.129	1.131 1.132 1.131	1.265 1.269 1.271
17	0.959 0.952 0.955	1.078 1.076 1.080	1.110 1.109 1.110	1.153 1.148 1.152	1.269 1.276 1.272
18	0.944 0.942 0.944	1.074 1.073 1.076	1.087 1.095 1.091	1.117 1.123 1.118	1.228 1.231 1.229
19	1.004 0.996 1.005	1.034 1.040 1.037	1.044 1.050 1.042	1.114 1.107 1.112	1.139 1.137 1.139
20	0.982 0.982 0.986	1.003 1.002 1.008	1.051 1.054 1.053	1.053 1.053 1.057	1.140 1.131 1.138
21	0.952 0.955 0.952	1.045 1.044 1.047	1.054 1.052 1.057	1.118 1.124 1.119	1.181 1.175 1.182
22	0.959 0.965 0.958	1.005 1.001 0.997	1.030 1.028 1.029	1.037 1.044 1.040	1.063 1.064 1.061
23	0.975 0.980 0.978	1.009 1.007 1.005	1.033 1.038 1.032	1.068 1.069 1.065	1.097 1.096 1.095
24	0.998 1.004 0.996	1.001 1.001 1.002	1.019 1.023 1.022	1.032 1.037 1.035	1.100 1.103 1.102

Table C.1 Titratable acidity of yoghurt samples during 21-day storage (Cont'd)

Sample Number	Titratable Acidity (% lactic acid)				
	Day 1	Day 4	Day 7	Day 14	Day 21
25	0.967 0.972 0.967	1.049 1.053 1.049	1.057 1.051 1.058	1.079 1.083 1.081	1.098 1.105 1.097
26	0.973 0.970 0.971	1.011 1.010 1.017	1.049 1.050 1.053	1.199 1.199 1.200	1.302 1.305 1.292
27	0.918 0.914 0.918	0.953 0.957 0.955	1.089 1.087 1.084	1.125 1.120 1.130	1.205 1.202 1.207
28	0.973 0.975 0.974	1.076 1.082 1.079	1.074 1.079 1.072	1.158 1.163 1.160	1.175 1.171 1.179
29	0.942 0.946 0.942	1.035 1.038 1.037	1.078 1.084 1.081	1.149 1.153 1.150	1.169 1.170 1.169
30	0.932 0.934 0.932	0.973 0.977 0.977	1.038 1.032 1.036	1.078 1.083 1.079	1.140 1.144 1.142
31	0.857 0.851 0.854	0.905 0.906 0.904	1.011 1.013 1.013	1.061 1.059 1.060	1.105 1.103 1.102
32	0.954 0.949 0.947	0.999 1.001 1.000	1.096 1.103 1.095	1.130 1.130 1.130	1.158 1.158 1.154
33	0.897 0.890 0.893	0.978 0.983 0.980	1.036 1.046 1.041	1.074 1.083 1.079	1.111 1.105 1.107

Table C.1 Titratable acidity of yoghurt samples during 21-day storage (Cont'd)

Sample Number	Titratable Acidity (% lactic acid)				
	Day 1	Day 4	Day 7	Day 14	Day 21
34	1.041 1.045 1.039	1.082 1.077 1.085	1.141 1.142 1.143	1.194 1.186 1.191	1.217 1.226 1.224
35	0.996 1.002 0.999	1.031 1.040 1.033	1.134 1.138 1.132	1.191 1.192 1.190	1.304 1.304 1.302
36	0.950 0.956 0.952	1.051 1.048 1.055	1.124 1.117 1.125	1.149 1.147 1.148	1.301 1.307 1.305
37	1.011 1.016 1.014	1.100 1.101 1.100	1.160 1.160 1.162	1.197 1.205 1.200	1.334 1.337 1.333
38	1.010 1.012 1.010	1.048 1.053 1.049	1.110 1.111 1.111	1.173 1.177 1.173	1.286 1.290 1.288
FD24	0.913 0.911 0.913	0.982 0.985 0.987	1.027 1.032 1.030	1.047 1.053 1.047	1.108 1.115 1.110
YCX11	0.930 0.937 0.935	1.018 1.021 1.018	1.068 1.070 1.067	1.119 1.116 1.115	1.146 1.156 1.150

APPENDIX D

WHEY SEPARATION AND HARDNESS RESULTS

Table D.1 Whey separation and hardness results of yoghurt samples

Sample Number	Whey Separation (mL/g)	Hardness (N)
1	0.361 0.359 0.365	176.098 269.104 184.323
2	0.373 0.374 0.370	270.369 227.276 227.135
3	0.353 0.357 0.352	208.506 200.000 176.942
4	0.279 0.284 0.278	278.946 248.647 284.359
5	0.331 0.336 0.331	242.109 249.069 235.220
6	0.203 0.209 0.204	259.543 345.378 200.492
7	0.375 0.384 0.371	202.460 191.494 184.183

Table D.1 Whey separation and Hardness results of yoghurt samples (Cont'd)

Sample Number	Whey Separation (mL/g)	Hardness (N)
8	0.382 0.382 0.377	182.483 128.567 143.610
9	0.281 0.284 0.283	267.276 221.863 186.011
10	0.312 0.313 0.306	158.934 185.365 177.422
11	0.300 0.303 0.304	270.762 280.884 267.248
12	0.332 0.335 0.338	94.405 174.399 130.044
13	0.328 0.328 0.325	218.684 228.596 238.929
14	0.315 0.316 0.316	266.264 234.633 264.366
15	0.278 0.279 0.276	272.660 263.944 260.078
16	0.323 0.323 0.322	247.426 170.316 244.333

Table D.1 Whey separation and Hardness results of yoghurt samples (Cont'd)

Sample Number	Whey Separation (mL/g)	Hardness (N)
17	0.306 0.303 0.308	227.744 149.369 268.935
18	0.310 0.313 0.310	223.245 213.967 190.068
19	0.352 0.351 0.351	131.918 137.752 135.222
20	0.300 0.306 0.304	196.015 189.830 136.487
21	0.430 0.439 0.431	169.350 121.265 225.308
22	0.295 0.297 0.304	212.561 269.286 236.249
23	0.371 0.371 0.376	90.117 161.184 145.649
24	0.277 0.275 0.281	317.717 326.363 321.513
25	0.324 0.326 0.323	310.828 288.054 260.851
26	0.330 0.330 0.332	189.961 203.037 318.616

Table D.1 Whey separation and hardness results of yoghurt samples (Cont'd)

Sample Number	Whey Separation (mL/g)	Hardness (N)
27	0.341 0.342 0.338	176.533 168.940 160.574
28	0.307 0.308 0.301	192.351 204.162 187.641
29	0.290 0.294 0.291	328.670 338.934 247.399
30	0.295 0.293 0.289	207.888 232.424 269.755
31	0.332 0.332 0.330	121.306 101.205 75.482
32	0.332 0.332 0.330	108.374 115.824 135.292
33	0.307 0.303 0.304	102.611 104.579 79.067
34	0.287 0.295 0.292	190.744 190.182 177.250
35	0.293 0.292 0.295	210.319 249.613 217.138
36	0.264 0.261 0.269	225.151 246.802 229.791

Table D.1 Whey separation and hardness results of yoghurt samples (Cont'd)

Sample Number	Whey Separation (mL/g)	Hardness (N)
37	0.246 0.238 0.240	269.436 223.113 210.600
38	0.333 0.333 0.331	257.908 162.590 115.633
FD24	0.234 0.236 0.237	334.938 338.640 331.892
YC X11	0.278 0.277 0.277	219.012 222.808 215.006

APPENDIX E

GLUCOSE CURVE, EXOPOLYSACCHARIDE AND ACETALDEHYDE CONTENT OF YOGHURT SAMPLES

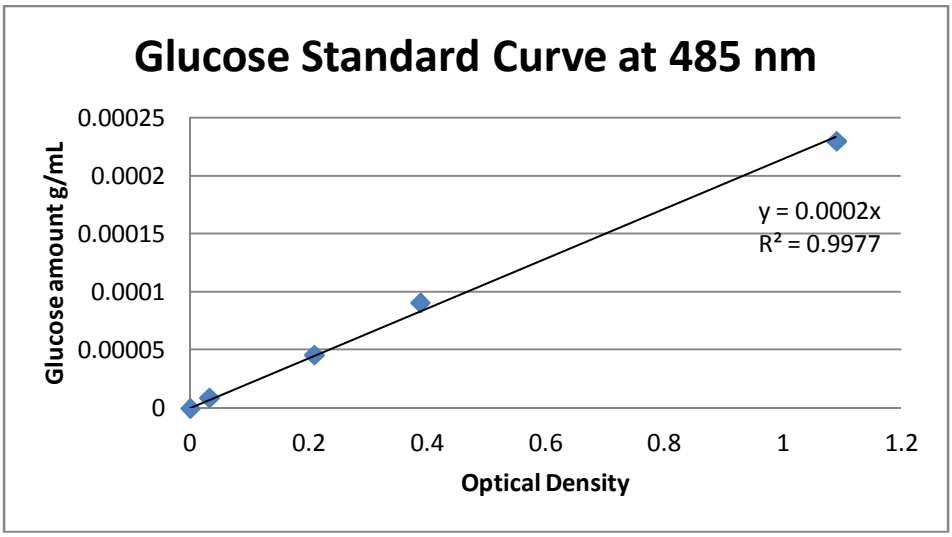


Figure E.1 Glucose curve used in EPS quantification

Table E.1 Exopolysaccharide and acetaldehyde content of yoghurt samples

Sample Number	EPS Content (g/mL)	Acetaldehyde Content (ppm)
6	0.0000504 0.0000580 0.0000567	30.708 30.345 30.986
9	0.0000308 0.0000362 0.0000434	29.194 29.587 28.598
11	0.0000390 0.0000316 0.0000347	30.584 25.287 30.375
15	0.0000444 0.0000529 0.0000586	29.333 35.518 30.755
24	0.0000684 0.0000772 0.0000726	49.953 33.911 44.211
25	0.0000308 0.0000350 0.0000382	42.463 37.931 37.465
27	0.0000222 0.0000392 0.0000306	25.882 22.617 25.071
29	0.0000896 0.0000766 0.0000843	44.767 38.900 42.230
30	0.0000769 0.0000680 0.0000874	64.390 58.278 59.404
FD24	0.0000667 0.0000604 0.0000744	47.685 40.741 46.550

Table E.1 Exopolysaccharide and acetaldehyde content of yoghurt samples (Cont'd)

Sample Number	EPS Content (g/mL)	Acetaldehyde Content (ppm)
YCX11	0.0000526 0.0000484 0.0000507	48.32856 49.07856 47.77887

APPENDIX F

EFFECT OF SPECIES ON POST-ACIDIFICATION OF YOGHURT SAMPLES

Table F.1 ANOVA Table for the effect of species on post-acidification of yoghurt samples

```
Factor  Type   Levels  Values
Lb      fixed       3  MRSK1-43. MRSK2-1. MRSN4-3
St      fixed       3  M17K1-14. M17N8-2. M17S1-3

Analysis of Variance for pH. using Adjusted SS for Tests
Source  DF    Seq SS    Adj SS    Adj MS      F      P
Lb       2  0.259074  0.259074  0.129537  74.23  0.000
St       2  0.051674  0.051674  0.025837  14.81  0.000
Error   22  0.038393  0.038393  0.001745
Total   26  0.349141

S = 0.0417746   R-Sq = 89.00%   R-Sq(adj) = 87.00%

Unusual Observations for pH

Obs       pH      Fit   SE Fit  Residual  St Resid
 11  4.47000  4.38037  0.01798   0.08963      2.38 R

R denotes an observation with a large standardized residual.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable pH
All Pairwise Comparisons among Levels of Lb
Lb = MRSK1-43  subtracted from:

Lb         Lower  Center   Upper  ---+---------+---------+---------+---
MRSK2-1  0.05057  0.1000  0.1494  (------*------)
MRSN4-3  0.18946  0.2389  0.2883                    (------*------)
                               ---+---------+---------+---------+---
                                0.070     0.140     0.210     0.280
```

Table F.1 ANOVA Table for the effect of species on post-acidification of yoghurt samples (Cont'd)

```
Lb = MRSK2-1  subtracted from:

Lb         Lower  Center   Upper  ---+---------+---------+---------+---
MRSN4-3  0.08946  0.1389  0.1883        (------*------)
                                  ---+---------+---------+---------+---
                                   0.070     0.140     0.210     0.280

Tukey Simultaneous Tests
Response Variable pH
All Pairwise Comparisons among Levels of Lb
Lb = MRSK1-43  subtracted from:

         Difference       SE of           Adjusted
Lb         of Means  Difference  T-Value   P-Value
MRSK2-1      0.1000     0.01969    5.078    0.0001
MRSN4-3      0.2389     0.01969   12.131    0.0000

Lb = MRSK2-1  subtracted from:

         Difference       SE of           Adjusted
Lb         of Means  Difference  T-Value   P-Value
MRSN4-3      0.1389     0.01969    7.053    0.0000


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable pH
All Pairwise Comparisons among Levels of St
St = M17K1-14  subtracted from:

St         Lower    Center     Upper   --+---------+---------+---------+----
M17N8-2  -0.0050   0.04444   0.09388                       (------*------)
M17S1-3  -0.1117  -0.06222  -0.01279         (------*------)
                                    --+---------+---------+---------+----
                                   -0.140    -0.070     0.000     0.070

St = M17N8-2  subtracted from:

St         Lower   Center     Upper   --+---------+---------+---------+----
M17S1-3  -0.1561  -0.1067  -0.05723   (------*------)
                                     --+---------+---------+---------+----
                                    -0.140    -0.070     0.000     0.070
```

Table F.1 ANOVA Table for the effect of species on post-acidification of yoghurt samples (Cont'd)

```
Tukey Simultaneous Tests
Response Variable pH
All Pairwise Comparisons among Levels of St
St = M17K1-14  subtracted from:

          Difference       SE of           Adjusted
St          of Means  Difference  T-Value   P-Value
M17N8-2      0.04444     0.01969    2.257    0.0836
M17S1-3     -0.06222     0.01969   -3.160    0.0121


St = M17N8-2  subtracted from:

          Difference       SE of           Adjusted
St          of Means  Difference  T-Value   P-Value
M17S1-3      -0.1067     0.01969   -5.417    0.0001
```

APPENDIX G

SENSORY ANALYSIS RESULTS

Table G.1 ANOVA Table for the effect of appearance on the consumer choice

ANALYSIS OF VARIANCE ON appearance

SOURCE	DF	SS	MS	F	p
overall	3	4.154	1.385	1.74	0.165
ERROR	95	75.805	0.798		
TOTAL	98	79.960			

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95% CI'S FOR MEAN BASED ON POOLED STDEV
2	4	4.2500	0.9574	(----------*----------)
3	31	3.9032	1.1062	(-----*-----)
4	44	4.3182	0.7400	(----*-----)
5	20	4.4000	0.8208	(-------*-------)

POOLED STDEV = 0.8933

3.50 4.00 4.50 5.00

Table G.1 ANOVA Table for the effect of appearance on the consumer choice (Cont'd)

```
Tukey's pairwise comparisons

    Family error rate = 0.0500

Individual error rate = 0.0104

Critical value = 3.70

Intervals for (column level mean) - (row level mean)

               2         3         4

     3   -0.8949

          1.5884

     4   -1.2887   -0.9630

          1.1523    0.1331

     5   -1.4301   -1.1671   -0.7121

          1.1301    0.1735    0.5484
```

Table G.2 ANOVA Table for the effect of odor on the consumer choice

```
ANALYSIS OF VARIANCE ON odor
SOURCE     DF        SS        MS        F        p
overall     3    32.012    10.671    18.22    0.000
ERROR      95    55.624     0.586
TOTAL      98    87.636
                                  INDIVIDUAL 95% CI'S FOR MEAN
                                  BASED ON POOLED STDEV
 LEVEL      N      MEAN     STDEV  -+---------+---------+---------+-----
     2      4    3.5000    1.2910  (----------*----------)
     3     31    3.3548    0.9504       (---*---)
     4     44    4.2273    0.7108                 (--*---)
     5     20    4.9000    0.3078                          (----*----)
                                  -+---------+---------+---------+-----
POOLED STDEV =   0.7652           2.80      3.50      4.20      4.90
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0104
Critical value = 3.70
Intervals for (column level mean) - (row level mean)
               2         3         4
     3   -0.9184
          1.2088
     4   -1.7728   -1.3419
          0.3182   -0.4030
     5   -2.4965   -2.1193   -1.2126
         -0.3035   -0.9710   -0.1328
```

Table G.3 ANOVA Table for the effect of flavor on the consumer choice

```
ANALYSIS OF VARIANCE ON flavor
SOURCE     DF        SS        MS        F        p
overall     3    13.969     4.656     5.66    0.001
ERROR      95    78.213     0.823
TOTAL      98    92.182
                                  INDIVIDUAL 95% CI'S FOR MEAN
                                  BASED ON POOLED STDEV
 LEVEL      N      MEAN     STDEV  -+---------+---------+---------+-----
     2      4    3.2500    1.5000  (--------------*--------------)
     3     31    3.2581    1.0945            (----*-----)
     4     44    3.9773    0.7621                      (---*----)
     5     20    4.1500    0.7452                      (------*------)
                                  -+---------+---------+---------+-----
POOLED STDEV =   0.9074          2.40      3.00      3.60      4.20
Tukey's pairwise comparisons
    Family error rate = 0.0500
Individual error rate = 0.0104
Critical value = 3.70
Intervals for (column level mean) - (row level mean)
               2         3         4
     3   -1.2693
          1.2531
     4   -1.9670   -1.2759
          0.5125   -0.1626
     5   -2.2002   -1.5728   -0.8129
          0.4002   -0.2111    0.4675
```

	Sample 6	Sample 11	Sample 15	Sample 24	Sample 25	Sample 27	Sample 29	Sample 30
Appearance								
Odor								
Flavor								
Overall								

5- Very good 4- Good 3- Normal 2- Bad 1- Very bad

Figure G.1 Sensory analysis questionnaire

APPENDIX H

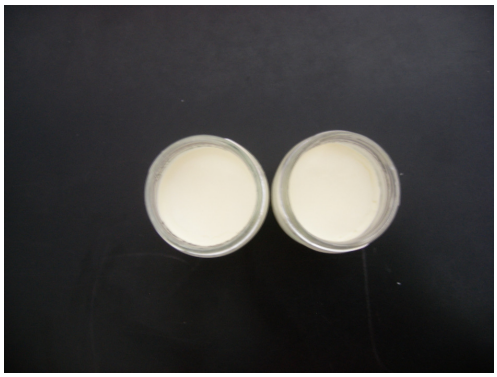
EFFECT OF EPS CONTENT ON HARDNESS OF YOGHURT SAMPLES

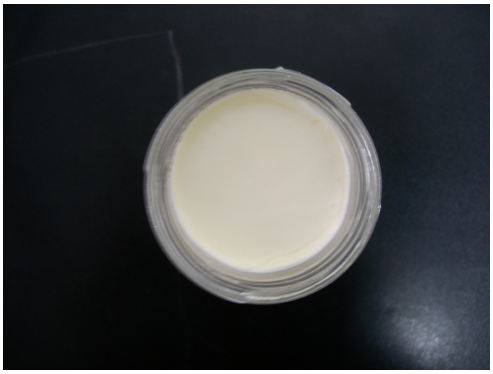
Table H.1 ANOVA Table for the effect of EPS content on hardness of yoghurt samples

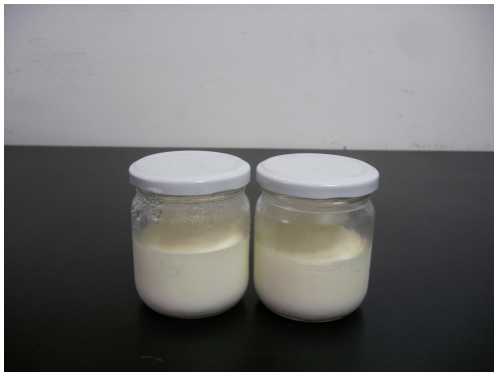
```
Source       DF     SS    MS     F      P
EPS content  24  72068  3003  5.02  0.179
Error         2   1197   598
Total        26  73264
S = 24.46   R-Sq = 98.37%   R-Sq(adj) = 78.77%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
0.111  1  176.53      *   (----------*---------)
0.154  3  282.2  24.46                 (-----*-----)
0.158  1  280.88      *              (---------*----------)
0.173  1  260.08      *           (----------*----------)
0.177  1  260.85      *            (---------*----------)
0.186  1  247.40      *          (----------*---------)
0.191  1  288.05      *              (----------*---------)
0.195  1  270.76      *             (---------*----------)
0.196  1  168.94      *  (----------*---------)
0.217  1  221.86      *        (---------*----------)
0.222  1  272.66      *             (---------*----------)
0.252  1  259.54      *           (----------*---------)
0.258  1  267.25      *            (----------*---------)
0.271  1  321.51      *                  (---------*----------)
0.290  1  345.38      *                    (----------*---------)
0.293  1  263.94      *             (---------*----------)
0.340  1  207.89      *      (----------*---------)
0.342  1  317.72      *                 (----------*---------)
0.364  1  200.49      *      (---------*----------)
0.383  1  338.93      *                   (----------*---------)
0.386  1  326.36      *                 (----------*---------)
0.389  1  160.57      *  (---------*----------)
0.416  1  186.01      *    (----------*---------)
0.437  1  232.42      *         (---------*----------)
0.448  1  328.67      *                 (----------*---------)
                         ----+---------+---------+---------+-----
                           100       200       300       400
Pooled StDev = 24.46
```

APPENDIX I

PHOTOS OF YOGHURT SAMPLES







APPENDIX J

ACIDIFICATION ACTIVITY AND ACETALDEHYDE PRODUCTION PROPERTIES OF SELECTED *L.DELBRUECKİİ* SPP. *BULGARICUS* AND *S.THERMOPHILUS* STRAINS

Table J.1 Acidification activity of selected *S.thermophilus* strains (Altay Dede, 2010)

S.thermophilus Strains	ΔpH at 6th hour
M17 K1-14	1.58
M17 N2-3	1.66
M17 N8-2	1.65
M17 N5-7	1.74
M17 N6-6	1.65
M17 S1-3	1.53
M17 Dan TA040-1	1.41
M17 Dan-Yo-Mix410-1	1.82

Table J.2 Acidification activity and acetaldehyde production properties of selected *L.delbrueckii* spp. *bulgaricus* (Altay Dede, 2010)

L.delbrueckii spp. *bulgaricus* Strains	ΔpH at 6th hour	Acetaldehyde production (µg/g)
MRS K1-43	1.81	15.20
MRS M2-16	1.79	11.12
MRS M2-23	1.14	-
MRS N6-2	1.70	9.08
MRS N4-3	1.66	13.05
MRS K2-1	1.73	11.54
MRS Visby-2	1.78	-