DETERMINATION OF AROMA COMPOUNDS AND EXOPOLYSACCHARIDES FORMATION BY LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL YOGURTS

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ABSTRACT

DETERMINATION OF AROMA COMPOUNDS AND EXOPOLYSACCHARIDES FORMATION BY LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL YOGURTS

Yogurt, consumed widely around the world, is a fermented milk product as a result of the lactic acid fermentation by addition of starter cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The most important criteria for yogurt production is the selection of starter cultures since each culture affects the end-product quality differently. Our natural flora and traditional flavor have been continously changing due to the introduction of imported commercial starter cultures. Because of the necessity to preserve our natural starter cultures and to increase the availability of them for industrial use, these cultures must be isolated from artisanal yogurts, genetically characterized and investigated regarding their technological properties. Aims of our study were to determine the technological and organoleptic properties of starter cultures previously isolated and genetically characterized and to assess the feasibility of near-infrared (NIR) spectroscopy for determining the yogurt quality.

Total of 20 different yogurt samples were produced using combinations of 5 *Streptococcus thermophilus* and 4 *Lactobacillus delbrueckii* ssp. *bulgaricus* strains. Yogurt samples were stored at 4 °C for 21 days. Chemical, physical, microbial and organoleptic analyses for samples were conducted at predetermined days.

As a conclusion, it was found that 4 *Streptococcus thermophilus* and 4 *Lactobacillus delbrueckii* ssp. *bulgaricus* yogurt isolates have potential to be used in dairy industry regarding their high technological and organoleptic characteristics. In addition, NIR spectroscopy technique could be used successfully on yogurt samples to predict total solids, fat, pH, syneresis, and microbial counts of yogurts based on the calibration models (Genetic Algoritm).

ÖZET

GELENEKSEL YOĞURTLARDAN İZOLE EDİLEN LAKTİK ASİT BAKTERİLERİNİN AROMA BİLEŞİKLERİ VE EKZOPOLİSAKKARİT ÜRETİMLERİNİN BELİRLENMESİ

Dünyada yaygın olarak tüketilen yoğurt, süte katılan *Streptococcus thermophilus* ve *Lactobacillus bulgaricus* starter kültürlerinin laktik asit fermentasyonu sonucu oluşan fermente bir üründür. Her bir kültürün son ürün kalitesini farklı etkilemesi sebebiyle yoğurt yapımındaki en önemli husus, kullanılacak kültürün seçimidir. Doğal floramızı ve Anadolu'ya özgü damak tadımızı kaybetmemizin nedeni endüstriyel yoğurt üretiminde çoğunlukla yurtdışından ithal edilen starter kültürlerin kullanılmasıdır. Coğrafyamıza özgü floramızı korumak ve bunun endüstriyel çapta kullanımını arttırmak için, geleneksel yöntemle yapılmış yoğurtlardan söz konusu bakterilerin saflaştırılıp, genetik olarak tanımlanması ve teknolojik özelliklerinin belirlenmesi gerekmektedir. Bu bilgiler ışığında, çalışmamızın amaçları, geleneksel yöntemle yapılmış yoğurtlardan önceden izole edilmiş ve genetik olarak tanımlanmış yoğurt kültürlerini hem teknolojik hem de organoleptik özelliklerini incelemek ve Yakın İnfrared (NIR) Spektroskopi yönteminin yoğurt kalitesini belirlemede kullanılabilirliğini belirlemektir.

Toplam 5 adet *Streptococcus thermophilus* ve 4 adet *Lactobacillus bulgaricus* kültürün kombinasyonlarıyla 20 çeşit yoğurt örneği yapılmıştır. Örnekler 21 gün boyunca 4 °C'da depolanmıştır. Örneklerde kimyasal, fiziksel, mikrobiyolojik ve duyusal analizler önceden belirlenmiş günlerde yapılmıştır.

Bu araştırmanın sonucunda, 4 adet *Streptococcus thermophilus* and 4 adet *Lactobacillus bulgaricus* yogurt izolatının yoğurt endüstrisinde kullanılabilir teknolojik ve duyusal özelliklere sahip oldukları görülmüştür. Ayrıca bu çalışmada kullanılan NIR spektroskopi yöntemi, kuru madde, yağ, serum ayrılması, pH ve mikrobiyal sayım gibi bazı analizlerin yapılmasına gerek kalmadan başarılı ve güvenilir olarak kullanılabileceğini göstermiştir.

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CHAPTER 1

INTRODUCTION

1.1. Definition and History of Yogurt

Yogurt is a fermented milk product containing a mixture of *Streptococcus salivarius* ssp. *thermophilus* (*Streptococcus thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lactobacillus bulgaricus*) which convert lactose into lactic acid (Tamime and Marshall 1997). In some countries, less traditional bacteria such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis* are used with the main starter culture (McKinley 2005).

There are no records available stating the origin of yogurt, but it is believed that its beneficial influence on human health and nutrition has existed in many civilizations for a long period of time. It is thought that the origin of yogurt was the Middle East. In fact, the production of milk in the Middle East has always been seasonal. Intensive animal production has never really existed, so that, it was restricted to no more that a few months of the year. For this reason, the production of milk is limited. In addition, keeping milk fresh was difficult due to subtropical climate and contamination by microorganisms from air, animal, feeding stuff or hands of milker (Tamime and Robinson 1985).

Yogurt has been produced popularly in warmer regions around the Mediterranean and Middle Eastern for centuries. Moreover, yogurt is considered as belonging to nomadic people living in that part of the world. For instance, the use of yogurt by ancient Turks is recorded in books, “Divan-ı Lugati’t Türk” by “Kaşgarlı Mahmut” and “Kutadgu Bilig” by “Yusuf Has Hacib” which were written in the 11th century. The word “yogurt” is mentioned in different sections and its use by nomadic Turks is described in both books (Anon 2007a). In more recent times, yogurt became widespread in Europe used in clinically for diarrhoea treatment of Emperor Francis I of France by consuming yogurt (Tamime and Robinson 1985).

Nomadic people have gradually devised a fermentation process which brought under control the souring of milk. They started heating milk over an open fire in order to concentrate it slightly, to modify the properties of the casein, to eradicate any

pathogenic microorganisms present in milk, to encourage the fermentation of milk to take place at a slightly higher temperature and also to ensure a gradual selection of lactic acid bacteria capable of tolerating high levels of lactic acid, and of giving the product its distinctive flavor. At the end of this procedure, sour milk is produced which is named as yogurt. It is fact that, yogurt production was an intuitive process for the milk preservation (Tamime and Robinson 1985).

Thereafter, yogurt was kept in animal skin especially by nomadic people. They invented hanging yogurt in animal skin result in more concentrated product to consume in long period. However, it was not effectual because spoilage was the main problem. For this reason, salted yogurt became popular. Similar methods were used by the Turkish, Armenian, and Egyptians and as well as other societies. Each society found the best appropriate preservation methods for their needs, for instance, salting and drying, heating for a few hours over low fires of a special type of wood that called smoked yogurt, or keeping salted and dried yogurt in olive oil or tallow. Another method that Turkish, Lebanese, Syrian, Iranian and Iraqi used was mixing concentrated yogurt with wheat that is called kishk (Tamime and Robinson 1985).

After the refrigeration became widespread, these traditional methods lost popularity except among certain communities in Middle East. In the following years, new trends such as fruit yogurts gained interest. Thus, over the years, the method of yogurt production has changed little by the improvement of new technologies although the essential steps are still the same. In addition, as the health benefits of yogurt are proved, consuming is increased by the years (Tamime and Robinson 1985).

1.2. Manufacture of Yogurt

In dairy industry, there is not only one type of manufacturing process or incubation temperature and time because these depend on yogurt type as well as the factory conditions. However, no matter which method is employed, the fermented dairy product must be appropriate to national and international standards, additionally be healthy and has best organoleptic characteristics (Akın 2006). The flow diagram of manufacturing steps for yogurt production is given in Figure 1.1 and the basic manufacturing steps for any types of yogurts are as follows:

Filtration: Any cellular matter and other contaminants present in milk are separated by filtration.

Checking the presence of antibiotics: Presence of antibiotics is checked due to their negative effects on the starter bacteria.

Standardization of milk: Fat and solids-non-fat content in milk are standardized for good quality yogurt. For this purpose, fat content of milk reduced or increased for the type of the yogurt. Thus, solids-non-fat content in milk fortified to required level which is legal standards of the country. Generally, skim milk powder is added to milk or evaporation under vacuum is used in order to increase the solids-non-fat content.

Homogenization: Milk is homogenized to prevent lypolysis before heat treatment (Tekinşen 1997). Homogenization causes some chemical changes in milk. Firstly, fat globule size is reduced so cream line formation is prevented. Secondly, casein micelles are destroyed by the homogenization, thus, hydrophilicity and water binding capacity are increased. Then, reduced size of fat globules is adsorbed by casein micelles so the volume of non-water soluble substances is increased and consequently viscosity is increased. In addition, protein-protein interaction and salt balance are changed so as to decrease in the protein stabilization. At the end of the homogenization, milk became whiter and the yogurt made from that milk is more viscous and flavor is homogeneously distributed all over the container. However, as a result of phospholipids in the skim milk phase, pumping of yogurt milk may cause foaming in the incubation tank (Yaygın 1999).

Heat treatment (Pasteurization): The aim of this process is to eliminate the pathogens and other undesirable microorganisms, stimulate the starter bacteria and change the physicochemical properties of the milk constituents such as increasing the solid level of milk. In addition, heat treatment leads to production of some aroma compounds. On the other hand, sometimes heat treatment has some disadvantages due to the formation of some by-products which have an inhibitory effect on the growth of starter bacteria. Heat treatments at 80-85 ºC for 30 min or 90-95 ºC for 5 min are the most commonly used in dairy industry. By the heat treatment, pH and oxygen content of milk are reduced and serum proteins like β–lactoglobulin and α–lactalbumin are denaturated. Subsequently, hydrophilicity of casein increases and syneresis decreases. As a result, it is important to choose most appropriate heat treatment for the type of yogurt process (Akın 2006).

Inoculation: After heat treatment, milk is cooled to 40-45 °C and inoculated with the starter bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in 1:1 ratio. Although inoculation level varies between 1-4%, the optimum level is 2%. When

the inoculation level is less than 2%, the production of acidity slows down and the fermentation takes longer than the usual and there may be contamination risk because of the unsuitable conditions. On the other hand, immoderate inoculation levels result in fast and too much acidity production that causes unwanted aroma formation as well as the texture of yogurt breaks.

Inoculation types may differ depending on the production capacity of yogurt manufacturing plants. The first type is the inoculation of starter culture into yogurt vessels one by one in small-scale plants. This is the most common method used in Turkey due to cream formation on the surface of the yogurt. Nevertheless, this method needs too much manpower, time and may cause any problems such as miss inoculation, contamination or process conditions which are not the same in all vessels. The second one is the inoculation of starter cultures in large milk tanks and then filled to yogurt vessels which leads to the production of more homogenous yogurts. Another method is the direct injection of yogurt starter cultures in sterile milk tanks and immediately filled into vessels (Akın 2006, Yaygın 1999).

Incubation (Fermentation): After inoculation, incubation takes place at optimum temperature of 43 ºC in incubation room or cabinet and process ends between 2.5 and 3 hours. Rarely, at lower temperatures around 30-38 ºC prolong the incubation time up to 7-8 hours which mostly prevent post-acidification and increase the formation of aroma compounds as well as viscosity. As a result, incubation time affects the quality of final product (Tamime and Robinson 1985).

Cooling and Storage: If yogurts are not cooled immediately at the end of the fermentation, starter cultures continue to grow. The acidity continues to decrease and causes syneresis on the surface of yogurts. Yogurts are generally cooled by two different ways. These are one-phase cooling and two-phase cooling. In the first one, yogurts are cooled to 5 or 10 ºC just after fermentation and stored until distribution to the market. In the second one the temperature decreases to 37 ºC and then 10 ºC. Finally, all yogurts are stored at 4 ºC for 1-2 days before sale due to maturation of viscosity and aroma of yogurt (Akın 2006).

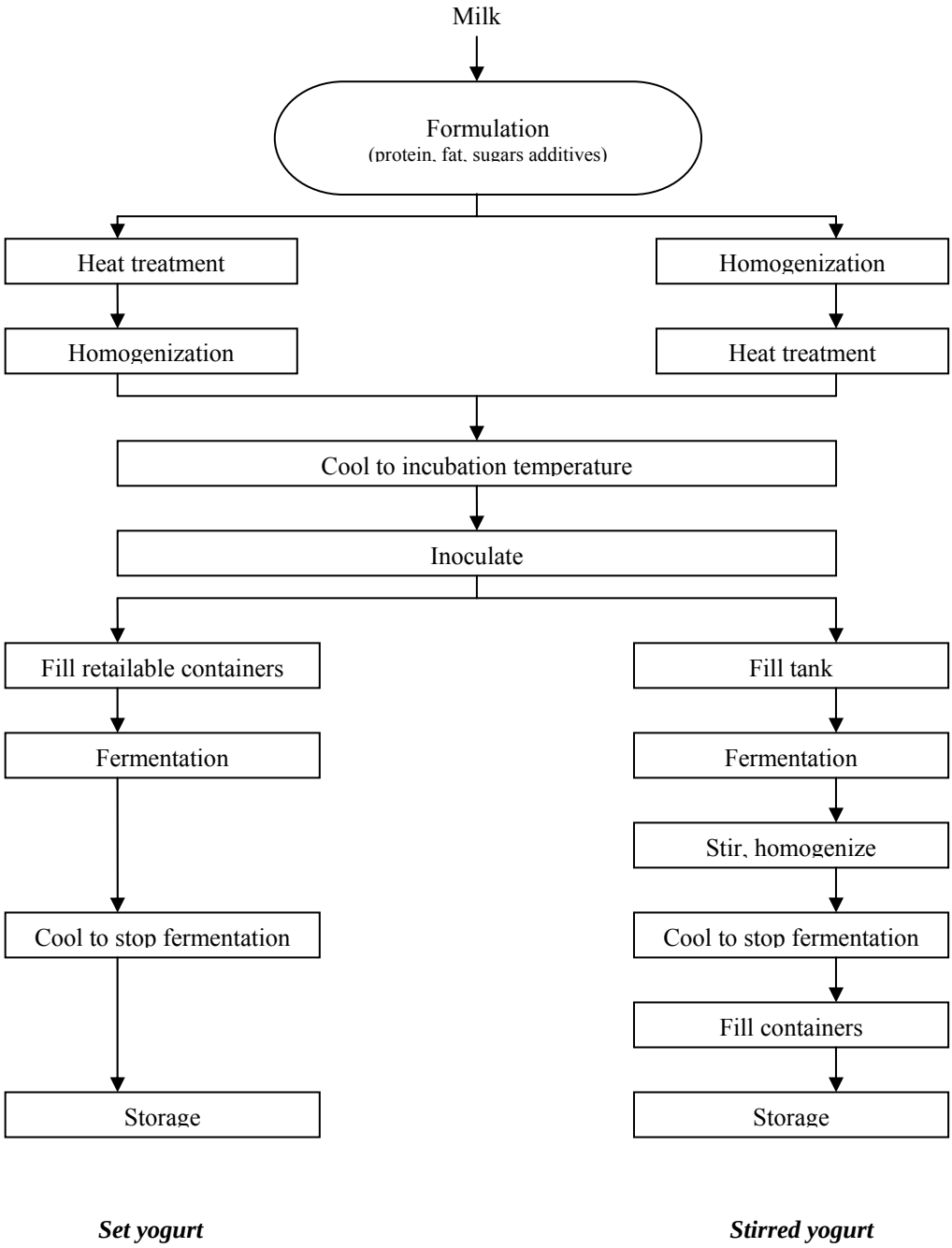


Figure 1.1. Process for yogurt manufacturing
(Source: Duboc and Mollet 2001)

1.3. Yogurt Types

Yogurt is mainly classified based on its chemical composition (full-fat, reduced-fat and low-fat), manufacturing type (set and stirred yogurt), flavor type or post-incubation process. Other types of yogurts found in the market are lactose hydrolysed yogurt, drinking yogurt, concentrated yogurt, frozen yogurt, carbonated yogurt,

dried/instant yogurt, dietetic/therapeutic yogurt and soy-milk yogurt (Tamime and Robinson 1985).

Probiotic yogurts gained high popularity during the last two decades due to their health benefits. Probiotic bacteria are the live microbial feed supplements which beneficially affect the host animal by improving their intestinal microbial balance. *Lactobacillus acidophilus* and *Bifidobacterium* spp. are most commonly used probiotics in dairy industry. They secrete lactic and acetic acids, which lower the pH of the colonic content, help to inhibit the development of invasive pathogens such as *E. coli*, and also compete successfully for space and nutrients against pathogenic or putrefactive bacteria. In addition, high population levels may stimulate the immune system of the host (Akın 2006).

1.4. Yogurt Fermentation

The use of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is partly historical in origin as they have frequently been isolated from natural yogurt produced in Middle East where the high ambient temperature has led to the selection of thermophilic microflora in fermented dairy products. Continuing with the tradition is reasonable because of synergistic interaction of these organisms (Robinson 1999).

As the bacteria grow, they use lactose as an energy source and produce lactic acid which lowers the pH and makes the yogurt's taste as sour. Initially *S. thermophilus* ferments the lactose. *L. bulgaricus,* which is more acid tolerant, continues to ferment the remaining lactose. During this process the pH drops from 6.5 to around 4.5. This inhibits the growth of spoilage microbes. The presence of lactic acid causes the structure of the milk protein to change which gives yogurt its special thickened texture. The lactic acid also gives the yogurt its sharp taste. Other products of lactic acid fermentation such as acetaldehyde give the yogurt its characteristic aroma (Tamime and Robinson 1985).

1.5. Composition of Yogurt

Nutritional content of yogurt is similar to the nutritional content of milk (McKinley 2005). However, variations in the quality of yogurt depend on the type of milk. In yogurt production, usually fresh cow's milk and also sheep milk are used as a

raw material. For yogurt production the solids-non-fat (SNF), lactose, protein and mineral contents of cow's milk is approximately 8.5-9.0 %, 4.5 %, 3.3%, and 0.7 %, respectively. Chemical composition of different milk source used in production of yogurt is given in Table 1.1. The average nutritional contents of full-fat, reduced-fat and non-fat yogurts are given in Table 1.2.

Table 1.1. Chemical composition of different milk sources used in production of yogurt. (Source: Akın 2006)

Milk Source	**Fat (%)**	**Water (%)**	**Total Solids (%)**	**Protein (%)**	**Lactose (%)**	**Ash (%)**
Cow	3.7-3.9	87.4	12.7	3.3-3.4	47-4.8	0.7
Goat	4.5	87.0	12.3	2.9-3.3	4.1-4.6	0.6-0.8
Sheep	7.4	81.7	19.3	4.5-5.6	4.4-4.8	0.9-1.0
Buffalo	8.0	82.1	17.9	4.2	4.9	0.8

Table 1.2. The average nutritional contents of full-fat, reduced-fat, and non-fat yogurts. (Source: Akın 2006)

Contents	**Full-Fat Yogurt**	**Reduced-Fat Yogurt**	**Low-Fat Yogurt**
Milk solid non fat (%)	-	≥ 8	≥ 8
Energy kcal	70	84	76
KJ	293	351	318
Water (g)	87.0	78.9	80.0
Protein (g)	3.8	4.0	3.5
Fat (g)	3.8	0.9	0.1
Lactose (g)	4.6	6.3	5.5
Other sugars (g)	0	9.0	10.0
Ash (g)	0.8	0.9	0.9
Calcium (Ca) (mg)	120	130	120
Phosphorus (P) (mg)	92	110	100
Iron (Fe) (mg)	0.46	0.2	0.1
Sodium (Na) (mg)	48	60	60
Potassium (K) (mg)	157	150	150
Vitamin A (IU)	100	32	0
Vitamin B_1 (mg)	0.04	0.05	0.03
Vitamin B_2 (mg)	0.18	0.20	0.15
Niacin (mg)	0.1	0.1	0.1

Proteins and minerals (calcium and phosphorus) increase the basic structure of gel. Therefore, lactose is the main energy source for the starter culture (Robinson 1999). Nevertheless, composition of fresh milk varies from day to day due to several factors such as feeding type, age and health of animal, climatic conditions and also season of the year. Hence, milk has to be standardized in order to overcome these compositional variations of milk (Tamime and Robinson 1985).

Due to nutritional similarity between yogurt and milk, yogurt is the best alternative in human diet being an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B_2), thiamin (vitamin B_1), vitamin B_{12} and also folate, niacin, magnesium and zinc (McKinley 2005).

1.5.1. Total Solids Content

In general, high levels of solid in yogurt increase viscosity of the end-product. As a result, milk is fortified before yogurt production. Optimum total solids content in yogurt should be 15-16%. There are several methods for the fortification of milk. Traditionally, skim milk powder (SMP) is used to enrich the milk before fermentation. However, availability of other dairy ingredients, such as whey protein concentrates (WPCs), may provide a cost-effective alternative to skim milk powder. Evaporation of milk is commonly used method to obtain the desirable total solids content. The removal of water from the milk under vacuum improves the stability of the coagulum and reduces syneresis during storage. Moreover, evaporation of goat's milk not only improves the consistency, but also reduces the goaty flavor of the end-product (Tamime and Robinson 1985).

1.5.2 Carbohydrates

Lactose is the dominant disaccharide in milk comparing to other mono- and disaccharides present in yogurt. The yogurt fermentation uses only a small proportion of the milk lactose and, as a result, yogurt normally has low lactose content (3-4%). The presence of (initially) viable starter cultures in yogurt can be beneficial to lactose utilization since lactose in milk provides as the energy source for yogurt starter cultures (Tamime and Robinson 1985).

Lactose is utilized by the starter cultures especially *Streptococcus thermophilus*. The amount of lactase (β-galactosidases) activation of *Streptococcus thermophilus* is 3 fold more than *Lactobacillus bulgaricus*' lactase activation and it increases during incubation. In human body, lactose is hydrolyzed into glucose and galactose by the intracellular β-galactosidases in small intestine. However, hydrolysis rate of lactose is slower than other sugars so that lactose cannot be hydrolyzed completely.

1.5.3. Lactic Acid

Lactic acid is the end product of lactose hydrolysis. Lactose can hydrolyze by homofermentative and heterofermentative ways. Differences in the amount of lactic

acid produced depend on the starter culture, milk type, and manufacturing and storage conditions.

Furthermore, lactic acid is produced in D(-) and L(+) forms. L(+) lactic acid is produced during the early fermentation. In contrast, D(-) lactic acid production starts from about the second hour of fermentation and increases continuously. High fermentation temperature and long time storage can cause an increase in the concentration of D(-) lactic acid, thus, the ratio of L(+) / D(-) decreases. On the other hand, World Health Organization (WHO) reported that there is no limit for the consumption of L(+) lactic acid. On the contrary, the concentration of D(-) lactic acid that could be taken per day should be 0-100 mg/kg body weight and should not be used in infant and children diet due to low digestion (Akın 2006).

It is agreed that the typical yogurt flavor is caused by lactic acid which imparts an acidic and refreshing taste (Chaves, et al. 2002). Besides, more important trait is that its increasing effect on regulation of hydrolysis of casein, and adsorption of some amino acids, peptides, lactose and minerals as well. Other organic acids present in yogurt are mainly citric and acetic acids, fatty acids and hypuric acid which is present in skim milk (Akın 2006).

1.5.4. Protein

Lactic acid bacteria partially hydrolyze proteins and the amount of free amino acids in fermented dairy products increase. Moreover, pre-hydrolysis of these proteins may be useful for people who are lacking of digestion enzymes. Therefore, proteins of yogurt are more digestible than the proteins found in milk even though the protein contents of milk and yogurt are similar. This makes yogurt more preferable than liquid milk (Akın 2006).

Protein in milk plays an important role in the formation of coagulum. As a result, viscosity of the product is directly proportional to the level of protein present (Tamime and Robinson 1985). Heat treatment in the manufacturing of yogurt may cause protein denaturation which induces the reduction of immunological disease. In addition, decrease in the amount of the urea in the manufacturing is beneficial to nutritional value of yogurt (Akın 2006).

1.5.5. Fat

The fat content of yogurt varies from 0.1% to 10% depending on the yogurt standards described by each country in the World (Tamime and Robinson 1985). Either homogenization of milk or lypolysis eases the digestion of fat present in acidic milk products. Yogurt manufacturing using cow's milk leads to an increase in saturated fatty acid content, but a decrease in total acid content. The contents of stearic, oleic, linoeic, and palmitic acids decrease. Volatile fatty acids have an important effect on organoleptic properties of yogurt, but have limited nutritional value. The volatile fatty acid content of raw milk and yogurt are given in Table 1.3.

Table 1.3. Volatile fatty acid content of raw milk and yogurt.
(Source: Akın 2006)

Volatile Fatty Acids	**Raw Milk**		**Yogurt**	
	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
Categlutaric 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 aid	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
Lauric acid	1.72	0.7	2.58	0.3

1.5.6. Vitamins and Minerals

Vitamins act as co-factors in metabolic reactions. Fermented dairy products are rich sources of vitamins. The yogurt starter cultures utilize some vitamins present in milk during fermentation for their growth. This factor contributes to a reduction of the nutritional properties of the product. However, the quantities consumed depend on the rate of the inoculation, the strain of yogurt starter cultures and the fermentation conditions (Tamime and Robinson 1985).

Folic acid (Vitamin B9) is the most important vitamin for some lactic acid bacteria. Folic acid produced by *S. thermophilus* in milk fermentation, is subsequently used for growth of *L. bulgaricus*. By selecting high folic acid-producing strains or by using relatively high amounts of *S. thermophilus* compared to *L. bulgaricus*, manufactured yogurts could contain high folic acid (Tamime and Robinson 1985). Since folic acid is an essential component in human nutrition, it is important to get enough folic acid in diet. However, it is conspicuously absent or present at very low levels in many food products, so yogurts manufactured using high folic acid producing strains have critical importance.

Concentration of Vitamin B is generally higher in fermented milk products, but yogurt made from skim milk may sometimes have less Vitamin B. Although milk contains low levels of ascorbic acid (vitamin C), it is lost almost completely during manufacturing as well as transportation. The vitamin content of yogurt depends on milk type, animal feeding, medium composition, manufacturing process, fermentation conditions and starter culture activation (Tamime and Robinson 1985). The vitamin content of milk and yogurt is given in Table 1.4.

Generally, the mineral content of yogurt is similar to milk. Yogurt is an excellent calcium source for people suffering from lactose intolerance. Moreover, calcium supplied by yogurt may be better absorbed and utilized than calcium made available in other forms (McKinley 2005).

The acidity of yogurt is thought to increase the absorption of certain minerals including calcium, phosphorous and magnesium compared with other dairy products and may reduce the inhibitory effect of some compounds such as phytic acid which is known to interfere with mineral absorption (particularly calcium). Yogurt is also good source of phosphorus which serves many functions in the body and is necessary for healthy bones and teeth as well as energy production, cell membrane structure, tissue

growth and regulation of pH levels in the body. Magnesium, potassium, zinc, selenium, iron, iodine and chloride are also found in yogurt (The Dairy Council 2007).

Table 1.4. Vitamin contents of milk and yogurt.
(Source: Tamime and Robinson 1985)

Vitamins (Units/100 g)	**Milk**		**Yogurt**	
	Whole	**Skim**	**Full Fat**	**Low Fat**
Vitamin A(IU)	148	-	140	70
Thiamin (B_1)(µg)	37	40	30	42
Riboflavin(B_2)(µg)	160	180	190	200
Pyridoxine(B_6)(µg)	46	42	46	46
Cyanocobalamine(B_{12})(µg)	0.39	0.4	-	0.23
Vitamin C(mg)	1.5	1.0	-	0.7
Vitamin D(IU)	1.2	-	-	-
Vitamin E(IU)	0.13	-	-	Trace
Folic acid(µg)	0.25	-	-	4.1
Nicotin acid(µg)	480	-	-	125
Pantothenic acid(µg)	371	370	-	380
Biotin(µg)	3.4	1.6	1.2	2.6
Choline(mg)	12.1	4.8	-	0.6

CHAPTER 2

STARTER CULTURES AND FERMENTATION OF YOGURT

2.1. Yogurt Starter Culture

Lactic acid bacteria are able to lower pH rapidly by acid production. They produce many flavor compounds which are commonly used in food and feed industry. Lactic acid bacteria used for yogurt production are thermophilic, such as *Streptococcus salivarius* ssp. *thermophilus* (*Streptococcus thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lactobacillus bulgaricus*). In some countries, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis* are sometimes mixed with the starter culture (McKinley 2005). Yogurt is generally inoculated with 1:1 ratio of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Tamime and Robinson 1985). The growth association between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is termed symbiosis. The rate of acid production in mixed culture is greater than the rate of acid production using single strain (Tamime and Robinson 1985). The growth of yogurt starter bacteria is given in Figure 2.1.

These organisms are claimed to impart nutritional and health benefits to consumers due to their probiotic activities which are able to proliferate or even survive for a long period of time in human gastrointestinal tract (Gardini, et al. 1999). "The main role of yogurt starter culture in the production of yogurt is acidification through the conversion of lactose into lactic acid, creation of the viscous texture by the production of exopolysaccharides, and development of the typical yogurt flavor" (Chaves, et al. 2002).

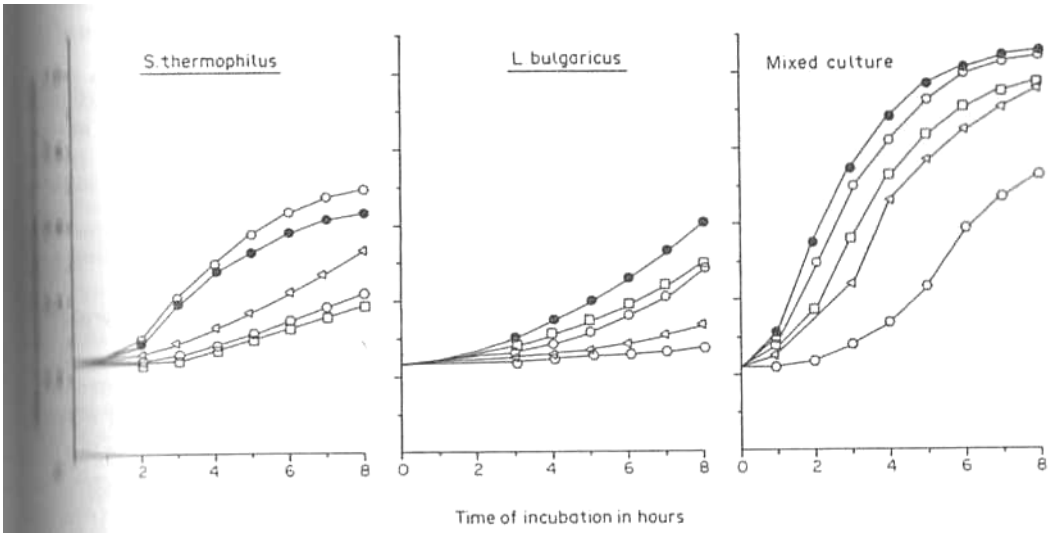


Figure 2.1. The growth of Yoghurt Starter Bacteria.
(Source: Tamime and Robinson 1985)

2.1.1. *Streptococcus salivarius* ssp. *thermophilus* (*Streptococcus thermophilus*)

Streptococcus thermophilus (*S. thermophilus*) belongs to the thermophilic group of lactic acid bacteria. It is normally in association with one or several *Lactobacillus* species. *S. thermophilus* is Gram-positive, spherical to ovoid, nonmotile coccus, 0.7-0.9 μm in diameter, occurring in pairs and chains. The bacterium has an optimum growth temperature of 37-42 ºC. It ferments a limited number of sugars including lactose, fructose, sucrose and glucose. *S. thermophilus* is highly adapted to the dairy environment and in the wild, can only be isolated from milk. It is found at low levels in raw milks obtained from a variety of animals. *S. thermophilus* shares many phenotypic and genetic properties of the other lactic acid bacteria mostly *S. salivarius*, although there is a difference in the species level by DNA-DNA hybridization (Kılıç 2001).

S. thermophilus is a homofermentative bacterium, fermenting lactose via the Embden-Meyerhof-Parnas pathway (EMP) to L(+) lactic acid. When it is grown in milk, lactose is transported into the cell in association with the removal of galactose via an antiport system. Lactose is hydrolyzed by β-galactosidase, but only glucose is metabolized further via the EMP to L(+) lactic acid.

Although *S. thermophilus* has a protein-rich habitat, its proteolytic activity is limited. *S. thermophilus* strains require free amino acids for growth. These include

glutamic acid, histidine, methionine, cysteine, valine, leucine, isoleucine, tryptophan, arginine and tyrosine. These bacteria grow well on media containing hydrolyzed protein (Robinson 1999).

S. thermophilus tends to have fewer plasmids than other lactic acid bacteria. The plasmid-free state is more common and observed plasmids are small. In contrast to the mesophilic lactococci where plasmids determine metabolic functions critical for use in dairy fermentations, *S. thermophilus* plasmids are usually cryptic. The largest *S. thermophilus* plasmid described is 25.5 Kb (Salminen, et al. 2004).

S. thermophilus is traditionally paired with *L. bulgaricus* in the manufacturing of yogurt. The typical yogurt flavor is due to the lactic acid in combination with various carbonyls and other compounds produced by thermal degradation of lipids, lactose and proteins during heat treatment of milk. A major metabolite of *S. thermophilus* and contributor to yogurt flavor is acetaldehyde. The acetaldehyde/acetone ratio in the yogurt is important in determining flavor balance (Tamime and Robinson 1985).

2.1.2. *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lactobacillus bulgaricus*)

Lactobacillus bulgaricus (*L. bulgaricus*) is first defined by Bulgarian scientist Grigoroff in 1900. Owing to similarities like G+C ratio in DNA structure, lactic acid production and concentration, cell wall structure, almost same with *Lactobacillus delbrueckii* spp. *lactis*, it is hard to differentiate *L. bulgaricus* morphologically. However, *L. bulgaricus* cannot utilize maltose unlike *L. lactis*.

L. bulgaricus is isolated from cheese and fermented milk products and ferments the smallest range of carbohydrates (glucose, lactose and fructose). Like *S. thermophilus*, it is Gram-positive, but occurs in milk as short rods 0.5-0.8 μm x 2.0-9.0 μm with rounded ends. It has a homofermentative metabolism and gives D(-) lactic acid. The optimum growth temperature is 42-45 ºC (Robinson 1999).

L. bulgaricus is galactose-negative. When growing in milk it transports the lactose into the cell in association with the expulsion of galactose via an antiport system, similar to *S. thermophilus*. Inside the cell, lactose is hydrolyzed by β-galactosidase with only glucose being fermented to D(-) lactate via the EMP.

L. bulgaricus is more proteolytic than *S. thermophilus* and milk provides a range of amino acids that stimulate the growth of *S. thermophilus* strains. The growth of *L.*

bulgaricus in milk benefits from the stimulation of formic acid and possibly carbon dioxide and pyruvate produced by *S. thermophilus* (Robinson 2002).

2.1.3. Fermentation

In the beginning of fermentation, there is a balance in the number of cells between *S. thermophilus* and *L. bulgaricus*. However, in the following stages of fermentation the bacterial counts of *S. thermophilus* are higher than *L. bulgaricus*. This is because of dominant characteristics of *S. thermophilus*.

S. thermophilus, which grows more rapidly than *L. bulgaricus*, produces lactic acid and CO_2 by the conversion of urea in milk and also produces formic acid. Additionally, it depletes the oxygen in the medium as serving an anaerobic medium favorable for *L. bulgaricus*. All these metabolites stimulate the growth of *L. bulgaricus*. On the other hand, *S. thermophilus* growth is inhibited by the accumulation of lactic acid. *L. bulgaricus* with high proteolytic activity hydrolyzes casein to form the essential amino acids especially valine for *S. thermophilus*. At the end of fermentation, lactose is converted into lactic acid (1.2-1.4 %), pH decreases to 4.2-4.3, bacteria counts increase to 2 x 10^7 cfu/ml, some aroma compounds and extracellular polysaccharides are synthesized in 3-4 hours (Robinson 1999). The relationship between yogurt starter bacteria is given in Figure 2.2.

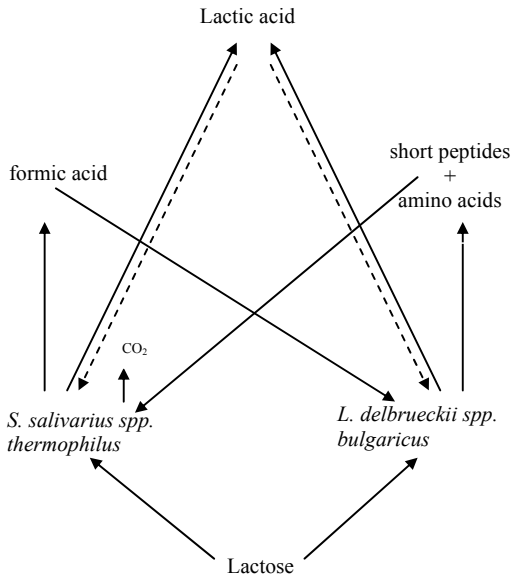
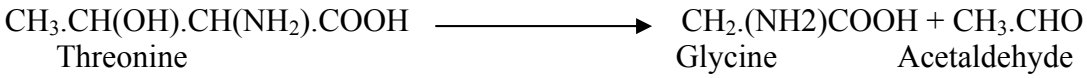


Figure 2.2. Relationship between starter bacteria in milk fermentation
(Source: Tekinşen 2000)

These two organisms give a characteristic flavor to yogurt which any other fermented milk products have. It is due to mainly acetaldehyde production by *S. thermophilus* and more extendedly *L. bulgaricus*. There are two possible pathways for the production of acetaldehyde. First one is the conversion of threonine to glycine by threonine aldolase. Second metabolic pathway may be the transformation of pyruvate by α-carboxylase that results in acetaldehyde production. The rate of formation of acetaldehyde by these two organisms depends on not only many chemical and environmental factors but also strain type. Other compounds such as diacetyl, acetone, free fatty acids, amino acids, keto and hydroxy acids produced by starter cultures contribute to the flavor of yogurt as well.



In addition to flavor compounds, yogurt starter cultures produce extracellular polysaccharides like glucans or polymers consist of glucose, galactose and rhamnose in different ratio. These by-products enhance the texture of yogurt.

CHAPTER 3

TEXTURE

3.1. Texture of Yogurt

The texture of yogurt is a crucial attribute for consumer acceptance (Britten and Giroux 2001). The texture of yogurt is based on the amount and functionality of the individual components comprising yogurt gel, and on the interaction between the components as well as on the technological steps in the production process. Moreover, milk composition and process influence yogurt stability and rheological properties such as firmness, consistency, adhesiveness, and cohesiveness (Tamime and Robinson 1985). To improve texture and prevent syneresis, stabilizers and polysacharide-producing cultures have been used (Escalante, et al. 1998).

Denaturation of protein during heat treatment of milk is an advantage in yogurt production because it increases the binding between water and protein molecules. As a result, texture of yogurt improves. However, fat solids reduction in yogurt has been associated with poor texture, where the fat removed is commonly substituted by skim milk powder, sodium caseinate, or whey protein concentrates (WPCs) (Tamime and Robinson 1985). Addition of milk solids is a routine method to improve the texture of milk products. As an alternative method to improve texture, whey protein concentrates (WPCs) may provide increased quality. Even though the effect of WPCs on texture and physical properties of yogurt is inconsistent due to opposite results by some authors, one of the studies reported that WPCs enrich the water-binding capacity of yogurts (Sodini, et al. 2005). Therefore, a novel process transglutaminase (TG) addition in fermentation is used to strengthen the gel formation of yogurt providing the cross-linking of protein molecules (Bönisch, et al. 2007).

Ünal et al. (2003) investigated the addition of polysaccharide such as locust bean gum (LBG) into milk and its effect on physical properties by response surface methodology. The researchers found that the amount of LBG and dry matter content were critical because overdose could cause decrease in viscosity and increase in syneresis.

Syneresis (whey separation) on the surface of set type yogurt is considered as a defect. Using ropy-EPS (ropy-exopolysaccharide) producing starter cultures, syneresis could be overcome since non-EPS starter cultures had the highest level of syneresis (Amatayakul, et al. 2006).

Many processing problems such as low viscosity and high syneresis, which occur during yogurt production, may be solved by increasing the total solids of milk or addition of stabilizers such as LBG. However, fortification of milk by these ingredients may affect the taste and aroma of the final product adversely. Moreover, the use of some stabilizers are restricted or prohibited in some European countries (Amatayakul, et al. 2005). For this reason, EPS-producing starter cultures are preferred as they improve the rheological properties of fermented milk. The gel structure and viscosity of the products are affected by the gel formation conditions, as well as the amount and the type of the EPSs produced. Ropy EPS-producing strains also increase the viscosity of yogurt when compared to yogurt made with non-ropy cultures and improve the texture (Marshall and Rawson 1997). The rheological behavior of the polysaccharides is also related to their three-dimensional structure. In addition to the viscosifying effect of the polysaccharides, the interactions between the EPSs and the milk proteins, e.g. caseins, also play a role in the improvement of the texture. The microorganisms and/or the EPSs that they produce may affect the protein aggregation, thereby affecting the physical properties of the milk gel. One of the recent studies showed that the rheological properties of stirred yogurt were affected by the type of EPS producing strains used, suggesting an effect due to the interaction between the polymer and milk proteins (Marshall and Rawson 1999).

3.2. Polysaccharides

Polysaccharides are defined as high molecular-weight polymers composed of saccharide subunits. The chemical reactions and pathways resulting in the formation of polysaccharides, polymers of more than 10 monosaccharide residues are joined by glycosidic linkages (Telefoncu 1992). In the major classification, there are two types of polysaccharides synthesized by organisms. These are intracellular polysaccharides and extracellular polysaccharides. Intracellular polysaccharides are produced by plants (starch, inulin), animals (glycogen), and microorganisms (glycogen). Extracellular

polysaccharides (exopolysaccharides EPS) occur widely among bacteria and microalgae and less frequently among yeasts and fungi (Degeest and De Vuyst 2000).

3.3. Exopolysaccharides

Like any other bacteria, lactic acid bacteria are able to produce several types of polysaccharides that are classified according to their location in the cell (Degeest, et al. 2001). Microbial exopolysaccharides occur as capsules (capsular exopolysaccharides CPS) which are covalently bounded to the cell surface or secreted as slime form in cell environment. Some bacteria produce only capsular EPS, but some produce only slime (ropy) form, whereas, in some cases, bacteria can produce both forms of EPSs (Yang, et al. 1999, Broadbent, et al. 2003)**.** The mesophilic heteropolysaccharide producing lactic acid bacteria have much more widespread ability to form CPS than the thermophilic heteropolysaccharide producing ones. However, synthesis of CPS and ropy behavior is almost strain dependent (Mozzi, et al. 2006).

Bacterial exopolysaccharides (EPSs) are long-chain polysaccharides consisting of branched, repeating units of sugar derivates which are mainly glucose (D-glucose), galactose (D-galactose), rhamnose (L-rhamnose), mannose, N-acetylglucosamine, N-acetylgalactosamine, D-glucuronic acid etc. in different ratios (Welman and Maddox 2003, Vaningelgem, et al. 2004a).

3.4. Chemical Composition of EPS Produced by Lactic Acid Bacteria

In the beginning of the EPS studies, it was found that EPS was a molecule like protein. The following studies had showed that EPS had a carbohydrate structure with α- and β-linkages in different types. That difference depends on the formation of main carbohydrate molecules (D-galactose, D-glucose and L-rhamnose) present in different ratios. Moreover, EPS-producing lactic acid bacteria can produce not only one type of polysaccharide but also different types of polysaccharides due to fermentation conditions. In addition, it is possible that same strain is able to produce high-molecular-mass and low- molecular-mass EPS fractions which do not differ in monomeric composition (De Vuyst and Degeest 1999).

The basic structures of EPS units from some type of *S. thermophilus* and *L. bulgaricus* are given in Figure 3.1.

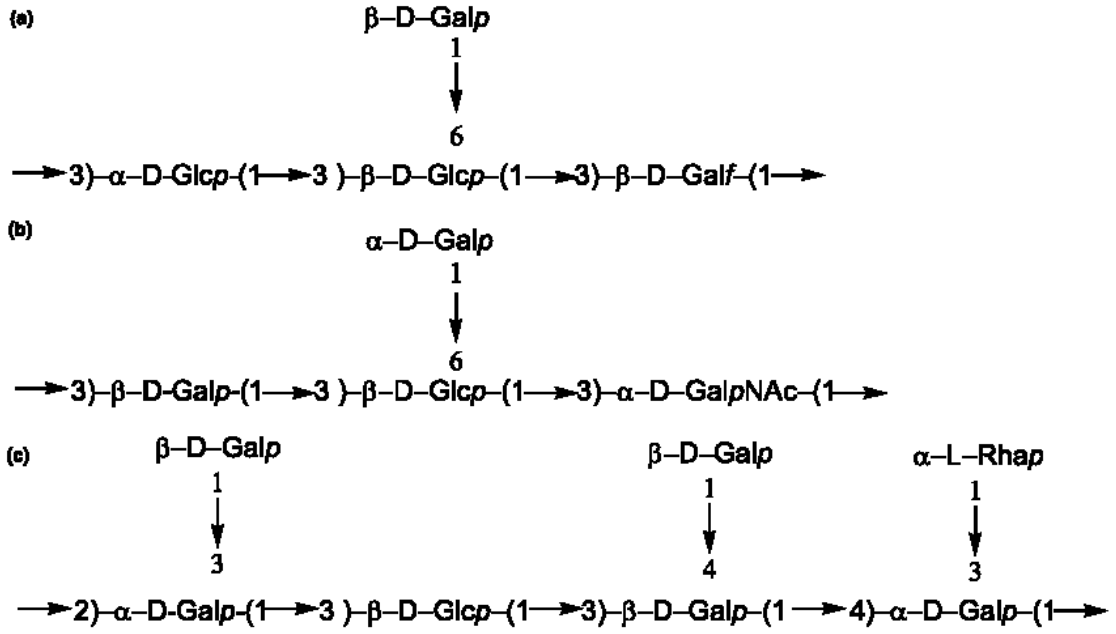


Figure 3.1. Structure of the EPS Subunits (a) Structure of the EPS subunit from *Streptococcus thermophilus* SY89 and SY102. (b) Structure of the EPS subunit from *Streptococcus thermophilus* IMDO1, IMDO2, IMDO3, NCFB 859 and strain `21'. (c) Structure of the EPS subunit from *Lactobacillus delbrueckii* subsp. *bulgaricus* strains LY03, strain `24' and strain `25' (Source: Marshall, et al. 2001)

3.5. Classification of EPS Produced by Lactic Acid Bacteria

Depending on their composition and mechanism of biosynthesis, bacterial EPSs can be divided into two classes: homopolysaccharides (HoPS) and heteropolysaccharides (HePS).

3.5.1. Homopolysaccharides

Homopolysaccharides are the polymers composed of one type of monosaccharide (Vaningelgem, et al. 2003). HoPS are subdivided into four groups: α-D-glucans such as dextrans composed of mainly α-1,6-linked glucose molecules and generally produced by *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, *Streptococcus mutans* and *Streptococcus sobrinus* , β-D-glucans composed of β-1,3-linked glucose molecules and produced by *Pediococcus* spp. and *Streptococcus* spp., fructans, composed of β-2,6-linked D-fructose molecules like levan produced by *S. salivarius* and finally others that are mainly polygalactans

composed of structurally identical repeating units with different glycosidic linkages. HoPSs are synthesized in the presence of a specific substrate such as sucrose. By the way, the energy needed is provided by the hydrolysis of sucrose (De Vuyst and Degeest 1999).

3.5.2. Heteropolysaccharides

Heteropolysaccharides are the polymers of repeating units that are composed of two or more types of monosaccharides (Degeest, et al. 2001, Welman and Maddox 2003, Vaningelgem, et al. 2003). They often differ from HoPSs by monosaccharide composition, linkage types between polymer units, branching, molecular weight, monosaccharide charge and also by the ability to interact with milk proteins (Vaningelgem, et al. 2004a). HePS synthesis is different from HoPS synthesis due to formation of intracellular precursor repeating units and having isoprenoid glycosyl carried lipids in the process (Ruas-Madiedo and Reyes-Gavilan 2005). The structure of the repeating unit of a LAB heteropolysaccharide produced by *S. thermophilus* was first determined. The molecular mass of HePS varies from 10^4 to $9x10^6$ Da (Vaningelgem, et al. 2004a).

HePS are mainly produced by mesophilic lactic acid bacteria such as *Lactococcus lactis*, *Lactobacillus sakei*, *Lactobacillus rhamnosus*, and *Lactobacillus casei* and thermophilic lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, and *Streptococcus thermophilus* (Yang, et al. 1999, Vaningelgem, et al. 2004a). Although HePSs are strain dependent, their production are influenced by the environmental conditions like bacterial growth phase, medium composition (carbon and nitrogen source), pH and temperature (Vaningelgem, et al. 2004b, Aslım, et al. 2006). As EPS production is growth associated in thermophilic LAB strains, the EPS biosynthesis pathway is very complex (De Vuyst, et al. 1998). As a result, it is necessary to elucidate either which factor has a considerable effect or in which step of the EPS biosynthesis leads to a production of different polymer product. Furthermore, the EPS produced by lactic acid bacteria is unstable as a result of environmental factors.

Cerning et al. (1992) studied the influence of the carbon source on the production of EPS by ropy strains of *L. lactis* ssp. *lactis* CNRZ in milk and reported that incubation at 25 °C instead of 30 °C and addition of 5% glucose stimulated EPS

production although the cell numbers were low. Some other researchers optimized the fermentation conditions for *S. thermophilus* LY03. According to the results of this study, fermentation temperature of 42 °C, constant pH of 5.5 and peptone and yeast extract as a nitrogen source equilibrated with carbon source are the most important fermentation parameters that best EPS yield (352 mg polymer dry mass (PDM) / L) was obtained under these conditions (De Vuyst, et al. 1998). Similar results were determined by Degeest and De Vuyst (2000), but additionally it was reported that any of the carbohydrate source had an effect on the monomer composition of EPS produced by *S. thermophilus* LY03. Moreover, the enzymes such as α-phosphoglucomutase, UDP-galactose 4-epimerase, and UDP-glucose pyrophosphorylase involved in the biosynthesis of EPS were stimulated by the certain type of carbon source (glucose for *S. thermophilus* LY03) which was depended on the strain. As a conclusion, under these circumstances the amount of EPS production was increased.

3.6. EPS Phenotypes

EPS phenotype is described as the bacteria having ropy or slime characteristics, for instance, EPS-producing bacteria is generally known as ropy strains. There are many ways to determine the ropiness of the bacteria. One of them is the measurement of macroscopic appearance of ropy EPS-producing lactic acid bacteria on the surface of agar plates. By this method, the resistances to flow through serological pipettes and structure of viscous strands during free fall from pipette tips are measured**.** Another method is to determine the ropiness of strain grown on ruthenium red milk agar by its color since ruthenium red stains the bacterial cell wall and the colonies grown on the agar surface are pink in nonropy strains. In contrast, white colonies are ropy strains (Stingele, et al. 1996). However, in the study of *Lactobacillus delbrueckii* spp. *bulgaricus* CNRZ 1187 showed that white (ropy) colonies produce less EPS than pink colonies (Bouzar, et al. 1996). As a result, ruthenium red is not able to differentiate all types of EPS-producing strains. Moreover, ruthenium red method was developed for ropy strains of *Streptococcus thermophilus* and also valid for certain types of EPS (Bouzar, et al. 1996). Confocal scanning laser microscopy (CSLM), scanning electron microscopy (SEM) techniques are not only used for the determination of the EPS phenotype but also used for the determination of rheological properties of fermented milk product (Hassan, et al. 2002, Goh, et al. 2005a).

3.7. Biosynthesis of EPS by Lactic Acid Bacteria

EPS biosynthesis is explained by the sugar conversion (gylcolysis) like other cell polysaccharides which is generally used for the cell wall. EPSs are synthesized by the bacteria in the cytoplasm. In addition, the EPS biosynthesis is an energy demanding process. The polymerization of the repeating unit is complex and involves several enzymes and proteins consecutively. Phosphoglutomutase (PGM) is an enzyme which has an important role in the conversion of glucose-6-phosphate to glucose-1-phosphate, between the catabolic and anabolic pathways of sugar conversion. The sugar nucleotides are formed by Glucose-1-phosphate due to serve as a branch point which is necessary for monosaccharide polymerization. Subsequently, the monosaccharide repeating unit is carried by several EPS specific enzymes. At last, several gene products on the EPS gene cluster (glycosyl-transferases) provide sugars to link so as to form the repeating unit. However, the mechanism of polymerization of the repeating unit in LAB, and its subsequent export from the cell, is unclear (Marshall, et al. 2001, Welman and Maddox 2003).

The total yield of EPS produced by lactic acid bacteria strains varies between 0.150 to 0.600 g/L under optimum growth conditions (Degeest, et al. 2001). In addition, growth conditions and medium composition have effects on the total yields of EPS. However, EPS producing ability is not a stable characteristic by some lactic acid bacteria because of the loss of plasmids especially in the mesophilic strains (Bouzar, et al. 1997).

Although there are many researches about the determination of amount of EPS, monomer composition and also the EPS production ability of single strain lactic acid bacteria, there were limited number of studies about mixed strains behavior. Bouzar et al. (1997) reported that EPS in mixed-strain cultures was produced faster than in single-strain cultures and that the composition of the EPS is very different. EPS biosyntesis diagram is given in Figure 3.2.

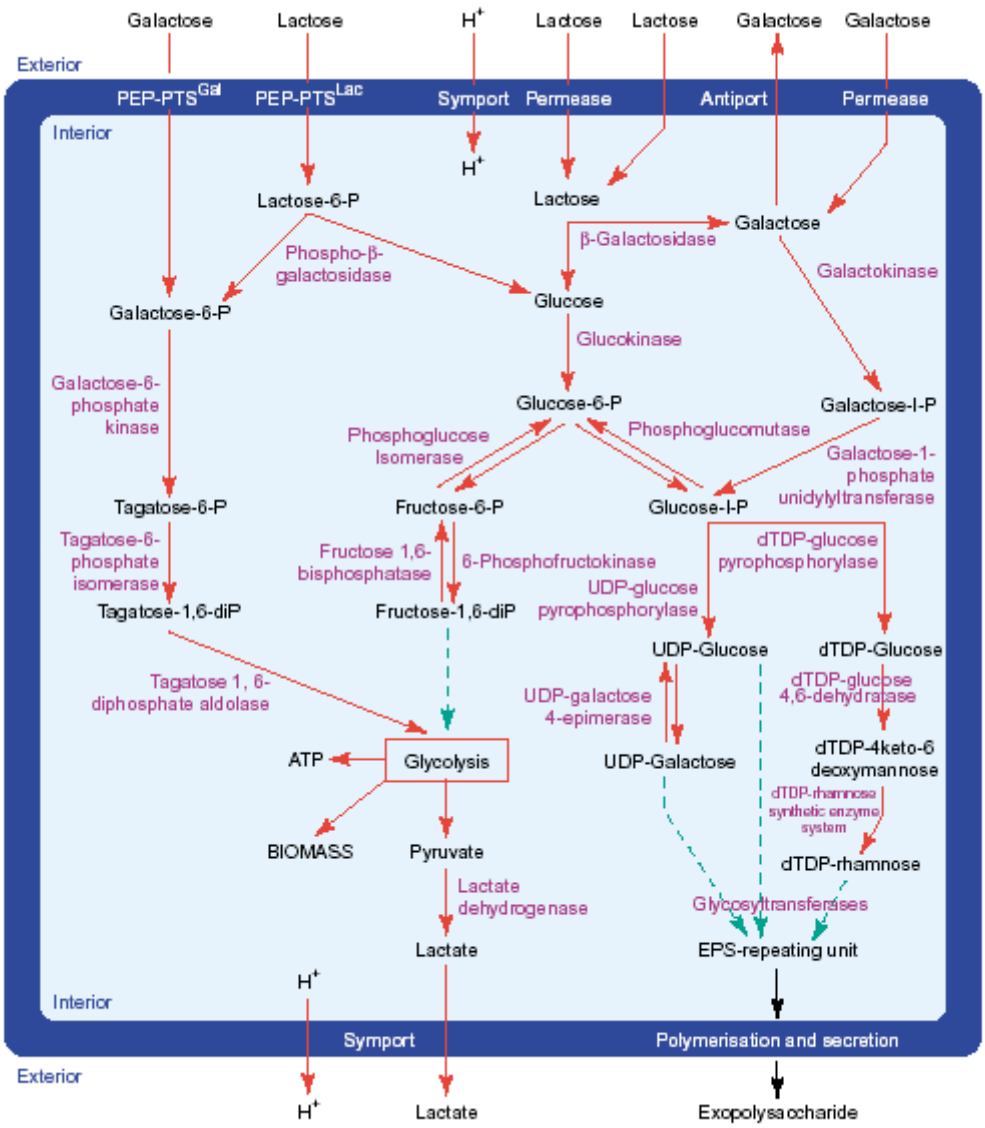


Figure 3.2. Diagram of the conversion of lactose, galactose and glucose to EPS and to glycolysis in lactic acid bacteria (glucose uptake not shown). In lactose utilizing galactose negative strains (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus*), galactose is not metabolized and is expelled from the cell via a lactose/galactose antiport system.
(Source: Welman and Maddox 2003).

3.8. Isolation and Quantification Methods of EPS

Lactic acid bacteria fermentation in milk results in formation of weak gel due to aggregation of casein micelles and also complex medium comprised bacterial cells, lactose, lactic acid and other components mainly EPS. For this reason, careful isolation

and purification is necessary so as to separate EPS from non-EPS components in fermentation medium (Goh, et al. 2005b).

There are several EPS isolation methods present and choosing the most appropriate method for EPS isolation and purification depends on the complexity of the culture medium. Among them, dialysis against water is the simplest one. In this method, bacterial cells are centrifuged and then ethanol or acetone precipitation for protein removal may be used to concentrate the EPS produced by thermophilic and mesophilic lactic acid bacteria strains. If the medium is more complex, additional purification is needed to reduce protein content and other components that have to be removed. For this purpose, generally trichloroacetic acid (TCA) (De Vuyst, et al. 1998, Marshall and Rawson 1999, Amatayakul, et al. 2006), some type of enzymes such as pronase E from *Streptomyces griseus* (Bouzar, et al. 1996, Bouzar, et al. 1997) or membrane filtration techniques such as microfiltration, ultrafiltration (UF) and diafiltration are used (Yang, et al. 1999).

In the TCA method, EPS is concentrated with ethanol or sometimes with acetone (Stingele, et al. 1996, De Vuyst, et al. 1998). The aim of enzyme digestion is to hydrolyze or precipitate the proteins. Heat treatment is sometimes followed after pronase treatment and then UF or evaporation is used. In this method, TCA and enzyme precipitation may be used consecutively. Filtration methods have centrifugation, ethanol precipitation steps and also filtration through synthetic membrane due to purification of EPS. In addition, in some cases, other treatments such as ion-exchange columns, Dnase digestion (Shihata and Shah 2002), SDS-PAGE protein removal, size exclusion chromatography are used because highly pure EPS is required (Ruas-Madiedo and Reyes-Gavilan 2005).

After the isolation step, EPS production can be expressed as the dextran equivalent miligrams of dextran per milliliter. Even though dextran and phenol-sulphuric acid methods are commonly used, proteins and other carbohydrates which are not EPS could be determined using these methods. Although these methods are simple and cheap, they always have a risk of determination of low molecular weight carbohydrates. In order to decrease inaccuracy, HPLC method is used. Recently, near-infrared spectroscopy (NIRS) method is used for the lactose, lactic acid and EPS yields in culture media. The results of this method show high correlation coefficients with HPLC and phenol sulphuric acid method. NIRS method could be useful for rapid monitoring of EPS and lactic acid production (Ruas-Madiedo and Reyes-Gavilan 2005).

The monomer composition, the sequence and ring size of the constituting monosaccharides, the location of the glycosidic linkages and the type of noncarbohydrate structure constituents determine the primary structure of EPS molecule. Therefore, the qualitative and quantitative determinations of EPS monosaccharides are determined by HPLC (Degeest, et al. 2002), high-performance anion-exchange chromatography pulse amperometric detection (HPAEC-PAD) and gas chromatography/mass spectrometry (GC/MS) (Ruas-Madiedo and Reyes-Gavilan 2005). HPLC involves separation of monosaccharides by ion-exchange columns. HPAEC-PAD is used for the identification and quantification of mono- and oligopolysaccharides. Moreover, it is very sensitive and selective for the analysis of sugar compounds although having the low resolving power is the main disadvantage (Ruas-Madiedo and Reyes-Gavilan 2005).

The molar mass of EPS is generally determined by gel permeation chromatography or alternatively fast protein liquid chromatography (FPLC) in which EPS concentration determined by phenol sulphuric acid and than molar mass and radius gyration (Rg) simultaneously determined by gel permeation chromatography using a multiangle laser light scattering detector coupled online with the refractive index (RI) detector in the HPLC system (Ruas-Madiedo and Reyes-Gavilan 2005).

As a conclusion, researchers have been combining all these methods in order to isolate, purify and quantify the EPS. For this purpose, Goh et al. (2005b) tried to improve the EPS assay that gave reproducible results with higher accuracy. This assay was based on the comparison of the methods that were widely used for EPS isolation. This assay includes the Flavourzyme for protein hydrolysis, optimum ethanol concentration for maximum dextran recovery and most appropriate centrifugation regime to minimize EPS loss.

3.9. Effects of EPS Production on Rheological Properties of Yogurt

The use of EPS-producing cultures is commonly used as a substitute for commercial stabilizers in yogurt manufacturing due to reduction of syneresis and improvement of product texture and viscosity. Some researchers reported that some EPS-producing (ropy) lactic acid bacteria showed a higher viscosity and a lower degree of syneresis compared with non-EPS-producing lactic acid bacteria (Bouzar, et al. 1996, Folkenberg, et al. 2006). In contrast, several studies reported that no direct correlation

was found between the amounts of EPS produced by strain and the rheological properties of fermented milk products. However, rheology of fermented milk product depends not only on the quantity of EPS present, but also on the structure and apparent molecular mass of the polymer and the physical state of the proteins, particularly caseins (Hassan, et al. 2003, Petry, et al. 2003, Welman and Maddox 2003). Therefore, the increase in viscosity may be related to the changes in physical properties of the milk proteins due to the pH decline (Aslım, et al. 2006).

3.10. Applications of EPSs Produced by Lactic Acid Bacteria

The exact functions of EPS for the bacterial cells have not been completely elucidated. It is known that EPSs not only play a role in the protection of the microbial cells against desiccation, phagocytosis and phage attact, antibiotics or toxic compounds, but also have lots of benefits for human health and for applications in dairy industry. Some studies have indicated that EPS may have immunostimulatory, antitumoral, antiulcer effects or cholesterol-lowering activity (Aslım, et al. 2006, Lin and Chang Chien 2007).

Many polysaccharides and stabilizers are used in food industry as viscosifying, water binding, gel forming and thickening agent (Looijesteijn, et al. 2000). These are mostly plant carbohydrates (starch, gum, pectin and alginate) or animal hydrocolloids (gelatin and casein) (Vaningelgem, et al. 2004b). In addition, xanthan produced by *Xanthomanas campestris* was used firstly as a microbial EPS (De Vuyst, et al. 1998). Nevertheless, its use is not allowed in most European countries and U.S. EPS producing lactic acid bacteria are gained popularity of being safe and used as natural stabilizers. Moreover, recently there is an increasing interest for EPS producing dairy cultures because of high consumer demand for smooth and creamy yogurt products. EPS formation by lactic acid bacteria during the production of fermented milk products either acts as a viscosifying, emulsifying agent or imparts favorable rheological properties. Furthermore, it was reported that EPS from food grade organisms, particularly lactic acid bacteria, has potential as food additives and functional food ingredients with both health and economic benefits (Welman and Maddox 2003).

CHAPTER 4

NEAR-INFRARED SPECTROSCOPY TECHNIQUE

4.1. Near-Infrared (NIR) Spectroscopy

Recently, NIR Spectroscopy is a popular method for simultaneous chemical analysis and is being studied extensively in a number of fields, such as biotechnology, pharmaceutical industry and process monitoring due to its potential as an on-line, nondestructive and noninvasive analysis (Wittrup and Norgaard 1998). The NIR Spectroscopy covers the wavelength range between 780-2500 nm (Burns and Ciurczak 2001). Samples are illuminated with a near-infrared light source and the reflected or transmitted light is recorded. The energy difference between the illuminating radiation and the reflected or transmitted radiation at each wavelength, constitutes the absorbance spectrum of the sample (Asimopoylos, et al. 2004). Most of the absorption bands observed in this region are due to overtones and combinations of the fundamental mid-IR molecular vibrational bands. The most commonly observed bands arise from the C-H, O-H or N-H bonds in the molecules, eventough all the fundamental vibrational modes can have overtones (Burns and Ciurczak 2001).

Computers and automation technology have many advantages such as being fast which make it possible to produce hundreds of spectra in few minutes for a given sample that contains multiple components. In addition, it is important to choose the most suitable calibration method. Especially multivariate calibration deals with data of instrument responses measured on multiple wavelengths for a sample that usually contains more than one component (Özdemir and Öztürk 2006).

There are many multivariate calibration methods used in NIR Spectroscopy such as inverse least squares (ILS), genetic inverse least squares (GILS), classical least squares (CLS), partial least square (PLS). ILS is a method that is based on the inverse of Beer's Law where the concentration of an analyte is modeled as a function of absorbance. GILS is modified version of original ILS method in which a small set of wavelengths is selected from a full spectral data matrix. By the development of optimum solution, genetic algorithm (GA) has been applied to a number of wavelength selection problems (Özdemir and Öztürk 2006).

CLS method is founded in using the Beer-Lambert Law to extend the calculation of the absorptivity coefficients across a much larger portion of the spectrum than the much simpler Least Squares Regression method.

$$\mathbf{A = K \,.\, C + E_A} \qquad \text{(Eq. 4.1)}$$

where **A** is the absorbance of a single sample of known concentration**, K** is the constant, **C** is the known concentration and $\mathbf{E_A}$ is the errors in the calculation of the absorbance (Thermo Scientific 2007).

CLS method has a major disadvantage which all the interfering species must be known and their concentrations are included in the model However, ILS method can eliminate this problem by modeling the concentration of a component as a function of absorbance (Özdemir and Öztürk 2006).

The ILS model for *m* calibration samples with n wavelengths for each spectrum is described by :

$$\mathbf{C = AP + E_C} \qquad \text{(Eq. 4.2)}$$

where **C** is the *m* x *l* matrix of the component concentrations, **A** is the *m* x *n* matrix of the calibration spectra, **P** is the *n* x *l* matrix of the unknown calibration coefficients relating *l* component concentrations to the spectral intensities and $\mathbf{E_C}$ is the *m* x *l* matrix of errors in the concentrations not fit the model. In the calibration step. ILS minimizes the squared sum of the residuals in the concentrations.

The biggest advantage of ILS is that equation (1) can be reduced for the analysis of single component at a time since the analysis is based on an ILS model invariant with respect to the number of chemical components in the analysis. The reduced model is given as :

$$\mathbf{c = Ap + e_c} \qquad \text{(Eq. 4.3)}$$

where **c** is the *m* x *l* vector of concentrations for the component that is being analyzed, **p** is *n* x *l* vector of calibration coefficients and $\mathbf{e_c}$ is the *m* x *l* vector of concentration residuals unfit for the model. During the calibration step, the least-squares estimate of **p** is :

$$\hat{\mathbf{p}} = (\mathbf{A}' \mathbf{A})^{-1}\mathbf{A}' \cdot \mathbf{c} \qquad \text{(Eq. 4.4)}$$

where $\hat{\mathbf{p}}$ is the vector of estimated calibration coefficients.

Once $\hat{\mathbf{p}}$ is calculated, the concentration of the analyte of interest can be predicted with the equation below.

$$\hat{c} = \mathbf{a}' \cdot \hat{\mathbf{p}} \qquad \text{(Eq. 4.5)}$$

where $\hat{c}$ is the scalar estimated concentration and **a** is the spectrum of the unknown sample. Due to the ability to predict one component at a time without knowing the concentrations of interfering species, ILS has became one of the most frequently used calibration methods (Özdemir 2007).

On the other hand, ILS has a major disadvantage of being restricted by the number of wavelengths in a spectrum which are much more than the calibration samples. In this situation, several wavelength selection strategies, such as stepwise wavelength selection and all possible combination searches are avaliable to built an ILS model in order to fit the data best (Özdemir 2007).

Recently, GA is global search and optimisation methods which are based upon the principles of natural evolution and selection as developed by Darwin. GA is simple and consist of five basic steps including initialization of a gene population, evaluation of the population, selection of the parent genes for breeding and mating, crossover and mutation, and finally replacing parents with their offspring as given in Figure 4.1. The names of the steps are taken from the biological foundation of the algoritm (Özdemir and Öztürk 2006).

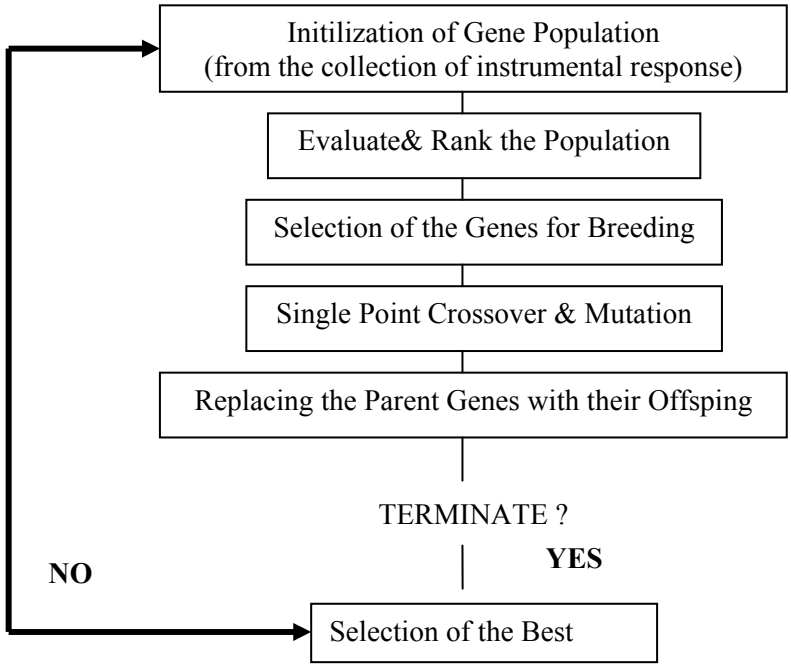


Figure 4.1. GA optimization method steps
(Source: Özdemir and Öztürk 2006)

GA is implemented to GILS for selecting wavelengths in order to build multivariate calibration models with reduced data set. GILS follows the same basic steps as other GAs model, but it is unique in the way it encodes genes. The term gene is used to describe the collection of instrumental responses in GILS method. The term population is used to describe the collection of individual genes in the current generation.

4.2. Applications of NIR Spectroscopy in Food Analysis

Most samples are analyzed by conventional chemical analyses, such as the Kjeldahl procedure for total N or ammonium-N or Gerber method for fat. These procedures can be time consuming and expensive, and also generate chemical wastes (Reeves and Van Kessel 2000).

Due to the fact that, routine analytical methods used for food products such as dairy products are destructive, expensive, time and labor consuming, the NIR spectroscopy has been used to measure the content of various constituents in milk, milk

powder, whey, cheese and yogurt (Tsenkova, et al. 1999). The NIR spectroscopy is widely used in the food industry as a quality control tool.

Tsenkova et al. (1999), investigated the potential of NIR spectroscopy for measurement of fat, total protein and lactose in unhomogenized milk. Researchers found that spectral region and sample thickness were the significant factors for the determination of milk fat and total protein. This study showed that the NIR spectroscopy was an adequate method for determination of milk constituents.

Shao et al. (2007) examined the feasibility of using Visible NIR (Vis/NIR) spectroscopy to detect the sugar and acid contents of yogurt through using a hybrid model called PC-ANN, which combined principle component analysis (PCA) with artificial neural network. At the same time, the models established via chemometrics partial least square (PLS) regression were also established, in order to find sensitive wavelengths corresponding to sugar and acidity contents of yogurt through the regression coefficient of PLS. The sugar content was measured by a sugar content meter and acidity was measured by a pH meter analytically. Their study indicated that it is possible to use a non-destructive technique to measure the sugar contents and acidity of yogurt using Vis/NIR spectroscopy. By the use of PC-ANN hybrid model, they established a correlation between the absorbance spectra and the parameters of sugar content and acidity. The results showed an excellent prediction performance and were encouraging with a correlation coefficient of 0.92 and 0.91, the root mean square errors of calibration (RMSEP) of 0.33 and 0.04 for sugar content and acidity. On the other hand, the results showed that it was difficult to select one or a few wavelengths for accurate prediction of the sugar content or acidity of yogurt and it is necessary to use a wider spectrum or even an entire spectrum for sugar content and acidity prediction.

He et al. (2007), studied sugar content of yogurt by Vis/NIR spectroscopy with PLS model. Their sdudy showed 0.934 correlation coefficient between predicted and measured values of 25 yogurt samples. Hence, the spectroscopy technique was reliable and acceptable in predicting the sugar content of yogurt. In the studty of Sultaneh and Rohm (2007), it was evaluated that the application of NIR spectroscopy was undoubtedly necessary for performing rapid methods in the determination of solids and protein content in cheese curd. Partial least squares (PLS) regression was used to build statistical models between curd moisture or curd protein content and the predictors and the calibration models resulted in coefficients of determination of $R^2 = 0.994$ for total solids and $R^2 = 0.985$ for protein content for unhomogenized curd.

CHAPTER 5

AROMA

5.1. Aroma Compounds

Flavor is a crucial characteristic of foods as the sensory characteristics play an important role in product acceptance by consumers. In fermented dairy products, flavor perception is strongly based on the volatile components (Kalviainen, et al. 2003). Most commercial yogurts are produced by the action of two microorganisms, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, which grow simultaneously to give the desired flavor, mouthfeel and texture to the yogurt. Several flavor compounds have been isolated from natural yogurts. The most prominent ones are mainly lactic acid which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetaldehyde, ethanol, acetone, diacetyl and 2-butanone. However, among them, acetaldehyde is considered as the major flavor compound for the typical yogurt aroma reported by several researchers (Chaves, et al. 2002, Ott, et al. 1997). Both the ratio and balance between these flavor compounds must be taken into account due to their effect on yogurt flavor, for instance, the ratio between acetaldehyde and acetone of 2.8 is considered optimum (Gardini, et al. 1999, Chaves, et al. 2002). On the other hand, a greater number of volatile organic compounds identified in yogurt are not only produced by starter culture but also originated from milk (Beshkova, et al. 1998).

5.2. Flavor Formation

Flavor formation is accumulated by a series of biochemical processes in which the starter cultures provide the enzymes. There are three main pathways responsible for the formation that are identified as the conversions of lactose (glycolysis), fat (lipolysis), and caseins (proteolysis). In the case of glycolysis, firstly lactose converted to lactate and then a fraction of the intermediate pyruvate is converted to various flavor compounds such as diacetyl, acetoin, acetaldehyde, or acetic acid. Lipolysis caused by the formation of free fatty acids, which can be precursors of flavor compounds such as methylketones, secondary alcohols, esters and lactones. However, in some cases, in

fermented dairy products, mostly cheeses, the basis of the flavor formation depends on the enzymatic degradation of proteins (caseins) into peptides and amino acids that subsequently results in volatile aroma compounds. Formation and breakdown of casein is important because it prevents the accumulation of bittertasting peptides. Flavor forming pathways originating from proteins are shown in Figure 4.1.

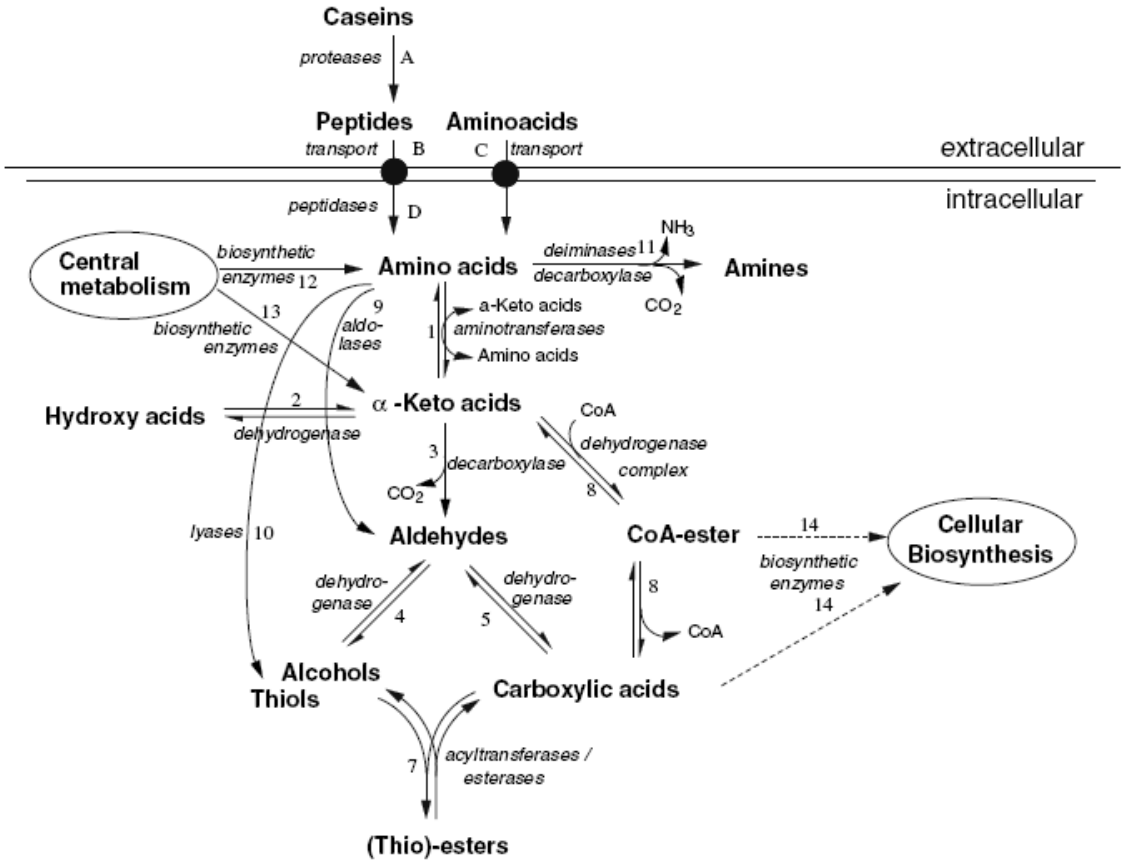


Figure 5.1. Overview of general protein conversion pathways relevant for flavor formation in dairy fermentations. (Source: Smit, et al. 2005)

Diacetyl (butanedione or 2,3-butanedione, $CH_3COCOCH_3$) is a natural byproduct of fermentation. Diacetyl is defined as the butter aroma for being an essential flavor component for many dairy products. It is produced during conversion of the citric acid to pyruvate in milk by specific citrate-utilizing LAB and pyruvate is further converted the precursor for diacetyl. Some authors reported that diacetyl was only produced by *S. thermophilus* although others reported that *L. bulgaricus* produced large amounts of diacetyl. In mixed cultured yogurt, active diacetyl production was measured during the first 4 h and also in the cooling step with maximum concentration in Bulgarian yogurt (Beshkova, et al. 1998). Diacetyl formation is given in Figure 4.2.

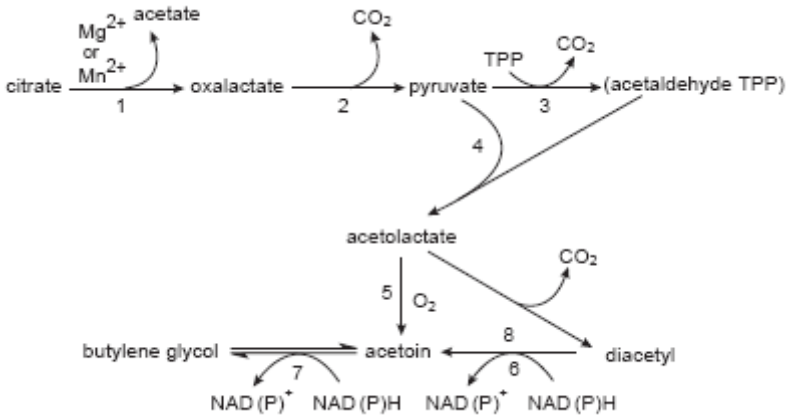


Figure 5.2. Pathway for the conversion of citrate to diacetyl and other compounds. The reactions are catalyzed by the following enzymes: (1) citrate lyase; (2) oxaloacetate synthase; (3) pyruvate decarboxylase; (4) acetolactate synthase; (5) acetolactate decarboxylase; (6) diacetyl reductase; (7) acetoin reductase. Reaction 8 is the oxidative conversion of a-acetolactate to diacetyl. (Source: Marsili 2002).

Butanone ($CH_3COC_2H_5$) is a ketone, also known as methyl ethyl ketone (MEK). It is a colorless liquid with a sharp and sweet odor. Acetone (also known as propanone, dimethyl ketone, 2-propanone, propan-2-one and β-ketopropane, CH_3COCH_3) is the simplest representative of the ketones (Anon 2007b).

Acetaldehyde, (CH_3CHO), is an organic chemical compound which is volatile at room temperature and pressure. In addition, it is colorless liquid with a fruity smell (Miyake and Shibamoto 1993). Acetaldehyde is produced by yogurt bacteria, *S. thermophilus* and *L. bulgaricus*, as being a major component of the typical yogurt flavor. The exact mechanism of acetaldehyde production has not been well established (Smit, et al. 2005).

Acetaldehyde can be derived from amino acid (especially threonine, methionine and valine), nucleotide, and pyruvate metabolism in bacteria. In the liver, the enzyme alcohol dehydrogenase converts ethanol into acetaldehyde, which is then further converted into harmless acetic acid by acetaldehyde dehydrogenase. Acetaldehyde is more toxic than ethanol and is responsible for many hangover symptoms (Smit, et al. 2005).

With regard to two major possibilities in acetaldehyde production, pyruvate is converted to Acetyl-Coenzyme A by the pyruvate formate lyase or the pyruvate dehydrogenase or threonine is converted by the threonine aldolase which results in

glycine and acetaldehyde. Besides, acetaldehyde can be produced by acetate conversion as part of the acetate utilization rescue pathway. It is reported that threonine aldolase (TA) is the major enzyme activity involved in acetaldehyde production in the yogurt bacterium *S. thermophilus* whereas high activation of TA in the lactobacilli than in the streptococci is also reported (Chaves, et al. 2002). The amount of acetaldehyde produced by the bacteria is between 17 and 41 mg/l during yogurt fermentation (Bongers, et al. 2004). Acetaldehyde formation is given in Figure 4.3.

Acetaldehyde production of *S. thermophilus* was investigated under control of specific enzyme serine hydroxymethyltransferase (SHMT) which had an important role in the production of acetaldehyde. Because SHMT possesses TA activity, acetaldehyde production during fermentation could be correlated with TA. In addition, supplementation of the growth medium with L-threonine resulted in an increase in acetaldehyde production. Nevertheless, the role of TA in acetaldehyde formation in mixed yogurt cultures has not been understood completely (Chaves, et al. 2002).

During milk fermentation, acetaldehyde production in mixed culture was occurred immediately after milk coagulation which was in log phase (Beshkova, et al. 1998). However, during storage the amount of acetaldehyde decreased because of the hydrolysis by microbial enzymes in order to form other substances such as ethanol (Güler-Akın 2005). Diacetyl and 2,3-pentanedione increased slightly in yogurt made by mixed cultures during storage at 4 °C due to basal metabolic activity of lactic acid bacteria (Ott, et al. 1999).

The two yogurt starter *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* are able to produce acetaldehyde. It has been reported by some authors that *L. delbrueckii* subsp. *bulgaricus* is a greater acetaldehyde producer than *S. thermophilus*, although other authors have reported the opposite (Ott, et al. 1999, Chaves, et al. 2002). In fact, the production of acetaldehyde by lactic acid bacteria is strain dependent (Chaves, et al. 2002).

Ott et al. (2000) reported that the yogurt made by only *L. bulgaricus* resulted in very similar to the yogurt made by both *L. bulgaricus* and *S. thermophilus*. In contrast, yogurt made by *S. thermophilus* was extremely different from the product obtained in the presence of *L. bulgaricus*. It had a higher butter, sweet, cooked creamy and cottage cheese aroma. Consequently, it is reported that *S. thermophilus* does not have a distinct effect on the production of yogurt flavor. It was also reported that there was a positive correlation between flavor and acidity. However, viscosity and ropiness were correlated

negatively with acidity and flavor (Ott, et al. 2000). In general, it was reported that non-polysaccharide-producing strains of yogurt bacteria produced high levels of acetaldehyde (37 mg/l). In contrast, ropy or viscous strains produce low levels of acetaldehyde (27.6 and 10.4 mg/l) (Ott, et al. 2000, Bongers, et al. 2004).

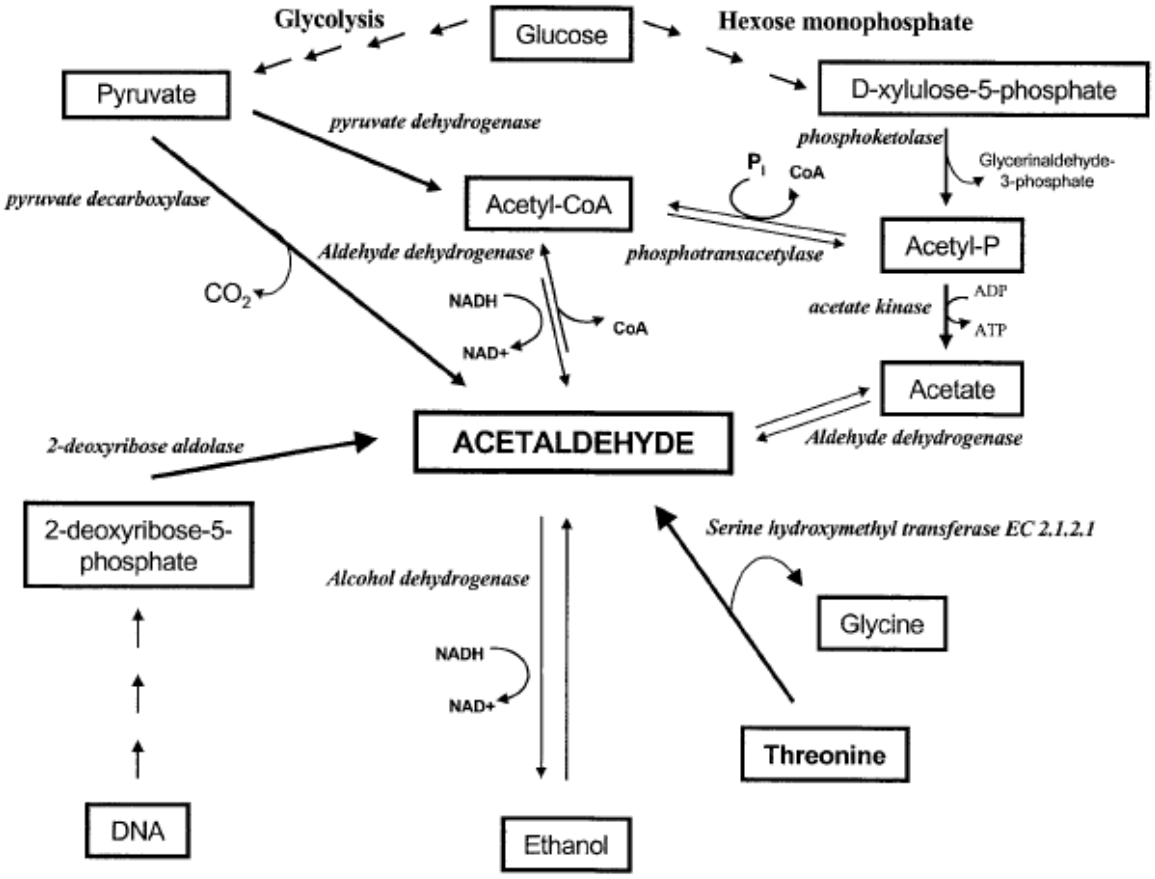


Figure 5.3. Overview of the different metabolic pathways in LAB that could lead to acetaldehyde formation. Acetyl-CoA, acetyl coenzyme A
(Source : Chaves, et al. 2002).

Özer and Atasoy (2002) had studied the acetaldehyde production ability of viscous and nonviscous yogurt starter cultures, the effects of supplementation with amino acids in viscous culture medium and also the effect of heat treatment of starter cultures. It was reported that yogurts made with nonviscous starter cultures showed significantly low acetaldehyde production (14.4 mg/l) than viscous starter culture (39.3 mg/l). However, fortification of viscous culture with threonine (22.7-31.0 mg/l), methionine (24.8-33.7 mg/l) and β-galactosidase (28.9 mg/l) led to an increase on acetaldehyde production. Heat treatment had also same effect on final product (20.7

mg/l) due to stimulating the activity of aldehyde dehydrogenase and more importantly TA.

Headspace and simultaneous distillation and extraction methods have been commonly used for the determination of yogurt flavor volatiles; however, these techniques can alter the composition of sensitive aroma compounds. Recently, a variety of low-temperature headspace procedures have been used to extract volatiles from yogurt. Using this technique, researchers detected some different volatiles in the headspace of yogurt. These odor impact chemicals are shown in Table 4.1. Therefore, acetaldehyde can be measured spectrophotometrically by using an acetaldehyde determination kit. The basis of the kit based on the acetaldehyde dehyrogenase reduction of NAD to NADH (Chaves, et al. 2002).

Gallardo-Escamilla et al. (2005) used the proton transfer reaction-mass spectrometry (PTR-MS) technique which allows analysis of volatile compounds present in a sample without the requirements for separation by gas chromatography. PTR-MS performs ionization of volatile compounds by proton transfer from hydronium ions (H_3O^+) followed by mass spectrometry. The various ions are separated in a mass analyzer due to their mass to charge ratio (m/z), and a detector counts the emerging ions. In this technique, absolute headspace concentrations can be calculated without calibration or need of standards on the basis of ion counts per second detected by the instrument and then transformed into parts per million (ppm), or parts per billion (ppb).

Table 5.1. Compounds contributing to yogurt aroma

(Source: Ott, et al.1999).

Retention index	**Compound**	**Odor descriptors**
716	Acetaldehyde	Fresh,green,plungent
757	Dimethyl sulphide	Milk,lactone-like,sulphury,warm
995	2,3-Butanedione	Butter,diacetyl,vanilla
1082	2,3-Pentanedione	Butter,vanilla,milk
1120	2-Methylthiophene	Gasoline,plastic,sytrene
1221	3-Methyl-2-butenal	Metalic,aldehydic,herbaceous
1322	1-Octen-3-one	Mushroom,earthy
1406	Dimethyl trisulphide	Sulphury,hydrogen sulphide,feacal
1424	1-Nonen-3-one	Mushroom,earthy
1462	Acetic acid	Plungent,acidic,vinegar
1479	Methional	Soup,cooked vegatables,plungent,sulphury
1551	(cis,cis)-Nonenal + 2-methyltetrahydrothiopen-3-one	Green,leather,sulphury
1680	2-Phenylacelaldehyde	Flowery
1684	3-Methylbutyric acid	Sweaty,cheese,soy sauce,flowery
1715	Unidentified	Flowery,warm,camel
1750	Unidentified	Metallic
1882	Caproic acid	Rancid.flowery
1896	Guaiacol	Bacon,phenolic,smoked,spicy
2002	Benzothiazole	Bumt,rubbery
2043	Unidentified	Hydrocarbon,chemical,bumt rubber

CHAPTER 6

SENSORY EVALUATION

6.1. Sensory Analysis

Sensory analysis involves the measurement of food characteristics such as taste, texture, appearance, odor, and after taste. Panelists are selected for their keen sense of taste, smell, and sight and are specially trained to provide consistent and accurate results. Tests are designed to obtain best answer questions about the product (Meilgaard, et al. 1999).

Lactic acid bacteria have been used to improve the preservation, sensorial characteristics and nutritional value of products like milk, meat and vegetables. A large variety of dairy products with different flavor, texture and health-promoting properties can be obtained from milk using different technologies and starter cultures. Rapid acidification, microbial preservation of milk, formation of specific flavors, texturing capacities and health benefits are the important features of the starter bacteria. During bacterial growth, fermentation of lactose causes both acidification of milk and production of antimicrobial compounds which inhibit the growth of pathogenic bacteria of possible contaminants in the fermented products. Moreover, acidification leads to neutralization of the negative charges on the milk proteins, resulting in coagulation. The acid also gives pleasant fresh and mild acid taste to fermented products, such as yogurt and cheese. Furthermore, by the conversion of sugars, organic acids, proteins or fats, typical aroma and flavor components are produced. Additionally, several lactic acid bacteria strains can contribute to improve the texture and viscosity of fermented products by the synthesis of exopolysaccharides (EPSs) (Ruas-Madiedo, et al. 2002).

The quality and the consumer acceptability of fermented dairy products like yogurt is generally determined by the sensory perception which is a complex process and influenced by many factors, such as the content of flavor compounds, texture, and appearance (Smit, et al. 2005). The composition and type of milk proteins particularly may have a great impact on the yogurt texture and on its flavor attributes (Janhoj, et al. 2006).

Attributes and definitions used for sensory profiling of set yogurts are:

- Serum: The volume of separated serum on the surface of yogurt (Amatayakul, et al. 2006).
- Ropiness (Ropiness of serum): The spoon is placed on the surface and pulled up, and the ropy property is evaluated; the longer the thread becomes before it breaks, the ropier the product (Meilgaard, et al. 1999).
- Clean cut: A spoonful of yogurt is removed, and the edges of the cut are evaluated. (absent = very rough; high = smooth) (Meilgaard, et al. 1999).
- Gel firmness: A spoonful of yogurt is placed on the untouched surface of the remaining yogurt, and a slight pressure is applied with the spoon; at high gel firmness, the yogurt breaks in flakes (resembling boiled egg white), whereas yogurt with low gel firmness is soft (Meilgaard, et al. 1999).
- Mouth thickness: Perceived as the degree of thickness when swallowing the yogurt at normal-high eating rate; high mouth thickness corresponds to a thick product that takes a long time to swallow (Meilgaard, et al. 1999).

Attributes and definitions used for sensory profiling of stirred yogurts are:

After twenty times stirring

- Shiny: The shininess is evaluated. (absent = gritty, rough; high = shiny, smooth) (Meilgaard, et al. 1999).
- Gel firmness: A spoonful of yogurt is placed on the untouched surface of the remaining yogurt, and it is observed for how long the structure is retained; high gel firmness corresponds to a long time of structural integrity (Meilgaard, et al. 1999).
- Ropiness: A spoonful of yogurt is pulled from the sample, and the ropy property is evaluated; the longer the thread becomes before it breaks, the ropier the product (Meilgaard, et al. 1999).
- Mouth thickness: Perceived as the degree of thickness when swallowing the yogurt at normal-high eating rate; high mouth thickness corresponds to a thick product that takes a long time to swallow (Meilgaard, et al. 1999).
- Creaminess: The intensity of creamy flavor (Meilgaard, et al. 1999).

Moreover, flavor and odor of yogurt have sensory attributes named as sour, sweet, salty, off-flavor, irritating, bitter, skim milk like overheated and butter (Jaworska, et al. 2005).

Overall sensory quality of the product can be evaluated using quantitative descriptive analysis (QDA) which describes all sensations perceived when evaluating a product sample. Sensory properties of each product, like appearance, odor, texture and flavor, are determined using several descriptors, which are then quantitatively measured using an appropriate scale. The multidimensional sensory image of a product is given by the resulting descriptive analysis. This method is also used for the comparison of many products as well. The results of QDA indicate sensory attributes, thus it differentiates samples from each other.

Jaworska et al. (2005) evaluated the influence of sensory and texture properties of natural yogurts on their QDA and acceptability. Their results suggested that sensory texture properties (thickness and smoothness) were not significantly correlated with consumer acceptability of natural yogurts. However, in analytical panel evaluation, smoothness (creaminess) appeared to be of more importance for overall sensory quality than thickness, as demonstrated by a higher and statistically significant correlation. Furthermore, viscosity correlated well with the sensory thickness of the yogurts tested. Negative sensory attributes (off-flavor and bitterness) were of critical importance for consumer acceptability of the product. Regardless of the texture properties, the acceptance of yogurts tested decreased strongly above the threshold value.

Janhoj et al. (2006) reported that creaminess was one of the key attributes for consumer acceptance of fermented low-fat dairy products. In stirred yogurt, both the presence of protein particles and the additional factors from the fluid milk (appearance, flavor) may be important for the creaminess. For this purpose, the researchers investigated the effect of protein content on creaminess using four different milk protein sources. It was found that the sensory perception of creaminess in low-fat yogurt was clearly dependent on protein source.

EPS-producing yogurt bacteria are used extensively for the yogurt production as an important texture modifying resource although it is not always correlated. For instance, Folkenberg et al. (2006) studied the texture profile analysis of EPS-producing bacteria compared to non-EPS-producing bacteria. They performed the sensory evaluation by a panel of four to five trained assessors. The assessors developed vocabularies including gel firmness, ropiness, creaminess and mouth thickness and focusing on texture properties by describing differences between samples during three training sessions. Finally, they reported that fermentation with EPS-producing strains resulted in yogurts with high ropiness, high mouth thickness, and high creaminess in

contrast to non-EPS-producing bacteria but low gel firmness and low spontaneous syneresis (Folkenberg, et al. 2006).

Syneresis or spontaneous whey separation on the surface of set yogurt is considered as a defect which could be reduced or eliminated by increasing the level of milk solids to < 15%. In addition to this, stabilizers like starch, gelatine and vegetable gum, or exopolysaccharide (EPS)-producing starter cultures are used as alternatives. Because of consumer awareness of natural products, the use of stabilizers is restricted in some countries (Amatayakul, et al. 2006).

CHAPTER 7

MATERIALS AND METHODS

7.1. Materials

7.1.1. Chemicals

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

7.1.2. Media

MRS and M17 media were used for activation and enumeration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* activation and enumeration, respectively. The single strains were grown in sterile milk (12% total solids and 3.5% fat) supplied from Pınar Dairy Company, Pınarbaşı, İzmir. Evaporated milk used for yogurt production (14-17% total solids) was supplied from Or-Köy Dairy Plant, Urla, İzmir.

7.1.3. Selection of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* isolates

In total 14 artisanal yogurt samples obtained from Toros and İskenderun regions of Turkey in 2005 (13 from "Toros" region and 1 from İskenderun) were used for the isolation of yogurt starter bacteria. Yogurts, which had been produced traditionally, were selected according to their organoleptic properties such as aroma, viscosity, and acidity. Total of 66 *Streptococcus thermophilus* and 72 *Lactobacillus bulgaricus* isolates were obtained. The isolates were genetically identified by Erkuş (2007). The isolates were stored at -80°C in 20% (v/v) glycerol stock solutions for further analysis. The glycerol stock solutions were prepared by mixing 0.5 ml of active cultures with 0.5 ml sterile MRS and M17 broth for *L. bulgaricus* and *S. thermophilus*, respectively, with 0.5 ml 40% glycerol in eppendorf tubes. Thus, the resulting suspension included 20% glycerol. The frozen stock cultures were prepared in triplicate and only one set was used

for the experiments for avoiding any contamination or loss of activity. The isolate codes were given in Tables 7.1 and 7.2. All isolates were tested for their yogurt producing properties which were coagulation, growth time, acid production, acetaldehyde and exopolysaccharide (EPS) formation.

Table 7.1. *Streptococcus thermophilus* isolates

29	68	95-1	TY20	TY41	TY65
38c	71	95-2	TY21	TY44	TY70
39a	74	97-1	TY23	TY45	TY71
47	77a	97-2	TY24	TY47	TY75
50	77b	TY8	TY25	TY53	TY77
52	78	TY9	TY26	TY55	TY78
60	79	TY10	TY27	TY57	TY79
62	85	TY12	TY29	TY61	TY81
65	90b	TY14	TY31	TY62	TY82
66a	94	TY15	TY32	TY63	
66b	94a	TY17	TY38	TY63-2	

Table 7.2. *Lactobacillus bulgaricus* isolates

16	48	79	TY15	TY36b	TY77a
22	49	TY5	TY16	TY40	TY77b
22b	51	TY5b	TY17	TY41	TY79
24	53	TY6	TY20	TY42	TY80
25	54	TY7	TY21	TY43	TY83
26	57	TY8	TY22a	TY45	TY85
30	62	TY9a	TY22b	TY65	TY86
30b	64	TY9b	TY23	TY68	TY87
33	69	TY10	TY24	TY69	TY88
33b	71	TY11	TY27a	TY70	TY90
34	76	TY14a	TY30	TY71	TY91
44	77	TY14b	TY34	TY73	TY92

7.2. Methods

7.2.1. Single Strain Analyses

7.2.1.1. Coagulation of Yogurt Isolates

S. thermophilus and *L. bulgaricus* were activated separately using M17 broth (pH 6.9) and MRS broth (pH 6.2), respectively. Inoculum of 1%, taken from stock cultures, was transferred to M17 or MRS broths depending on the culture and then incubated at 42 °C for 16 h. Then both bacteria, separately, were inoculated at 2% into sterile milk and incubated at 42 °C for 16 h. The isolates were selected based on their formation of coagulation (curd). The experiments were duplicated.

7.2.1.2. Acid Production of Yogurt Isolates

The ability of acid production of *S. thermophilus* was determined instead of *L. bulgaricus* since *S. thermophilus* initiates the fermentation process in yogurt manufacturing by increasing the acidity. Selected *S. thermophilus* strains (based on their coagulation formation) are initially cultured in M17 broth and incubated at 42 °C for 16 h. Then, activated cultures were inoculated into sterile milk individually at 2% and incubated at 42 °C for 7 h. The pH of the samples during 7 h of incubation was determined using a pH meter (Metrohm 744 pH meter, Switzerland) every hour. Lactic acid concentrations of the samples were also determined by following method 947.05 of the AOAC (1999) at every hour during 7 h of fermentation. *S. thermophilus* isolates having pH around 4.60-4.70 at 4-5 h of incubation were selected for further analyses.

7.2.1.3. Acetaldehyde Formation of Yogurt Isolates

The stock cultures of *S. thermophilus* and *L. bulgaricus* strains were activated as described in section 7.2.1.1. The activated cultures were inoculated at 2% into sterile milk and were incubated at 42 °C until pH 4.6. Then incubated samples were stored at 4 °C overnight. Acetaldehyde formation of *S. thermophilus* and *L. bulgaricus* strains were determined spectrophotometrically using an acetaldehyde determination kit (Boehringer

Mannheim, R-Biopharm AG, Darmstadt, Germany) based on the enzymatic (acetaldehyde dehydrogenase) reduction of NAD to $NADH^+$ (Chaves, et al. 2002).

7.2.1.4. EPS Purification and Quantification in Yogurt Isolates

The samples produced from separate yogurt isolates were prepared as described in section 7.2.1.3. The EPS purification was performed according to the method described by Goh, et al. (2005b). Chemical analysis for the determination of total carbohydrate amount was adapted from the phenol-sulphuric acid method described by Dubois et al. (1956).

7.2.2. Selection of Yogurt Isolates

The yogurt isolates that decrease pH to 4.60-4.70 up to 5 h, produce high amounts of acetaldehyde and EPS were selected. After this selection, the isolates were used to produce yogurt samples. The evaporated and pasteurized milk (%14-17 total-solids), obtained from Or-Köy Dairy Plant, and inoculated with 3% of *S. thermophilus* and *L. bulgaricus* strains in 1:1 ratio. The inoculated samples were incubated at 42 °C till the pH decreased to 4.60 value (approximately 3-4 h). Incubated yogurt samples were stored at 4 °C overnight. Then, they were tested for their organoleptic properties based on their appearance, syneresis, consistency with spoon, consistency in mouth, flavor, aroma, and overall acceptability by our research group. Consequently, yogurts having the best organoleptic properties were chosen for the further yogurt production.

7.2.3. Yogurt Analyses

Pasteurized milk, obtained from Or-Köy Dairy Plant, was inoculated with 3% of of *S. thermophilus* and *L. bulgaricus* strains in 1:1 ratio. The inoculated samples were incubated at 42 °C until pH 4.50-4.60 (approximately 3-4 h). The incubated yogurt samples were kept at 4 °C overnight. The yogurt samples were stored at 4 °C for 21 days and the following analyses were conducted. The combinations of selected isolates which were used for yogurt manufacturing are given in Table 7.3 and sample numbers were assigned for each different combination.

Table 7.3. Sample numbers given to each different combination of yogurt isolates

Combination of isolates	Sample No	Combination of isolates	Sample No
95-1 x 54	1	95-1 x TY30	11
TY24 x 54	2	TY24 x TY30	12
TY47 x 54	3	TY47 x TY30	13
TY55 x 54	4	TY55 x TY30	14
TY63-2 x 54	5	TY63-2 x TY30	15
95-1 x 25	6	95-1 x 22	16
TY24 x 25	7	TY24 x 22	17
TY47 x 25	8	TY47 x 22	18
TY55 x 25	9	TY55 x 22	19
TY63-2 x 25	10	TY63-2 x 22	20

7.2.3.1. Total Solids, Fat and Protein Contents of Milk and Yogurt Samples

Total solids of milk and yogurt samples at day 1 were measured gravimetrically (Turkish Standards Institute 1989, AOAC 1999). Fat content of milk and yogurt samples at day 1 were determined by butyrometers using the Gerber method (Turkish Standards Institute 1989). Protein content of yogurt samples at day 1 was assessed by Kjeldahl method 991.20 of the AOAC (1999) using 6.38 as the nitrogen conversion factor. The fat and protein contents of the samples were determined in duplicate and total solids were determined in triplicate.

7.2.3.2. pH and Titratable Acidity of Yogurt Samples

The pH of yogurt samples was measured using a digital pH meter (Metrohm 744, Switzerland) at days 1, 7, 14, and 21. Titratable acidity of yogurt samples was assessed in duplicate samples following method 947.05 of the AOAC (1999) at day 1. The titratable acidity was expressed as % lactic acid.

7.2.3.3. Syneresis of Yogurt Samples

Syneresis of yogurt samples at days 1, 7, 14, 21 was determined in triplicate as described by Rodarte et al. (1993). Each sample (10 ml) was centrifuged (Hettich-Universal 320R, Tuttlingen, Germany) at 5000 rpm for 20 min at 4 ºC. The clear

supernatant was decanted and measured. Syneresis was based on the volume of clear supernatant per 100 ml yogurt.

7.2.3.4. Apparent Viscosity of Yogurt Samples

Yogurts were produced in 600 ml beakers for the apparent viscosity measurements. Apparent viscosity of yogurt samples was measured at 10ºC using Brookfield DV-II-Pro Viscometer, USA with LV4 spindle at a speed of 100 rpm in circulating water bath at day 1 in triplicate. Yogurt samples were stirred for 20 s clockwise and 20 s counterclockwise. Results were recorded in centipoise.

7.2.3.5. Amount of EPS in Yogurts

Amount of EPS in yogurts were determined by the same assay used for the yogurts made by individual strains (Section 7.2.1.4). The amount of EPS in yogurt samples was determined at day 1.

7.2.3.6. Lactic Acid Bacteria Counts

The *S. thermophilus* and *L. bulgaricus* counts were determined using M17 (pH 6.9) and MRS (pH 6.3) agars, respectively, at days 1, 7, 14, and 21. The yogurt samples (1 ml) were decimally diluted in 9 ml sterile peptone water (0.1%) and 1 ml aliquot dilutions were pour plated and incubated anaerobically at 45 ºC 48 h for *L. bulgaricus* and aerobically at 42 ºC 48 h for *S. thermophilus*. Anaerobic conditions were created using AnaeroGen in plastic anaerobic jars (Oxoid). Plates having 25-250 colonies were counted and the results expressed as log colony forming units per milliliter (log cfu/ml).

7.2.3.7. NIR Spectroscopy of Yogurts

Spectra of yogurt samples were collected using a Bio-Rad Excalibur FTS 3000 NX Fourier Transform Near-Infrared Spectrometer (Bio-Rad Laboratories Europe Ltd., UK) between 4000 and 10000 cm^{-1} wave number. This spectrometer was equipped with a 250 W tungsten-halogen source, a calcium floride beam splitter and a lead selenide detector. Diffuse reflectance (DRIFT) measurement was done with 128 scan and 16

cm^{-1} resolution. All spectra were then transferred to a computer where data processing programs were installed. The GILS methods were written in MATLAB programming language using Matlab 5.3 (MathWorks Inc., Natick. MA). Yogurt samples were prepared in duplicate and three measurements were taken from each sample. The spectra of each sample were used to determine the total solids, fat, pH, syneresis, and lactic acid bacteria counts of the yogurt samples.

7.2.3.8. Analysis of Aroma Compounds

Pasteurized milk (10 ml) and 3 % starter culture were put into 20 ml glass vials (Agilent, USA) and sealed with 20-mm aluminum crimp caps (Agilent, USA) with dark gray septa (Agilent, USA) and shaken slightly to mix the content. All samples were incubated at 43 ºC for 3-4 h. The vials were kept at 4 ºC overnight and aroma profiling of the samples was determined at day 1 in duplicate. Yogurt samples were analyzed for acetaldehyde, ethanol, acetone, diacetyl and methyl ethyl ketone (MEK) using an automated headspace sampler (Agilent 7694, USA) followed by a gas chromatograph (GC) (Agilent 6890N, USA). The GC was equipped with a mass spectrometry (MS) detector (Agilent 5973Nms, USA) to identify and quantify these volatile compounds.

The stock standard solution was prepared with 20 ppm acetaldehyde (Fluka, Spain), 5 ppm ethanol (Merck, Germany), 1 ppm acetone (Merck, Germany), 5 ppm diacetyl (Merck, Germany) and 0.5 ppm MEK (Merck, Germany) in deionized water. Five calibration points were chosen and standard solutions were prepared which contain 1 µl, 2 µl, 4 µl, 6 µl, and 10 µl stock standard solution. Calibration curve was calculated by least-square regression from these five points and the R^2 values for the linearized calibration curves were 0.999.

In the static headspace method, yogurt samples were placed in a headspace vial and an aliquot of the closed airspace above the water phase was sampled directly to the gas chromatographic column with split injection. The samples were heated and shaken for 60 min in the headspace sampler to achieve volatilization of volatile compounds present in yogurt. The operating conditions for the headspace sampler and the GC/MS system are shown in Table 7.4. The column was temperature programmed to facilitate the separation of compounds which were then detected with the mass spectrometer.

Identification of the volatile compounds eluting from the GC column was accomplished by comparing their measured mass spectra and retention times to

reference spectra and retention times in a database (ChemStation, Agilent). Selective ion monitoring (SIM) program was employed to increase instrument sensitivity which is essential for yogurt samples since the concentrations are in the low µg/l range. As a result, impurities which may come from baseline were eliminated. Ions per compound were chosen for data acquisition, as presented in Table 7.5 along with the retention time for each compound.

Extrapolation of the calibration straight line to a zero area of the target peak gave the concentration of volatile compounds present in yogurt samples. In order to determine the amount of volatile compounds, the calibration straight line to a zero area of the target peak was extrapolated as it gave the concentration of volatile compounds present in the samples.

Table 7.4. GC-MS conditions

Instrument / Condition	**Description**
Headspace Autosampler	
Oven temperature	60 ºC
Loop temperature	160 ºC
Transferline temperature	190 ºC
GC Cycle time	70 min
Vial Equilibration time	60 min
Pressurizing time	0.15 min
Loop fill time	0.30 min
Loop Equilibration time	0.30 min
Inject time	0.30 min
Shake speed	high
Inlet	
Inlet	200 ºC
Splitless	
Constant flow	
Flow rate	1 ml/min
Oven	
35 ºC 6 min hold	23.50 min 300 ºC 4 min hold (post run)
10 ºC/min 200 ºC 1 min hold	

Table 7.5. Target volatile compound ions in SIM program

Compound	Retention Time (min)	Ion	Dwell (msec)
Acetaldehyde	0.00	41, 43	20
Ethanol, Acetone	1.58	31, 42, 43, 46, 58	20
Diacetyl, MEK	2.30	42, 43, 45, 60	20

7.2.3.9. Sensory Evaluation of Yogurts

The yogurt samples were evaluated at day 1 by a 10-member trained sensory panel. Panelists were chosen among the graduate students and faculty in the Food Engineering Department at IYTE (8 females, 2 males aged 23-32) and they were frequent yogurt consumers. Panelists were trained twice in a group discussion with reference samples having good and bad quality attributes. Verbal descriptors which is given in Table 7.6, from the Yogurt Standard (TS1330) of the Turkish Standards Institute (1989) had been discussed during training. Yogurt samples at day 1 were coded and presented to panelists at 8 °C in 200 ml commercial yogurt containers under typical daylight room conditions. Panelists independently evaluated each sample for appearance, consistency on the spoon, consistency in the mouth, odor, flavor, and overall acceptability using a descriptive hedonic scale of 1-5 (with 5 being the highest attribute score) as presented in Table 7.6. Samples were randomly ordered at the beginning of the panel and each panelist received the samples in the same order. Total of 8 samples were presented to the panelists in one session. Two replicate evaluation sessions were conducted.

7.2.3.10. Data Analysis

Two replications were conducted for all analyses and the sensory evaluation. Results of the total solids, fat, and protein contents, pH, titratable acidity, syneresis, apparent viscosity, EPS, lactic acid bacteria counts, aroma compounds and sensory scores were analyzed by one-way analysis of variance using MINITAB® release 13 (Minitab Inc., State College, USA) and Duncan significance test. Significance was accepted at $p<0.05$.

Table 7.6. Descriptive hedonic scale used for sensory evaluation of yogurt samples

Sensory Attributes	Descriptions of the attributes	Scores
Appearance	Glossy, having milk color, no whey(liquid) separation occurred, no cracks and gas bubbles, clean appearance, homogeneous	5
	Having milk color, no whey separation, no cracks and gas bubbles	4
	Dull, few cracks, some whey separation occurred, clean appearance	3
	Not having milk color, many cracks and gas bubbles, whey separation occurred, dirty appearance	1-2
Consistency on spoon	Thick on spoon, having normal texture, homogeneous, thick after stirring, no immediate whey separation occurred	5
	Thick on spoon, having normal texture, thick after stirring, some whey separation occurred	4
	Not too fluid on spoon, fluid after stirring, immediate whey separation occurred	3
	Free running from spoon, too fluid after stirring, immediate and much whey separation occurred	1-2
Consistency in mouth	Having very good texture, homogeneous	5
	Having good texture, homogeneous	4
	Fluid in the mouth, not having good texture	3
	Too fluid while squeezing one spoonful of the sample between tongue and palate	1-2
Odor	Having specific (normal) yogurt odor	4-5
	Not having normal yogurt odor	3
	Not having normal yogurt odor and having foreign odor	1-2
Flavor	Normal yogurt flavor, some sourness	4-5
	Sour, some bitterness, having foreign flavor	3
	Too sour, bitter, having foreign flavor	1-2
Overall Acceptability	Excellent	5
	Good	4
	Neither good or bad	3
	Bad	2
	Very Bad	1

CHAPTER 8

RESULTS AND DISCUSSION

8.1. Single Strain Analyses Results

8.1.1. Coagulation of Yogurt Isolates

Activated cultures were inoculated into 10 ml sterile milk and incubated for 12-16 h. Out of 57 *S. thermophilus* isolates in which coagulation was observed , 25 of them formed curd after 16 h of incubation and 7 *S. thermophilus* isolates did not form any curd. Coagulation occurred in all of the *L. bulgaricus* isolates. Curd formation of *S. thermophilus* isolates and *L. bulgaricus* isolates are given in Table 8.1 and Table 8.2.

Table 8.1. Curd formation of *S. thermophilus* isolates

29	+	68	-	95-1	++	TY20	+	TY41	++	TY65	+
38c	+	71	+	95-2	++	TY21	+	TY44	++	TY70	++
39a	+	74	+	97-1	+	TY23	+	TY45	++	TY72	++
47	-	77a	+	97-2	+	TY24	++	TY47	++	TY75	++
50	++	77b	+	TY8	++	TY25	++	TY53	++	TY77	+
52	++	78	-	TY9	++	TY26	+	TY55	++	TY78	+
60	-	79	++	TY10	++	TY27	++	TY57	++	TY79	-
62	+	85	-	TY12	+	TY29	++	TY61	++	TY81	-
65	++	90b	+	TY14	+	TY31	++	TY62	+	TY82	++
66a	++	94	++	TY15	+	TY32	+	TY63	+		
66b	++	94a	+	TY17	++	TY38	++	TY63-2	++		

(-) no curd
(++) curd formed in 12-16 h
(+) curd formed after 12-16 h

Table 8.2. Curd formation of *L. bulgaricus* isolates

16	++	48	+	79	++	TY15	+	TY36b	+	TY77a	++
22	++	49	+	TY5	++	TY16	+	TY40	+	TY77b	++
22b	++	51	++	TY5b	++	TY17	++	TY41	+	TY79	++
24	++	53	+	TY6	++	TY20	++	TY42	+	TY80	++
25	++	54	++	TY7	++	TY21	++	TY43	+	TY83	++
26	++	57	++	TY8	++	TY22a	++	TY45	+	TY85	+
30	++	62	+	TY9a	++	TY22b	++	TY65	+	TY86	+
30b	++	64	+	TY9b	++	TY23	++	TY68	++	TY87	+
33	++	69	+	TY10	++	TY24	++	TY69	++	TY88	+
33b	++	71	+	TY11	++	TY27a	++	TY70	++	TY90	++
34	++	76	+	TY14a	++	TY30	++	TY71	++	TY91	++
44	++	77	+	TY14b	++	TY34	+	TY73	+	TY92	++

(-) no curd
(++) curd formed in 12-16 h
(+) curd formed after 12-16 h

8.1.2. Acid Production of Yogurt Isolates

S. thermophilus isolates in which coagulation occurred were examined for acid production versus time. The most important criteria in order to choose a coccus strain for yogurt production for further studies was the acid production ability of *S. thermophilus* strains in 4-5 h. Out of 57, 38 of *S. thermophilus* strains decreased pH below 4.60. Among these, 18 isolates decreased pH to 4.60-4.70 in 4-5 h. These strains were selected for the organoleptic analyses. The acid production abilities of 18 *S. thermophilus* strains are given in Table 8.3.

Table 8.3. The acid production of *S. thermophilus* strains

Isolate number	Incubation Time						
	1 h	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**7 h**
95-1	6.34	5.83	4.96	4.68	4.48	4.29	4.20
TY24	6.27	5.76	4.92	4.61	4.41	4.23	4.14
TY47	6.29	5.85	4.98	4.68	4.49	4.24	4.17
TY55	6.33	5.80	4.96	4.68	4.47	4.26	4.15
TY63-2	6.39	5.89	5.10	4.74	4.50	4.28	4.18
50	6.36	6.21	5.41	4.85	4.64	4.44	4.28
52	6.40	6.18	5.28	4.81	4.62	4.45	4.33
66a	6.41	6.24	5.58	5.19	4.92	4.73	4.55
66b	6.36	6.15	5.40	4.92	4.69	4.53	4.42
77b	6.42	6.28	5.74	5.13	4.88	4.76	4.63
85	6.39	6.22	5.39	4.96	4.70	4.44	4.29
90b	6.32	5.88	5.08	4.75	4.51	4.31	4.22
94a	6.44	6.27	5.91	5.34	4.86	4.59	4.48
95-2	6.21	5.50	4.83	4.64	4.47	4.31	4.20
TY8	6.21	5.31	4.84	4.62	4.48	4.33	4.21
TY9	6.35	5.82	5.10	4.69	4.55	4.43	4.37
TY10	6.49	6.11	5.27	4.70	4.51	4.35	4.31
TY17	6.45	5.91	5.20	4.62	4.45	4.34	4.31
TY25	6.52	6.14	5.31	4.74	4.53	4.38	4.30
TY29	6.54	6.32	5.49	4.83	4.59	4.44	4.32
TY38	6.54	6.44	6.27	5.47	4.95	4.65	4.56
TY41	6.48	6.20	5.57	4.86	4.47	4.40	4.35
65	6.47	6.35	5.81	4.99	4.50	4.40	4.33
TY44	6.34	5.95	4.92	4.50	4.30	4.24	4.19
TY45	6.37	6.09	5.09	4.63	4.39	4.31	4.27
TY53	6.32	5.93	5.32	4.72	4.50	4.40	4.35
74	6.45	6.33	6.21	5.89	5.50	4.98	4.63
79	6.48	6.30	5.88	5.22	4.76	4.55	4.33
94	6.48	6.21	5.60	5.16	4.91	4.78	4.58
97	6.43	6.17	6.00	5.72	5.33	4.92	4.67
TY61	6.45	6.16	5.73	5.11	4.76	4.57	4.40
TY31	6.43	6.26	5.72	5.33	4.80	4.61	4.41
TY27	6.46	5.92	4.99	4.67	4.46	4.32	4.19
TY57	6.45	6.19	5.61	5.14	4.77	4.52	4.38
TY70	6.45	6.06	5.21	4.74	4.50	4.25	4.14
TY75	6.44	6.06	5.51	4.89	4.60	4.40	4.29
TY72	6.44	6.30	5.52	4.90	4.64	4.40	4.29
TY82	6.43	6.12	5.41	4.84	4.53	4.37	4.28

8.1.3. Acetaldehyde Production of Yogurt Isolates

L. bulgaricus isolates were examined for their acetaldehyde production. The level of acetaldehyde produced by 55 of *L. bulgaricus* strains were ranged in between 1.2-31.5 mg/l. On the other hand, 17 of *L. bulgaricus* strains and all *S. thermophilus* strains did not produce acetaldehyde in a remarkable level. Ayhan et al. (2005) reported that the domestic *S. thermophilus* and *L. bulgaricus* strains, which they studied, produced 25 to 55 mg/l and 27.28 to 47.74 mg/l acetaldehyde, respectively. Only 7 of *L. bulgaricus* strains which had the highest acetaldehyde production were chosen for the organoleptic analyses. The acetaldehyde production of *L. bulgaricus* strains are given in Table 8.4.

Table 8.4. The acetaldehyde production of *L. bulgaricus* strains

Isolate number	Acetaldehyde (mg/l)	Isolate number	Acetaldehyde (mg/l)	Isolate number	Acetaldehyde (mg/l)
16	22.80	**64**	6.50	**TY24**	28.10
22	26.17	**71**	1.50	**TY27a**	5.20
22b	24.10	**76**	5.20	**TY30**	26.80
24	24.10	**77**	16.56	**TY40**	3.00
25	26.95	**79**	20.80	**TY41**	6.00
26	20.60	**TY5b**	18.80	**TY68**	5.60
30	23.70	**TY7**	5.10	**TY69**	8.20
30b	21.40	**TY8**	4.00	**TY70**	8.60
33	22.20	**TY9a**	3.50	**TY71**	6.50
33b	23.50	**TY11**	8.50	**TY77a**	31.50
34	20.20	**TY14a**	10.40	**TY77b**	27.70
44	24.50	**TY14b**	17.30	**TY79**	4.20
48	14.40	**TY16**	16.20	**TY80**	14.50
49	6.80	**TY17**	5.40	**TY90**	16.10
53	23.60	**TY20**	15.00	**TY91**	6.50
54	30.80	**TY21**	1.20	**TY92**	8.00
57	16.00	**TY22a**	11.20	**TY43**	22.50
62	2.40	**TY22b**	5.30	**TY9b**	13.00

8.1.4. Amount of EPS in Yogurt Isolates

The EPS amounts of the yogurt isolates were determined and are given in Tables 8.5 and 8.6. The EPS production by lactobacilli strains during growth in milk was ranged from 2.56 to 94.45 mg/l, while streptococci strains' was between 3.40 and 45.32 mg/l. Aslım et al. (2006) reported that EPS production by lactobacilli strains and

streptococci strains during growth in milk were 36 to 314 mg/l and 24 to 140 mg/l, respectively. The researchers indicated that the EPS production by lactobacilli strains was lower than streptococci strains and similar results were obtained by Frengova et al. (2000). They reported that the polymer forming of thermophilic streptococci was lower than that of the lactobacilli and the quantities of EPS produced by different strains varied considerably. In fact, our findings were contradicting with the results of other researchers since in this study EPS produced by streptococci strains was lower than the EPS produced by lactobacilli strains. Even though the EPS amounts were determined; mainly acid production and acetaldehyde formation of the isolates were taken into account for choosing the isolates.

Table 8.5. Amount of EPS produced by *L. bulgaricus* isolates

Isolate Number	EPS (µg/ml)	Isolate Number	EPS (µg/ml)	Isolate Number	EPS (µg/ml)
16	37.75	**79**	7.26	**TY36b**	4.68
22	11.80	**TY5**	8.25	**TY40**	9.52
22b	8.77	**TY5b**	15.23	**TY41**	6.72
24	11.45	**TY6**	18.61	**TY42**	16.48
25	6.78	**TY7**	12.45	**TY43**	5.33
26	6.25	**TY8**	13.57	**TY45**	17.38
30	12.11	**TY9a**	18.23	**TY65**	14.99
30b	9.08	**TY9b**	5.46	**TY68**	10.99
33	2.56	**TY10**	6.21	**TY69**	12.57
33b	7.09	**TY11**	16.48	**TY70**	56.82
34	31.66	**TY14a**	11.19	**TY71**	72.59
44	4.21	**TY14b**	16.29	**TY73**	44.39
48	5.07	**TY15**	26.43	**TY77a**	3.40
49	11.13	**TY16**	16.07	**TY77b**	3.30
51	12.48	**TY17**	28.64	**TY79**	38.26
53	34.16	**TY20**	10.96	**TY80**	31.25
54	61.25	**TY21**	19.33	**TY83**	26.79
57	11.47	**TY22a**	14.23	**TY85**	11.64
62	11.39	**TY22b**	10.96	**TY86**	6.42
64	11.47	**TY23**	6.81	**TY87**	11.46
69	15.21	**TY24**	26.35	**TY88**	7.29
71	14.25	**TY27a**	5.38	**TY90**	14.11
76	13.75	**TY30**	94.45	**TY91**	7.59
77	4.75	**TY34**	31.47	**TY92**	11.99

Table 8.6. Amount of EPS produced by *S. thermophilus* isolates

Isolate Number	EPS (µg/ml)	Isolate Number	EPS (µg/ml)	Isolate Number	EPS (µg/ml)
29	11.99	**97-1**	23.48	**TY41**	5.07
38c	20.24	**97-2**	30.99	**TY44**	16.07
39a	12.11	**TY8**	26.43	**TY45**	2.56
50	11.29	**TY9**	9.49	**TY47**	4.21
52	75.26	**TY10**	3.40	**TY53**	9.08
62	31.22	**TY12**	4.15	**TY55**	17.07
65	37.75	**TY14**	4.43	**TY57**	14.23
66a	11.78	**TY15**	17.68	**TY61**	14.73
66b	8.17	**TY17**	10.19	**TY62**	9.55
71	17.26	**TY20**	16.28	**TY63**	4.41
74	6.24	**TY21**	9.29	**TY63-2**	21.36
77a	20.44	**TY23**	9.34	**TY65**	9.52
77b	6.42	**TY24**	37.51	**TY70**	5.53
79	14.11	**TY25**	7.87	**TY72**	7.84
90b	7.85	**TY26**	25.48	**TY75**	14.48
94	8.17	**TY27**	35.46	**TY77**	3.76
94a	9.22	**TY29**	9.13	**TY78**	16.94
95-1	5.08	**TY31**	45.32	**TY82**	19.70
95-2	22.62	**TY38**	31.58		

8.2. Selection of Yogurt Isolates

Combination of 7 *L. bulgaricus* and 18 *S. thermophilus* strains were used for the yogurt production and they were evaluated by our research group based on their organoleptic properties. The yogurt samples were evaluated based on their appearance, syneresis, consistency with spoon, consistency in mouth, flavor, aroma, and overall acceptability (data not shown). The results showed that yogurts made by combination of 4 *L. bulgaricus* and 5 *S. thermophilus* strains could be chosen for further studies.

8.3. Yogurt Analyses Results

8.3.1. Total Solids, Fat, and Protein Contents of Yogurt Samples

Total solids content of yogurt samples varied between 14.50% and 17.64%. This variation occurred due to the total solids content of pasteurized milk (14-17%) since no

skim milk or WPI was added to increase the total solids content. Total solids of yogurt are shown in Table 8.7.

Fat contents of yogurts were varied between 2.40% and 3.35% as given in Table 8.7. No additional cream was added during standardization of milk to increase its fat content. Since these lactic acid bacteria utilized for yogurt production were artisanal cultures, other bacteria coming from any other ingredients (cream, skim milk powder, etc.) were avoided. The reason for this variation could also be due to differences of milk composition in different seasons of the year.

Protein contents of yogurts given in Table 8.7 were ranged from 3.81% to 4.39%. Whereas Ayar and Akın (2001) determined the higher protein content of yogurt ranged between 4.68 and 6.02 in their research while our findings were found to be lower than theirs. In the Turkish Food Codex (2001), protein content of yogurt must be at least 4%. The results showed that samples 14, 16, 17, 18, 19 and 20 had acceptable amount of protein.

Table 8.7. Total solids, fat and protein contents of yogurts

Yogurt Sample	Total Solids (%) (w/w)	Fat (%) (w/w)	Protein (%) (w/w)
Sample 1	15.38 ± 0.18^{ef}	2.50 ± 0.10^{i}	3.87 ± 0.01^{bcdef}
Sample 2	15.16 ± 0.18^{efg}	2.40 ± 0.00^{j}	3.70 ± 0.20^{def}
Sample 3	15.03 ± 0.03^{efg}	2.45 ± 0.00^{j}	3.88 ± 0.00^{bcdef}
Sample 4	15.01 ± 0.06^{efg}	3.60 ± 0.00^{bc}	3.61 ± 0.05^{ef}
Sample 5	14.94 ± 0.15^{efg}	3.50 ± 0.00^{bcd}	3.81 ± 0.11^{bcdef}
Sample 6	16.53 ± 0.21^{cd}	3.35 ± 0.10^{bcde}	3.89 ± 0.01^{bcdef}
Sample 7	16.74 ± 0.27^{bc}	3.15 ± 0.10^{defg}	3.56 ± 0.02^{f}
Sample 8	16.81 ± 0.15^{abc}	3.20 ± 0.00^{cdef}	3.87 ± 0.02^{bcdef}
Sample 9	16.48 ± 0.16^{cd}	3.40 ± 0.00^{bcde}	3.82 ± 0.03^{bcdef}
Sample 10	16.59 ± 0.28^{cd}	2.75 ± 0.10^{ghij}	3.89 ± 0.05^{bcdef}
Sample 11	17.49 ± 0.26^{ab}	4.20 ± 0.00^{a}	3.79 ± 0.03^{cdef}
Sample 12	17.33 ± 0.35^{abc}	4.15 ± 0.10^{a}	3.99 ± 0.00^{bcd}
Sample 13	16.79 ± 0.43^{abc}	4.10 ± 0.00^{a}	3.95 ± 0.03^{bcde}
Sample 14	14.56 ± 0.58^{fg}	2.90 ± 0.00^{fghi}	4.05 ± 0.03^{abcd}
Sample 15	15.78 ± 0.29^{ed}	4.35 ± 0.10^{a}	3.96 ± 0.02^{bcde}
Sample 16	14.50 ± 0.09^{g}	2.80 ± 0.00^{fghij}	4.15 ± 0.06^{ab}
Sample 17	17.64 ± 0.14^{aj}	2.70 ± 0.00^{hij}	4.39 ± 0.39^{a}
Sample 18	17.60 ± 0.41^{ab}	2.65 ± 0.10^{hij}	4.35 ± 0.33^{a}
Sample 19	14.89 ± 0.71^{fg}	3.00 ± 0.00^{efgh}	4.39 ± 0.36^{a}
Sample 20	15.31 ± 0.74^{efg}	3.65 ± 0.10^{b}	4.14 ± 0.06^{ab}

[a-j] Column means having a different letter or letters differ ($P<0.05$).
Means ± SD of triplicate samples for total solids analyses and of duplicate samples for fat and protein content analysis

8.3.2. pH and Titratable Acidity of Yogurt Samples

When the initial pH of milk (pH 6.59) decreased to 4.60-4.50, fermentation was ended. The fermentation time to reach pH 4.50-4.60 for yogurts was 3-4 h which was varied with the starter cultures. The pH decrease during 21 days of storage is shown in Figure 8.1. The pH values for all the samples are given in Table 8.8. There was a

significant decrease of pH over time for each sample. The highest pH value obtained at day 1 was 4.56 (sample 18) and it decreased to pH 4.26 at day 21. The highest pH decrease was observed in Sample 13 (from pH 4.50 to pH 4.14).

The pH and titratable acidity (% lactic acid) were also measured for the samples used for the sensory analyses at day 1 as given in Table 8.9. The pH values ranged from 4.52 (samples 1 and 11) to 4.32 (samples 9 and 19). There was a significant difference among the samples 1, 5, 9, 11, 15, and 19 at day 1. The titratable acidity of yogurt samples varied between 8.64% and 9.05% and samples 1, 5, 17, 18, 19, and 20 were significantly different than sample 9 ($P<0.05$). The pH decrease could be responsible for the variability of titratable acidity. In addition, acid production is directly related to lactose metabolism by yogurt starters and amino acids (Özer and Atasoy 2002).

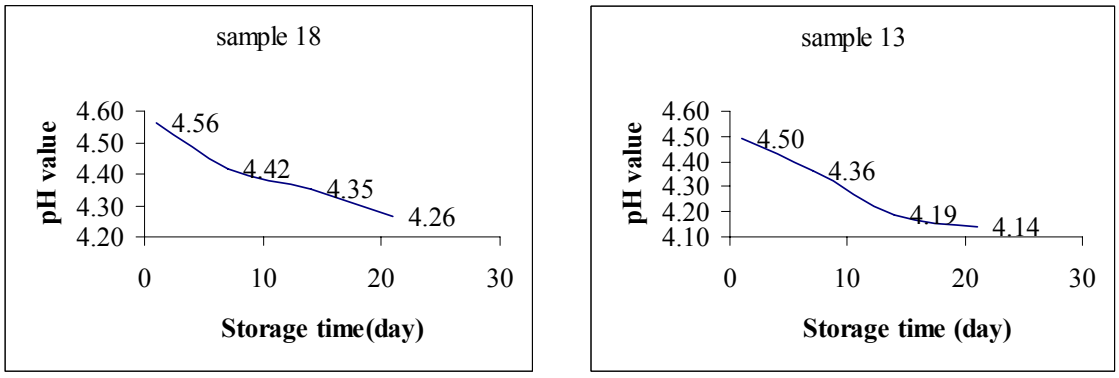


Figure 8.1. The pH decrease in samples 13 and 18 during 21 days of storage

Table 8.8. The pH values of yogurt samples during 21 days of storage

Yogurt Sample	Time (days)	pH	Yogurt Sample	Time (days)	pH
Sample 1	1	4.51 ±0.02 [a]	**Sample 11**	1	4.42 ±0.01 [a]
	7	4.38 ±0.04 [b]		7	4.30 ±0.00 [b]
	14	4.29 ±0.08 [c]		14	4.27 ±0.00 [c]
	21	4.29 ±0.00 [c]		21	4.28 ±0.00 [d]
Sample 2	1	4.48 ±0.01 [a]	**Sample 12**	1	4.45 ±0.01 [a]
	7	4.38 ±0.09[ab]		7	4.36 ±0.01 [b]
	14	4.33 ±0.07[bc]		14	4.25 ±0.00 [c]
	21	4.26 ±0.02 [c]		21	4.31 ±0.01 [d]
Sample 3	1	4.45 ±0.05 [a]	**Sample 13**	1	4.50 ±0.00 [a]
	7	4.36 ±0.03 [b]		7	4.36 ±0.02 [b]
	14	4.30 ±0.03 [b]		14	4.19 ±0.01 [c]
	21	4.34 ±0.08 [b]		21	4.14 ±0.02 [d]
Sample 4	1	4.42 ±0.01 [a]	**Sample 14**	1	4.37 ±0.05 [a]
	7	4.36 ±0.03 [b]		7	4.31 ±0.08[ab]
	14	4.25 ±0.01 [c]		14	4.20 ±0.10[bc]
	21	4.19 ±0.01 [d]		21	4.17 ±0.07 [c]
Sample 5	1	4.48 ±0.02 [a]	**Sample 15**	1	4.54 ±0.02 [a]
	7	4.40 ±0.00 [b]		7	4.43 ±0.00 [b]
	14	4.30 ±0.02 [c]		14	4.29 ±0.01 [c]
	21	4.20 ±0.02 [d]		21	4.26 ±0.00 [d]
Sample 6	1	4.30 ±0.01 [a]	**Sample 16**	1	4.47 ±0.07 [a]
	7	4.19 ±0.02 [b]		7	4.31 ±0.06 [b]
	14	4.20 ±0.00 [b]		14	4.21 ±0.05[bc]
	21	4.21 ±0.01 [b]		21	4.17 ±0.09 [c]
Sample 7	1	4.48 ±0.00 [a]	**Sample 17**	1	4.50 ±0.11 [a]
	7	4.31 ±0.00 [b]		7	4.36 ±0.05 [b]
	14	4.27 ±0.00 [c]		14	4.28 ±0.10 [b]
	21	4.19 ±0.01 [d]		21	4.23 ±0.02 [b]
Sample 8	1	4.47 ±0.00 [a]	**Sample 18**	1	4.56 ±0.02 [a]
	7	4.29 ±0.05 [b]		7	4.42 ±0.08 [b]
	14	4.23 ±0.01 [c]		14	4.35 ±0.10[bc]
	21	4.30 ±0.01 [d]		21	4.26 ±0.05 [c]
Sample 9	1	4.31 ±0.00 [a]	**Sample 19**	1	4.54 ±0.07 [a]
	7	4.19 ±0.01 [b]		7	4.31 ±0.03 [b]
	14	4.20 ±0.02 [c]		14	4.26 ±0.01[bc]
	21	4.31 ±0.01 [d]		21	4.23 ±0.05 [c]
Sample 10	1	4.46 ±0.00 [a]	**Sample 20**	1	4.52 ±0.00 [a]
	7	4.40 ±0.00 [b]		7	4.41 ±0.01 [b]
	14	4.31 ±0.01 [c]		14	4.31 ±0.02 [c]
	21	4.27 ±0.01 [d]		21	4.23 ±0.01 [d]

[a-d] Column means within each sample having a different letter or letters differ ($P<0.05$). Means ± SD of duplicate samples

Table 8.9. The pH values and titratable acidity of yogurt samples at day 1

Yogurt Sample	pH	Titratable acidity (% lactic acid)	Yogurt Sample	pH	Titratable acidity (% lactic acid)
Sample 1	4.52±0.02 [a]	9.05 ± 0.03 [a]	**Sample 11**	4.52 ± 0.02 [a]	8.90 ± 0.08[ab]
Sample 2	4.45±0.04[ab]	8.91 ± 0.08[ab]	**Sample 12**	4.45 ± 0.04[ab]	8.90 ± 0.05[ab]
Sample 3	4.44±0.06[ab]	8.89 ± 0.05[ab]	**Sample 13**	4.44 ±0.06[ab]	8.83 ± 0.01[ab]
Sample 4	4.42±0.02[ab]	8.85 ± 0.02[ab]	**Sample 14**	4.42 ± 0.02[ab]	8.89 ± 0.01[ab]
Sample 5	4.48±0.01 [a]	8.97 ± 0.05 [a]	**Sample 15**	4.48 ± 0.01 [a]	8.90 ± 0.03[ab]
Sample 6	4.41±0.02[ab]	8.83 ± 0.01[ab]	**Sample 16**	4.41 ± 0.02[ab]	8.82 ± 0.02[ab]
Sample 7	4.40±0.01[ab]	8.81 ± 0.03[ab]	**Sample 17**	4.40 ± 0.01[ab]	8.95 ± 0.02 [a]
Sample 8	4.39±0.01[ab]	8.79 ± 0.01[ab]	**Sample 18**	4.39 ± 0.01[ab]	8.97 ± 0. 01 [a]
Sample 9	4.32±0.07 [b]	8.64 ± 0.03 [b]	**Sample 19**	4.32 ± 0.07 [b]	8.93 ± 0.05 [a]
Sample 10	4.40±0.04[ab]	8.81 ± 0.05[ab]	**Sample 20**	4.40 ± 0.04[ab]	9.02 ± 0.00 [a]

[a-b] Column means having a different letter or letters differ (P<0.05).
Means ± SD of duplicate samples

8.3.3. Syneresis of Yogurt Samples

Syneresis of yogurt samples at days 1, 7, 14, and 21 are given in Table 8.10 and ranged between 42.0% and 55.8%. Syneresis was decreased in nearly all yogurt samples during 21 days of storage. This could be as a result of metabolic activity of yogurt starter cultures and decrease in net pressure in the protein matrix which causes decrease of syneresis (Güler-Akın and Akın 2007). The highest syneresis decrease was observed in sample 20 and the lowest one was occurred in samples 2 and 3. The average syneresis decrease was about 4-7 %. No significant difference was observed among the samples 1, 2, 3, 9, 14, 17, and 18 during 21 days of storage (P<0.05).

Table 8.10. Syneresis of yogurt samples

Yogurt Sample	Time	Syneresis (%) (v/v)	Yogurt Sample	Time	Syneresis (%) (v/v)
Sample 1	1	55.8±0.20	**Sample 11**	1	45.4±0.10[b]
	7	55.8±0.38		7	49.6±0.10[a]
	14	53.3±0.41		14	44.6±0.10[b]
	21	53.3±0.41		21	42.5±0.00[c]
Sample 2	1	55.0±0.00	**Sample 12**	1	45.0± 0.00[b]
	7	55.4±0.25		7	47.9±0.25[a]
	14	55.4±0.37		14	44.6±0.10[b]
	21	56.7±0.20		21	42.9±0.10[b]
Sample 3	1	56.7±0.41	**Sample 13**	1	50.0±0.00[a]
	7	57.1±0.33		7	47.9±0.10[b]
	14	52.9±0.25		14	45.0±0.00[c]
	21	55.0±0.00		21	45.0±0.00[c]
Sample 4	1	62.1±0.10[a]	**Sample 14**	1	57.5±0.27
	7	53.8±0.21[b]		7	55.0±0.00
	14	52.9±0.10[b]		14	54.2±0.13
	21	53.3±0.13[b]		21	55.0±0.55
Sample 5	1	60.0±0.00[a]	**Sample 15**	1	50.4±0.10[a]
	7	56.3±0.14[b]		7	50.0±0.00[b]
	14	54.2±0.13[c]		14	45.4±0.10[b]
	21	55.0±0.00[bc]		21	43.8±0.14[c]
Sample 6	1	50.0±0.00[a]	**Sample 16**	1	57.1±0.10[a]
	7	50.0±0.00[a]		7	54.9±0.14[b]
	14	50.4±0.10[a]		14	54.2±0.13[b]
	21	45.4±0.10[b]		21	53.8±0.14[b]
Sample 7	1	50.0± 0.00[a]	**Sample 17**	1	55.8±0.38
	7	50.0±0.00[a]		7	51.7±0.38
	14	50.0±0.00[a]		14	55.0±0.00
	21	47.9±0.10[b]		21	51.3±0.41
Sample 8	1	50.4±0.10[a]	**Sample 18**	1	54.2±0.47
	7	51.3±0.14[a]		7	52.5±0.22
	14	50.0±0.00[a]		14	53.8±0.41
	21	47.9± 0.10[b]		21	50.4±0.43
Sample 9	1	51.3±0.14	**Sample 19**	1	56.3±0.14[a]
	7	50.4±0.10		7	52.0±0.35[b]
	14	50.8±0.26		14	55.0±0.00[ab]
	21	49.2±0.13		21	54.2±0.13[ab]
Sample 10	1	55.0±0.00[a]	**Sample 20**	1	61.7±0.13[a]
	7	51.7±0.20[b]		7	54.6±0.10[bc]
	14	50.4±0.10[b]		14	56.7±0.13[b]
	21	50.0±0.00[b]		21	52.9±0.25[c]

[a-b] Column means within each sample having a different letter or letters differ ($P<0.05$). Means ± SD of triplicate samples

8.3.4. Apparent Viscosity of Yogurt Samples

The apparent viscosities of yogurt samples are shown in Table 8.11. The apparent viscosity measurements of yogurt samples ranged between 725.85 and 1321.75 cp. The highest apparent viscosity was obtained for sample 2 which was 1321.75±64.70 cp, while the lowest viscosity was obtained for the Sample 7 which was 725.85±26.15 cp. The results indicated that there was no correlation between the EPS production and viscosity. The highest apparent viscosity values did not correspond to the highest EPS yield. This result was also confirmed with the findings of Bouzar et al. (1996).

Table 8.11. Apparent viscosities of yogurt samples

Yogurt Sample	Viscosity (cp)	Yogurt Sample	Viscosity (cp)
Sample 1	1115.76±192.78^{cde}	**Sample 11***	1061.77±72.98^{de}
Sample 2*	1321.75±64.70^{a}	**Sample 12**	1276.73±64.21^{ab}
Sample 3	1161.75±62.04^{bcd}	**Sample 13**	1209.24±94.60^{abc}
Sample 4	1176.75±62.04^{abcd}	**Sample 14**	1099.77±54.94^{cde}
Sample 5	1112.76±94.60^{cde}	**Sample 15**	1205.74±32.19^{abcd}
Sample 6*	1003.79±86.71^{e}	**Sample 16***	1061.77±163.07^{de}
Sample 7*	725.85±26.15^{f}	**Sample 17**	1157.75±118.10^{bcd}
Sample 8*	843.82±93.77^{f}	**Sample 18**	1133.76±54.06^{bcde}
Sample 9*	986.79±123.0^{e}	**Sample 19***	1201.74±50.31^{abcd}
Sample 10*	829.82±71.57^{f}	**Sample 20**	1191.75±88.30^{abcd}

$^{a-f}$ Column means having a different letter or letters differ (P<0.05).
Means ± SD of triplicate samples
* indicates only one replication

8.3.5. Amount of EPS in Yogurts

The amounts of EPS in yogurt samples are given in Table 8.12. The highest EPS content of yogurt was found in sample 17 which was 62.63±3.18 μg/ml. The EPS contents of yogurts were significantly different from each other. The reason for this difference could be due to the varying EPS producing abilities of these cultures. In addition, total solids and protein contents as well as fermentation conditions could affect EPS yield. On the other hand, EPS production in yogurt samples was higher than EPS

production in yogurt isolates which could be due to stimulated growth of mix culture although it has not well established (Shihata and Shah 2002). Frengova et al. (2000) reported the maximum concentration of 720-860 mg EPS/l after full coagulation of milk.

Table 8.12. Amount of EPS in yogurts

Yogurt Sample	EPS (µg/ml)	Yogurt Sample	EPS (µg/ml)
Sample 1	41.35±5.29^{bcde}	**Sample 11**	25.01±2.72^{ef}
Sample 2	32.56±5.05^{def}	**Sample 12**	21.19±1.53^{f}
Sample 3	45.31±2.01^{bcd}	**Sample 13**	26.76±4.44^{ef}
Sample 4	39.34±3.22^{cde}	**Sample 14**	21.73±2.90^{f}
Sample 5	40.98±1.59^{bcde}	**Sample 15**	20.65±0.14^{f}
Sample 6	62.36±7.30^{a}	**Sample 16**	40.00±5.63^{cde}
Sample 7	54.60±3.60^{abc}	**Sample 17**	62.63±3.18^{a}
Sample 8	57.12±5.95^{ab}	**Sample 18**	56.66±6.31^{ab}
Sample 9	55.31±0.95^{abc}	**Sample 19**	39.87±3.64^{cde}
Sample 10	52.06±5.51^{abc}	**Sample 20**	46.22±1.13^{abcd}

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

8.3.6. Lactic Acid Bacteria Counts

Viable lactic acid bacteria counts of yogurt samples during storage are shown in Table 8.13. *S. thermophilus* counts were higher in almost all samples due to the stimulated growth of *Streptococcus* species. Both *L. bulgaricus* and *S. thermophilus* counts were decreased about 1 log cycle during storage. Similar results were reported by Birollo et al. (2000), Güler-Akın and Akın (2007). Figure 8.2 represents *L. bulgaricus, S. thermophilus,* and total lactic acid bacteria counts during 21 days of storage for sample 9.

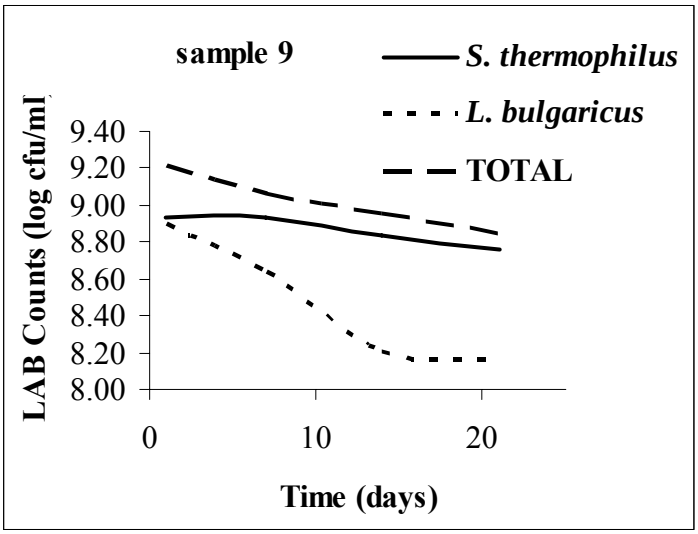


Figure 8.2. The lactic acid bacteria counts for sample 9 during 21 days of storage

Table 8.13. Viable lactic acid bacteria counts of yogurt samples during 21 days of storage

Yogurt Sample	Time	*S. thermophilus* (log cfu/ml)	*L. bulgaricus* (log cfu/ml)	Total LAB (log cfu/ml)	Yogurt Sample	Time	*S. thermophilus* (log cfu/ml)	*L. bulgaricus* (log cfu/ml)	Total LAB (log cfu/ml)
	1	9.23±0.07	8.29±0.15	9.28±0.07		1	9.13±0.01^{a}	9.00±0.08^{a}	9.38±0.03^{a}
Sample	7	9.27±0.16	8.31±0.09	9.32±0.14	**Sample**	7	8.93±0.03^{ab}	8.94±0.03^{a}	9.25±0.01^{b}
1	14	9.23±0.15	8.12±0.13	9.26±0.13	**6**	14	8.47±0.02^{b}	8.70±0.06^{b}	8.90±0.03^{c}
	21	9.38±0.22	8.12±0.23	9.40±0.22		21	8.54±0.59^{ab}	8.47±0.04^{c}	8.61±0.05^{d}
	1	9.01±0.11	8.34±0.42^{a}	9.07±0.09		1	9.06±0.02^{a}	8.91±0.05^{a}	9.30±0.02^{a}
Sample	7	9.15±0.06	8.01±0.32^{ab}	9.18±0.08	**Sample**	7	8.99±0.08^{b}	8.56±0.10^{b}	9.13±0.03^{b}
2	14	9.09±0.04	7.87±0.41^{ab}	9.13±0.05	**7**	14	8.88±0.12^{bc}	8.41±0.05^{c}	9.01±0.08^{c}
	21	9.02±0.16	7.06±0.64^{b}	9.03±0.16		21	8.78±0.02^{c}	8.51±0.07^{bc}	8.97±0.01^{c}
	1	8.99±0.01	8.28±0.14^{ab}	9.07±0.01		1	8.85±0.02^{a}	8.89±0.02^{a}	9.17±0.02^{a}
Sample	7	8.97±0.09	8.31±0.09^{a}	9.06±0.06	**Sample**	7	8.84±0.01^{a}	8.80±0.02^{b}	9.12±0.02^{a}
3	14	9.00±0.05	8.21±0.19^{ab}	9.07±0.06	**8**	14	8.72±0.10^{b}	8.60±0.03^{c}	8.97±0.06^{b}
	21	8.93±0.04	8.02±0.11^{b}	8.99±0.03		21	8.55±0.04^{c}	8.17±0.05^{d}	8.70±0.03^{c}
	1	9.27±0.01^{a}	8.94±0.05^{a}	9.44±0.01^{a}		1	8.94±0.01^{a}	8.90±0.08^{a}	9.22±0.04^{a}
Sample	7	9.13±0.02^{b}	8.86±0.03^{a}	9.31±0.02^{b}	**Sample**	7	8.93±0.08^{a}	8.64±0.11^{b}	9.07±0.08^{b}
4	14	8.92±0.02^{c}	8.67±0.05^{b}	9.11±0.02^{c}	**9**	14	8.83±0.04^{ab}	8.21±0.19^{c}	8.95±0.04^{bc}
	21	8.60±0.04^{d}	8.20±0.05^{c}	8.75±0.03^{d}		21	8.76±0.10^{b}	8.16±0.08^{c}	8.85±0.08^{c}
	1	9.08±0.04	8.91±0.03^{a}	9.30±0.01^{a}		1	8.79±0.05^{a}	9.09±0.04^{a}	9.27±0.01^{a}
Sample	7	8.96±0.02	8.64±0.09^{a}	9.13±0.04^{a}	**Sample**	7	8.61±0.08^{b}	8.68±0.51^{ab}	8.83±0.06^{b}
5	14	8.78±0.04	8.20±0.15^{b}	8.88±0.05^{b}	**10**	14	8.62±0.09^{b}	8.28±0.03^{bc}	8.79±0.06^{b}
	21	8.45±0.60	7.77±0.10^{c}	8.34±0.18^{c}		21	8.44±0.08^{c}	8.10±0.11^{c}	8.60±0.08^{c}

$^{a-d}$ Column means having a different letter or letters differ (P<0.05). **(cont. on next page)**

Means ± SD of triplicate samples

Table 8.13. (cont.) Viable lactic acid bacteria counts of yogurt samples during 21 days of storage

Yogurt Sample	Time	*S. thermophilus* (log cfu/ml)	*L. bulgaricus* (log cfu/ml)	Total LAB (log cfu/ml)	Yogurt Sample	Time	*S. thermophilus* (log cfu/ml)	*L. bulgaricus* (log cfu/ml)	Total LAB (log cfu/ml)
	1	9.19±0.02	8.98±0.04^{ab}	9.40±0.02^{a}		1	9.32±0.27	8.87±0.14^{a}	9.45±0.23^{a}
Sample	7	9.12±0.01	8.91±0.02^{ab}	9.33±0.01^{b}	**Sample**	7	9.18±0.13	8.54±0.36^{ab}	9.28±0.17^{ab}
11	14	9.22±0.51	9.04±0.49^{a}	9.19±0.01^{c}	**16**	14	9.15±0.07	8.78±0.51^{ab}	9.36±0.12^{ab}
	21	8.80±0.07	8.49±0.06^{b}	8.98±0.04^{d}		21	9.03±0.12	8.10±0.10^{b}	9.08±0.11^{b}
	1	9.18±0.03^{a}	8.82±0.06^{a}	9.34±0.03^{a}		1	9.06±0.24	8.90±0.02^{a}	9.30±0.15^{a}
Sample	7	8.94±0.02^{b}	8.68±0.04^{b}	9.13±0.01^{b}	**Sample**	7	8.92±0.11	8.19±0.08^{bc}	9.00±0.09^{b}
12	14	8.86±0.11^{bc}	8.48±0.06^{c}	9.01±0.06^{c}	**17**	14	9.11±0.07	8.29±0.03^{b}	9.17±0.06^{ab}
	21	8.73±0.04^{c}	8.06±0.07^{d}	8.81±0.03^{d}		21	9.07±0.03	7.90±0.29^{c}	9.10±0.04^{b}
	1	9.09±0.09^{a}	8.82±0.01^{a}	9.28±0.05^{a}		1	8.81±0.06	8.68±0.13	9.06±0.06
Sample	7	8.97±0.05^{a}	8.62±0.06^{a}	9.13±0.02^{b}	**Sample**	7	8.81±0.03	8.37±0.35	8.97±0.09
13	14	8.83±0.03^{b}	8.35±0.04^{a}	8.95±0.03^{c}	**18**	14	8.77±0.17	8.35±0.24	8.94±0.05
	21	8.65±0.06^{c}	7.76±0.51^{b}	8.74±0.05^{d}		21	8.78±0.24	8.36±0.06	8.93±0.16
	1	9.24±0.03^{a}	8.55±0.08^{a}	9.32±0.02^{a}		1	9.03±0.13^{a}	8.76±0.09	9.22±0.11^{a}
Sample	7	9.07±0.11^{b}	7.99±0.44^{ab}	9.13±0.07^{b}	**Sample**	7	8.95±0.12^{ab}	7.93±0.62	8.98±0.12^{ab}
14	14	9.05±0.05^{b}	7.60±0.00^{b}	9.06±0.05^{b}	**19**	14	9.02±0.03^{ab}	8.15±0.26	9.08±0.06^{a}
	21	9.00±0.08^{b}	7.68±0.35^{b}	9.03±0.06^{b}		21	8.73±0.23^{b}	8.10±0.41	8.79±0.20^{b}
	1	9.04±0.02^{a}	9.04±0.53^{a}	9.23±0.01^{a}		1	9.23±0.03^{a}	8.97±0.01^{a}	9.42±0.03^{a}
Sample	7	8.96±0.03^{b}	8.65±0.01^{a}	9.13±0.02^{b}	**Sample**	7	9.08±0.01^{b}	8.87±0.01^{b}	9.29±0.01^{b}
15	14	8.89±0.02^{c}	8.52±0.05^{ab}	9.04±0.02^{c}	**20**	14	8.52±0.05^{c}	8.72±0.05^{c}	8.93±0.05^{c}
	21	8.79±0.03^{d}	8.04±0.07^{b}	8.86±0.03^{d}		21	8.30±0.08^{d}	8.30±0.04^{d}	8.60±0.05^{d}

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

8.3.7. NIR Spectroscopy of Yogurts

The NIR spectra of total solids, fat, pH, syneresis, and microbial counts between 1000 nm and 2500 nm are shown in Figure 8.3. The reference data were randomly divided into two data sets: a calibration subset containing two-thirds of all data and a validation subset containing the remaining data (one-third). Hence, 30 of 38 samples were used to build calibration set and the remaining 8 samples were reserved for prediction set to test the performance of the models. Because of the random nature of the GILS method, the program was set to run 30 times with 20 genes and 50 iterations.

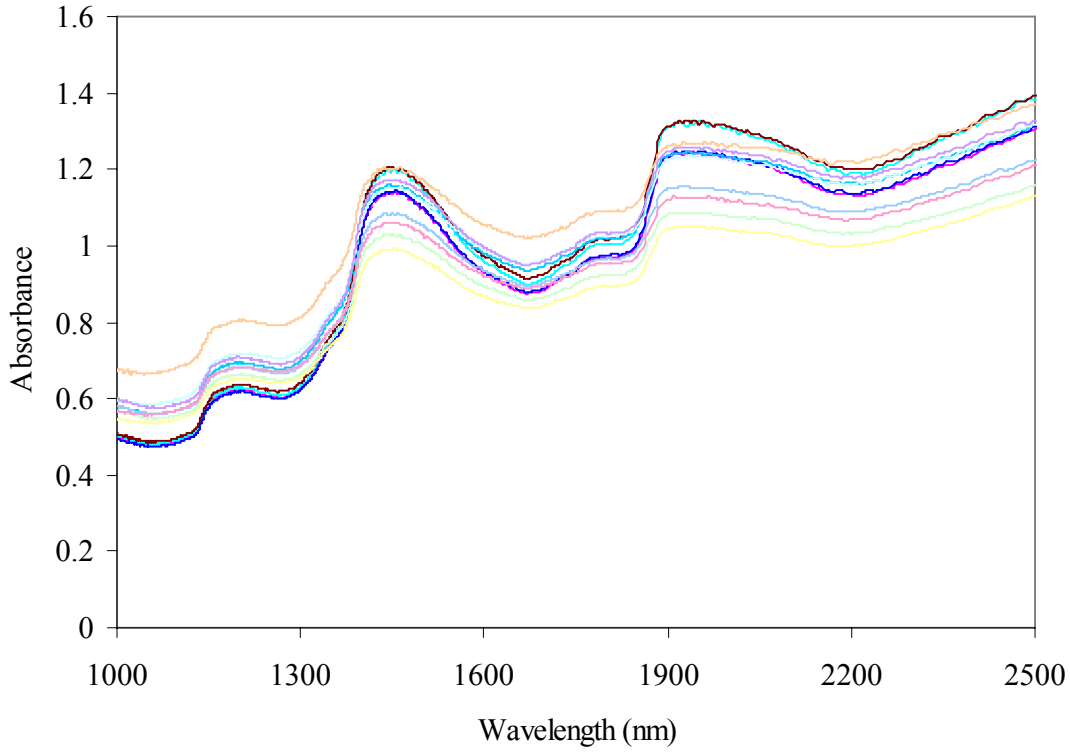


Figure 8.3. The NIR spectra of yogurt samples between 1000 and 2500 nm

The R^2 value of actual versus predicted actual graphs of total solids, fat, pH, syneresis, and microbial counts were 0.9499, 0.9954, 0.9282, 0.9747, and 0.9247, respectively, and are given in Figures 8.4 - 8.8. These meant that analyses results were calculated with least error by program. Standard error of calibration (SEC) and standard error of prediction (SEP) results are shown in Tables 8.14 and 8.15. The results of NIR

spectroscopy showed that NIR spectroscopy analysis was applicable on yogurt analysis as well.

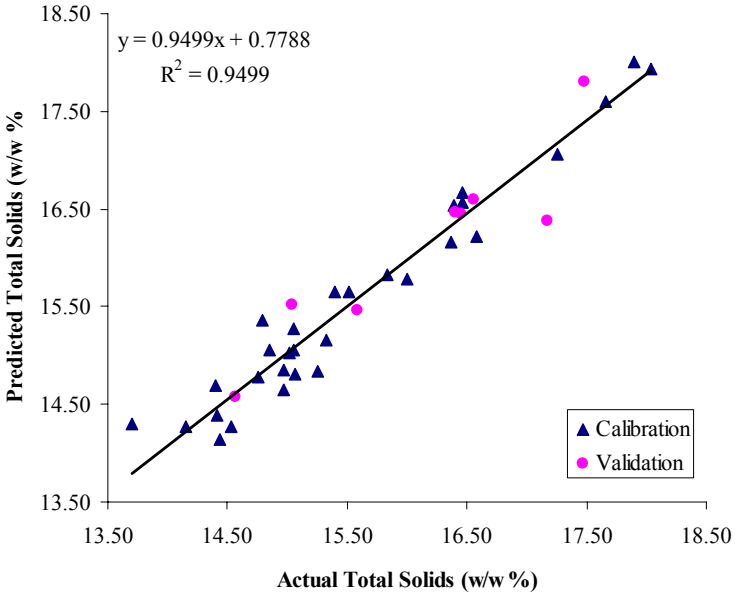


Figure 8.4. Actual versus genetic inverse least squares (GILS)-predicted total solids of yogurt samples

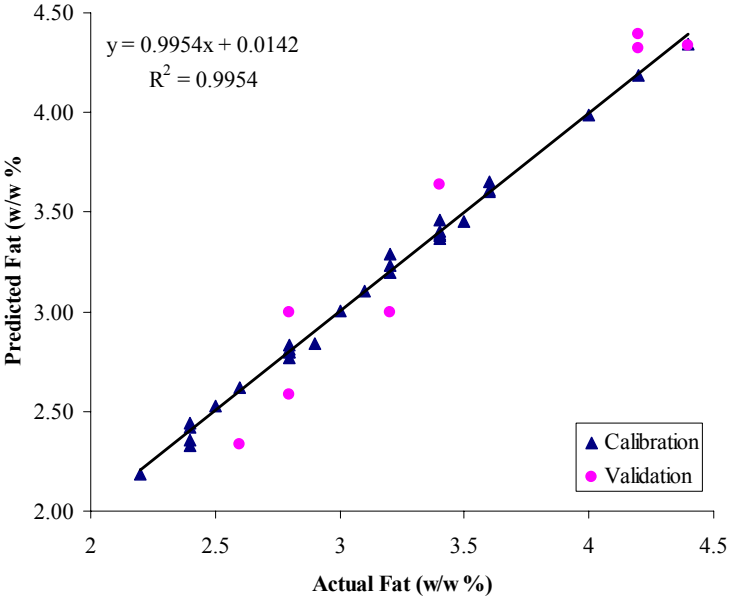


Figure 8.5. Actual versus genetic inverse least squares (GILS)-predicted fat contents of yogurt samples

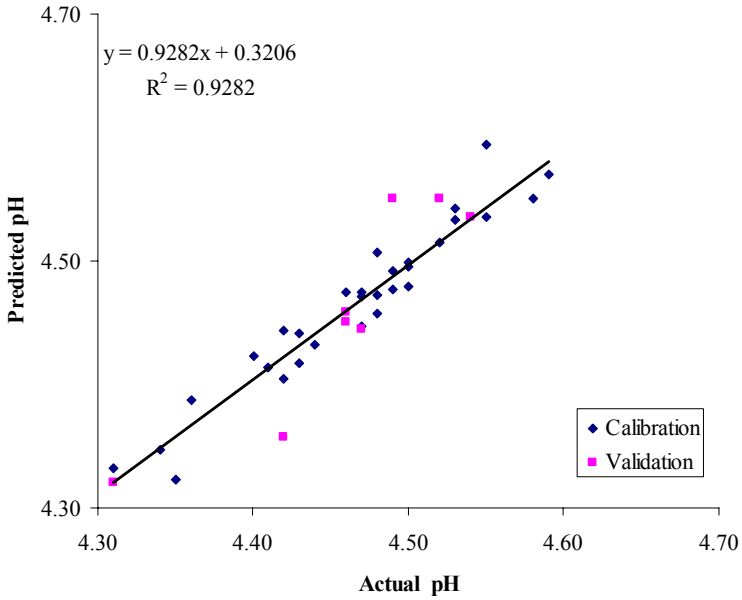


Figure 8.6. Actual versus genetic inverse least squares (GILS)-predicted pH values of yogurt samples

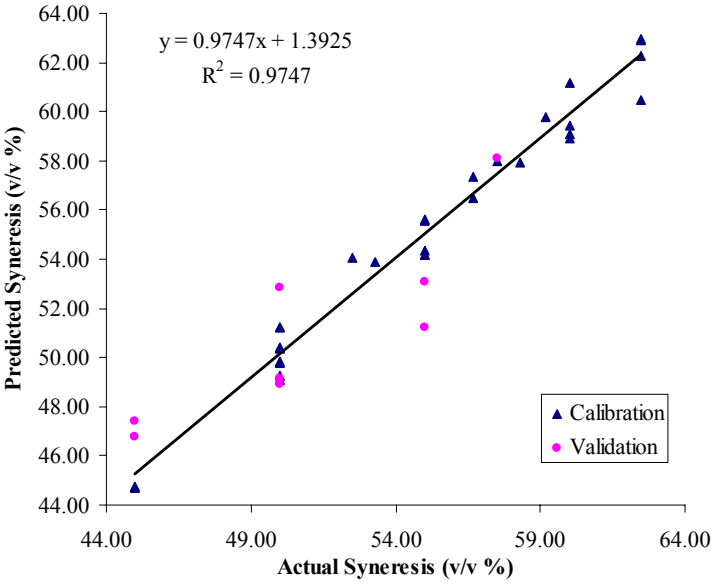


Figure 8.7. Actual versus genetic inverse least squares (GILS)-predicted syneresis of yogurt samples

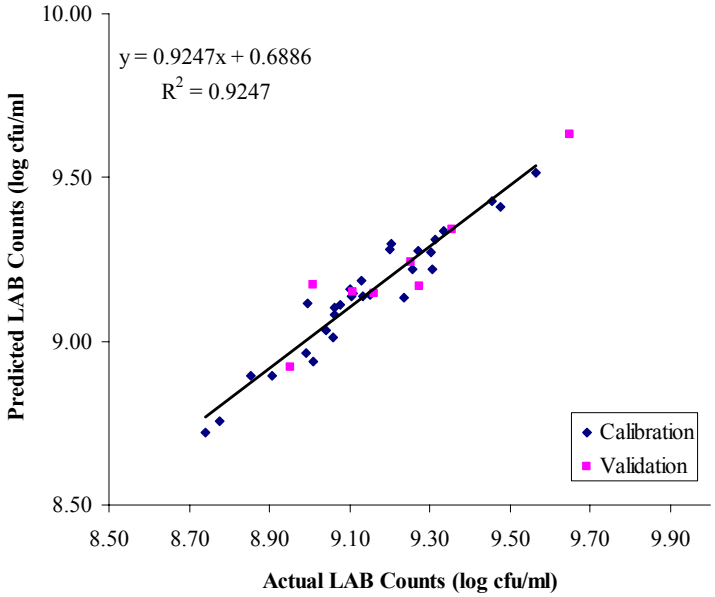


Figure 8.8. Actual versus genetic inverse least squares (GILS)-predicted lactic acid bacteria counts of yogurt samples

Table 8.14. Results of calibration sets for total solids, fat, pH, syneresis, and microbial counts determined previously with standart error of calibration (SEC)

Sample no	Calibration set									
	Total Solids (%) (w/w)		**Fat (%) (w/w)**		**pH**		**Syneresis (%) (w/w)**		**Microbial Counts (log cfu/ml)**	
	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	14.44	14.14	2.8	2.77	4.43	4.44	5.67	5.65	9.26	9.22
2	14.42	14.38	2.8	2.81	4.58	4.55	5.92	5.98	9.48	9.41
3	14.53	14.27	2.8	2.80	4.55	4.59	5.83	5.79	9.10	9.16
4	15.40	15.65	3.1	3.11	4.49	4.48	5.50	5.55	9.13	9.14
5	14.40	14.69	2.9	2.84	4.59	4.57	5.75	5.80	9.31	9.31
6	15.51	15.66	2.8	2.80	4.50	4.50	5.50	5.56	9.06	9.08
7	15.25	14.84	2.2	2.18	4.53	4.53	5.67	5.74	9.27	9.28
8	15.32	15.16	2.4	2.44	4.47	4.45	5.50	5.41	8.99	8.96
9	15.06	15.27	2.4	2.36	4.48	4.46	5.33	5.39	9.08	9.11
10	15.01	15.03	2.5	2.53	4.42	4.40	6.00	5.89	9.06	9.10
11	14.98	14.65	2.6	2.62	4.40	4.42	5.50	5.42	9.30	9.27
12	14.15	14.27	3.2	3.23	4.34	4.35	6.00	5.91	9.33	9.34
13	14.80	15.36	3.6	3.61	4.53	4.54	6.25	6.04	8.85	8.89
14	15.06	14.81	3.5	3.45	4.42	4.44	6.25	6.23	9.04	9.03
15	15.05	15.06	3.4	3.38	4.47	4.47	6.00	5.95	8.78	8.76
16	17.66	17.60	4.2	4.19	4.43	4.42	4.50	4.48	9.57	9.51
17	17.26	17.06	4	3.99	4.44	4.43	4.50	4.47	9.20	9.30
18	14.75	14.77	2.4	2.42	4.50	4.50	5.00	5.04	9.20	9.28
19	17.90	18.01	2.4	2.32	4.50	4.48	5.00	5.04	9.31	9.22
20	15.99	15.78	4.4	4.34	4.55	4.54	5.00	5.12	8.91	8.89
21	16.46	16.57	3.4	3.37	4.36	4.39	5.00	4.98	9.15	9.14
22	16.58	16.21	3.4	3.39	4.35	4.32	5.00	4.98	9.46	9.43
23	13.70	14.30	3.2	3.20	4.49	4.49	5.00	5.12	9.24	9.13
24	18.04	17.94	3.2	3.29	4.47	4.47	5.00	4.93	9.00	9.12
25	16.37	16.16	3.4	3.46	4.31	4.33	5.25	5.41	9.06	9.01
26	16.46	16.67	2.8	2.83	4.46	4.48	5.50	5.44	9.11	9.15
27	15.83	15.83	3.6	3.60	4.52	4.51	6.25	6.29	9.11	9.14
28	14.98	14.86	3.4	3.41	4.41	4.41	6.25	6.29	9.13	9.18
29	14.85	15.06	3.6	3.65	4.48	4.51	6.00	6.12	8.74	8.72
30	16.39	16.53	3	3.01	4.48	4.47	5.00	4.91	9.01	8.94
SEC		**0.26**		**0.04**		**0.02**		**0.08**		**0.05**

Pred.: Prediction

Table 8.15. Predicted total solids, fat, pH, syneresis, and microbial counts in the prediction set with standart error of prediction (SEP)

Sample no	Prediction set									
	Total Solids (%) (w/w)		Fat (%) (w/w)		pH		Syneresis (%) (w/w)		Microbial Counts (log cfu/ml)	
	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	14.56	14.57	2.8	2.6	4.52	4.55	5.75	5.81	9.65	9.63
2	15.04	15.51	2.8	3.0	4.49	4.55	5.50	5.30	9.25	9.24
3	17.48	17.80	4.2	4.4	4.42	4.36	4.50	4.74	9.11	9.15
4	17.18	16.38	4.2	4.3	4.46	4.46	4.50	4.68	9.16	9.15
5	15.58	15.47	4.4	4.3	4.54	4.54	5.00	5.28	8.95	8.92
6	16.56	16.59	3.2	3.0	4.47	4.45	5.00	4.89	9.36	9.34
7	16.45	16.45	2.6	2.3	4.46	4.45	5.50	5.12	9.01	9.17
8	16.40	16.46	3.4	3.6	4.31	4.32	5.00	4.91	9.28	9.17
SEP		**0.35**		**0.20**		**0.03**		**0.22**		**0.07**

Pred.: Prediction

8.3.8. Analysis of Aroma Compounds

The chromatogram of volatile compounds, acetaldehyde, ethanol, acetone, diacetyl, and methyl ethyl ketone (MEK) is given in Figure 8.8. The amounts these volatile compounds differed widely among yogurts produced with 20 different culture combinations**,** as given in Table 8.16. The acetaldehyde, ethanol, acetone, diacetyl, and MEK contents of the yogurt samples were varied between 13.442-25.44 mg/l, 1.488-7.022 mg/l, 0.280-0.541 mg/l, 0.425-1.789 mg/l, and 0.041-0.107 mg/l, respectively. The acetaldehyde was considered as the most prominent compound for the typical yogurt aroma (Gadrini, et al. 1999). Acetaldehyde was normally occurring in between 17 and 41 mg/l (Abrahamsen, et al. 1978, Tamime and Deeth 1980). Among 20 different yogurts, 11 of them showed acetaldehyde production over 20 mg/l and sample 20 had the highest acetaldehyde content (25.444 mg/l). Diacetyl was produced in large amounts by sample 16. Sample 10 showed the highest production of ethanol (7.022 mg/l) and acetone (0.541 mg/l). MEK was detected in only 7 samples with very low levels. The results also showed that sample 10 had the best aroma profile because of having nearly the highest levels of all volatile compounds.

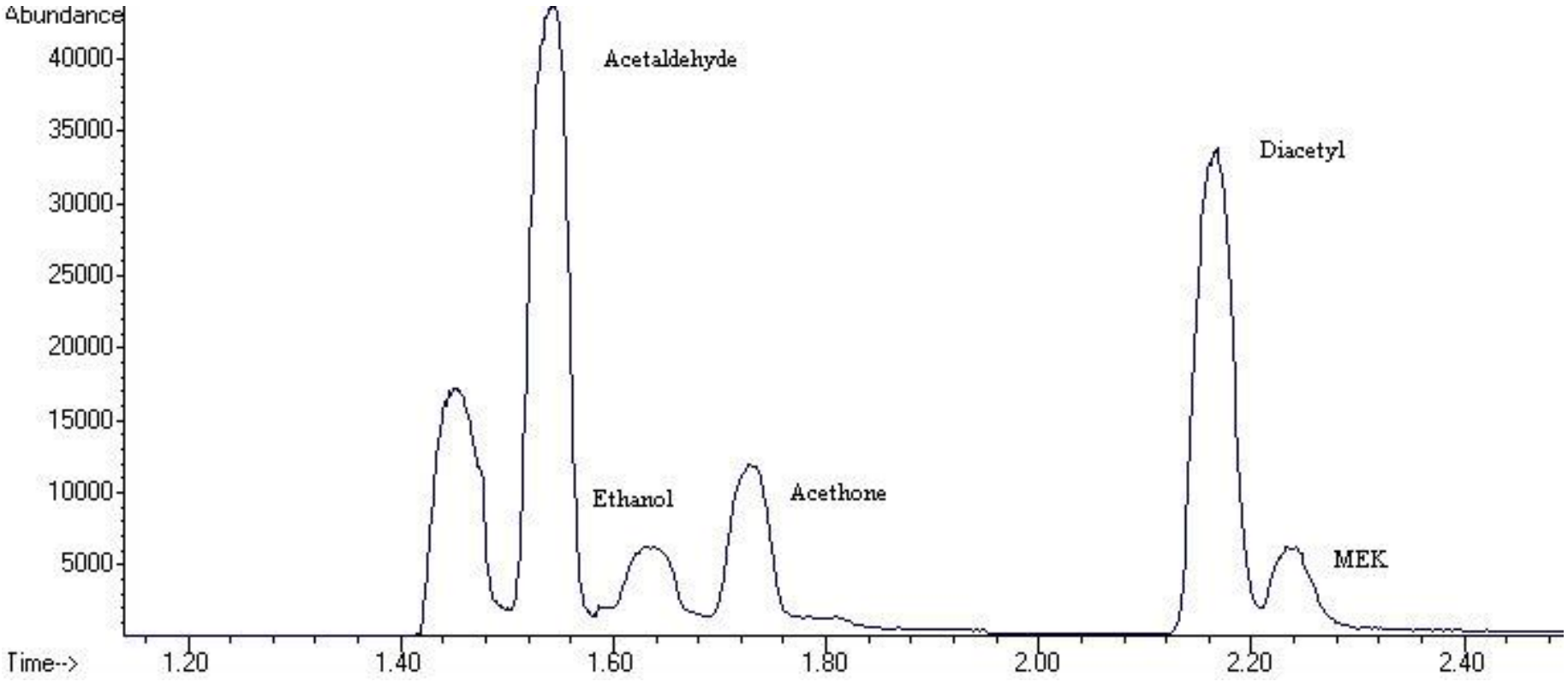


Figure 8.9. The chromatogram of volatile compounds in yogurt sample (acetaldehyde, ethanol, acetone, diacetyl, and methyl ethyl ketone (MEK)

Table 8.16. Contents of the volatile compounds in the yogurt samples

Yogurt Sample	Acetaldehyde (mg/l)	Ethanol (mg/l)	Acetone (mg/l)	Diacetyl (mg/l)	MEK (mg/l)
Sample 1	19.959±0.52^{abcd}	4.126±0.29^{cde}	0.557±0.02^{ab}	1.186±0.10^{abcd}	-
Sample 2	17.561±1.15^{abcd}	1.866±0.08^{ghı}	0.424±0.04^{ab}	1.306±0.02^{abc}	0.095±0.01^{b}
Sample 3	17.212±0.01^{abcd}	4.218±0.56^{cde}	0.497±0.01^{ab}	0.780±0.01^{bcde}	-
Sample 4	15.886±0.22^{abcd}	4.380±0.44^{dc}	0.495±0.01^{ab}	0.971±0.03^{abcde}	0.107±0.01^{b}
Sample 5	24.213±0.23^{ab}	2.050±0.09^{ghı}	0.489±0.03^{ab}	0.343±0.00^{e}	-
Sample 6	18.337±0.14^{abcd}	6.067±0.09^{ab}	0.394±0.01^{b}	1.394±0.03^{ab}	-
Sample 7	18.831±0.33^{abcd}	3.382±1.08^{defg}	0.418±0.07^{ab}	1.364±0.27^{ab}	0.061±0.01^{b}
Sample 8	21.715±2.84^{abc}	2.480±0.06^{fgh}	0.464±0.00^{ab}	0.856±0.05^{abcde}	-
Sample 9	22.894±1.34^{abc}	1.713±0.11^{ghı}	0.425±0.01^{ab}	0.425±0.02^{e}	-
Sample10	23.395±1.58^{ab}	7.022±0.99^{a}	0.541±0.04^{ab}	1.221±0.02^{abcd}	-
Sample11	23.698±0.01^{ab}	5.585±0.03^{abc}	0.511±0.01^{ab}	0.797±0.01^{bcde}	-
Sample12	15.800±0.86^{de}	2.637±0.05^{hı}	0.383±0.03^{a}	1.597±0.02^{abcde}	0.089±0.00^{ab}
Sample13	23.405±1.20^{e}	3.745±0.35^{ı}	0.499±0.02^{ab}	0.651±0.04^{bcde}	-
Sample14	22.707±4.62^{a}	4.374±0.27^{dc}	0.538±0.09^{ab}	0.571±0.08^{de}	0.070±0.04^{b}
Sample15	24.933±1.03^{bcde}	2.040±0.23^{hı}	0.441±0.02^{ab}	0.642±0.04^{cde}	-
Sample16	13.442±2.69^{cde}	3.789±0.26^{cdef}	0.389±0.07^{b}	1.789±0.032^{a}	-
Sample17	13.692±1.67^{bcde}	2.673±0.97^{efgh}	0.280±0.01^{b}	1.067±0.07^{abcde}	0.064±0.01^{b}
Sample18	23.495±0.12^{ab}	2.046±0.11^{ghı}	0.483±0.00^{ab}	0.684±0.05^{bcde}	-
Sample19	20.736±1.00^{abc}	1.488±0.09^{hı}	0.426±0.02^{ab}	0.732±0.05^{bcde}	0.041±0.01^{b}
Sample20	25.444±0.59^{a}	2.717±0.09^{efgh}	0.472±0.01^{ab}	1.176±0.01^{abcd}	-

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

8.3.9. Sensory Evaluation of Yogurts

The sensory scores given for each yogurt sample are shown in Table 8.17. The Yogurt Standard of the Turkish Standards Institute (1989) stated that the total score of a yogurt evaluated by panelists was required to be at least 20 based on the sum of the scores given to yogurt's appearance, consistency on spoon, consistency in mouth, odor and flavor. Total of 12 yogurt samples met this requirement having total score over 20. The sum of sensory scores for sample 2 was the highest among the other samples which was 22.7. This could be due to its highest apparent viscosity and caused the sample to obtain high scores of appearance, consistency on spoon and consistency in mouth. In fact, this sample had the lower amounts of volatile compounds, but the overall acceptance of the product, 4.75 ± 0.40, was the highest among the rest of the samples. This result showed that overall acceptance of the sample not only affected by taste, but also appearance and consistency on spoon and consistency in mouth could influence the consumers' preferences.

Table 8.17. Sensory scores for yogurt samples

Yogurt Sample	Appearance	Consistency on spoon	Consistency in mouth	Odor	Flavor	Overall Acceptance
Sample 1	3.75±0.90^{ab}	3.60±0.90^{cd}	3.45±0.70^{bc}	4.15±0.50^{abc}	3.65±0.70^{cde}	3.75 ±0.70^{bc}
Sample 2	4.25±0.80^{ab}	4.60±0.50^{a}	4.65±0.60^{a}	4.65±0.50^{a}	4.55±0.50^{a}	4.75±0.40^{a}
Sample 3	3.55±0.70^{ab}	3.60±0.80^{cd}	3.40±0.80^{bc}	4.30±0.60^{abc}	3.75±0.70^{cde}	3.70±0.70^{bc}
Sample 4	3.40±0.90^{b}	3.45±0.70^{cd}	3.35±0.70^{c}	3.95±0.50^{bc}	3.65±0.50^{cde}	3.55±0.50^{c}
Sample 5	4.40±0.80^{a}	3.80±1.00^{abcd}	3.60±0.80^{bc}	4.35±0.50^{abc}	4.05±0.60^{abcde}	4.05±0.50^{abc}
Sample 6	3.55±0.80^{ab}	3.75±0.70^{abcd}	3.55±0.70^{bc}	3.95 ±0.60^{bc}	3.85±0.70^{abcde}	3.7 0±0.60^{bc}
Sample 7	3.70±0.70^{ab}	4.30±0.70^{abc}	3.80±0.60^{bc}	4.20±0.60^{abc}	4.10±0.80^{abce}	4.15±0.70^{abc}
Sample 8	4.20±0.80^{ab}	4.50±0.60^{ab}	4.10±0.50^{abc}	4.50±0.50^{abc}	4.15±0.50^{abcd}	4.35±0.60^{ab}
Sample 9	4.10±0.80^{ab}	4.10±0.90^{abcd}	3.90±0.60^{abc}	4.15±0.60^{abc}	4.15±0.70^{abcd}	4.10±0.60^{abc}
Sample 10	3.70±0.60^{ab}	4.10±0.60^{abcd}	4.10±0.60^{abc}	4.25±0.60^{abc}	4.00±0.70^{abcde}	4.15±0.70^{abc}
Sample 11	4.35±0.70^{a}	3.80±0.60^{abcd}	3.80±0.80^{bc}	4.20±0.50^{abc}	3.85±0.60^{abcde}	4.00±0.60^{bc}
Sample 12	4.15±0.90^{ab}	4.30±0.70^{abc}	4.20±0.80^{ab}	4.55±0.60^{ab}	4.50±0.50^{ab}	4.40±0.60^{ab}
Sample 13	3.60±0.80^{ab}	3.75±0.80^{abcd}	3.60±0.40^{bc}	4.10±0.80^{abc}	3.50±0.80^{de}	3.60±0.60^{abc}
Sample 14	3.60±0.90^{ab}	3.40±1.00^{d}	3.35±1.00^{c}	3.90±0.70^{c}	3.35±0.70^{e}	3.45±0.80^{c}
Sample 15	3.50±0.80^{ab}	4.05±0.70^{abcd}	4.15±0.70^{abc}	4.45±0.50^{abc}	4.25±0.60^{abc}	4.05±0.50^{abc}
Sample 16	4.15±1.00^{ab}	3.65± 1.00^{bcd}	3.60±0.80^{bc}	4.20±0.80^{abc}	3.80±0.90^{bcde}	4.05±0.90^{abc}
Sample 17	3.40±0.80^{b}	4.20±0.80^{abcd}	3.90±0.60^{abc}	4.15±0.40^{abc}	4.20±0.40^{abcd}	4.10±0.40^{abc}
Sample 18	4.35±0.90^{a}	4.20±0.40^{abcd}	3.90±0.70^{abc}	4.25±0.40^{abc}	4.05±0.50^{abcde}	4.15±0.60^{abc}
Sample 19	4.00±0.90^{ab}	4.30±0.70^{abc}	4.05±0.60^{abc}	4.15±0.40^{abc}	3.90±0.60^{abcde}	4.15±0.60^{abc}
Sample 20	3.55±0.90^{ab}	3.80±0.80^{cd}	3.75±0.60^{bc}	4.25±0.60^{abc}	4.05±0.70^{abcde}	4.15±0.70^{abc}

[a-e] Column means having a different letter or letters differ (P<0.05).
Means ± SD of duplicate samples

CHAPTER 9

CONCLUSION

Objective of this study is to determine the technological and organoleptic properties of *L. bulgaricus* and *S. thermophilus* yogurt starter bacteria isolated and identified. Hence, curd formation, titratable acidity, acetaldehyde and EPS production of yogurt bacteria were determined in order to select the cultures having best properties and manufactured yogurts using them for the observation of these culture's bioavailability with respect to yogurt analyses. At the end of the study:

- Out of 64 *S. thermophilus* isolates, 57 of them formed curd. Out of 57 *S. thermophilus* isolates, 38 of them decreased the pH below 4.60 in 7 h. Among these, 18 of *S. thermophilus* isolates decreased the pH below 4.60-4.70 in 4-5 h. These strains were selected for the organoleptic analyses. All 72 *L. bulgaricus* isolates formed curd, but 54 of them produced a remarkable level of acetaldehyde, ranged between 1.2 and 31.5 mg/l. The EPS production by *S. thermophilus* and *L. bulgaricus* in milk was 3.40 to 45.32 mg/L, 2.56-94.45 mg/L, respectively. Results indicated that 18 *S. thermophilus* and 7 *L. bulgaricus* could be used for the manufacturing of yogurt.
- Yogurt samples made by the combination of the selected isolates had total solids content of 14.50-17.64%, fat contents of 2.40-3.35%, and protein contents of 3.81-4.39%.
- Titratable acidities of day 1 yogurts were varied from 8.64 to 9.05%. There was a significant decrease of pH over time (from day 1 to day 21) for each sample. Syneresis of yogurt samples ranged from 42.0 to 55.8% during 21 days of storage.
- The apparent viscosities were in between 725.85 and 1321.75 cp, and the EPS amounts were in between 20.65 and 62.63 μg/ml. Although the highest apparent viscosity was obtained in sample 2, this sample had low amount of EPS production. In contrast, sample 17 had the highest amount of EPS and high apparent viscosity. As a result, it was observed that there was no correlation between EPS production and apparent viscosity of the isolates used for yogurt manufacturing.

- During 21 days of storage, viable lactic acid bacteria counts of yogurt samples were decreased about 1 log cycle.
- NIR Spectroscopy analysis was successfully carried out for total solids, fat, pH, syneresis, and microbial counts with the R^2 of predicted versus actual graph values; 0.9499, 0.9954, 0.9282, 0.9747, and 0.9247, respectively.
- The volatile aroma compounds of yogurt samples were obtained as 13.442-25.44 mg/l of acetaldehyde, 1.488-7.022 mg/l of ethanol, 0.280-0.541 mg/l of acetone, 0.425-1.789 mg/l of diacetyl and 0.041-0.107 mg/l of MEK. It was confirmed that acetaldehyde was the most noticeable aroma compound in yogurt. Among yogurt samples, sample 10 had the best aroma profile because it contained almost the highest levels of all volatile compounds. It was also observed that sample 17 produced low level of acetaldehyde (13.69 mg/l) but the highest level of EPS (62.63 µg/ml) and sample 15 with high level of acetaldehyde (24.94 mg/l) but the lowest amount of EPS (20.65 µg/ml). It could be concluded that the strains used in sample 17 could be polysaccharide-producing strains and the strains used in sample 15 could be non-polysaccharide-producing strains.
- Out of 20 yogurt samples, 12 of them had the total of sensory scores over 20 which was required by Yogurt Standard of the Turkish Standards Institute (1989).
- In general, sensory evaluation results showed that out of 12 samples liked by panelists, the starter culture isolates used in 6 of these samples (sample 2, 5, 10, 11, 15 and 18) could be used in dairy industry for their following characteristics;
    - Sample 2: highest sensory score, highest apparent viscosity
    - Sample 5: sensory score over 20, high level of acetaldehyde
    - Sample 10: sensory score over 20, almost highest level of all aroma compounds
    - Sample 11: sensory score over 20, high level of acetaldehyde
    - Sample 15: sensory score over 20, high level of acetaldehyde, high level of apparent viscosity
    - Sample 18: sensory score over 20, high level of acetaldehyde, high level of apparent viscosity, high level of EPS production
- The isolate used in samples 5, 10 and 15 was the same *S. thermophilus* isolate (TY63-2), the isolate used in samples 15 and 18 was the same isolate *L.*

bulgaricus isolate (22) and the isolate used in samples 2 and 5 was the same *L. bulgaricus* isolate (54). It was interesting that TY63-2 *S. thermophilus* yogurt isolate had a bioavailability with all types of *L. bulgaricus* isolates.

- In conclusion, among *S. thermophilus* isolates TY63-2, 95-1, TY47 and TY24, and all *L. bulgaricus* isolates 22, 25, 54 and TY30 have potential to be used as starter cultures in dairy industry. Further studies are needed to investigate their preservation, and enhancement of EPS and acetaldehyde production.

REFERENCES

Abrahamsen, R.K, A. Svensen, G.N. Tufto. 1978. Some bacteriological and biochemical activities during the incubation of yogurt from goat's and cow's milk. *20th International Dairy Congress*, Paris, IE, 828.

Akın, N. 2006. *Modern Yoğurt Bilimi ve Teknolojisi*. Selçuk Üniversitesi Ziraat Fakültesi, Gıda Mühendisliği Bölümü, Konya.

Amatayakul, T., A.L. Halmos, F. Sherkat, N.P. Shah, 2005. Physical characteristics of yogurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. *International Dairy Journal* 16:40-51.

Amatayakul, T., F. Sherkat, N.P. Shah. 2006. Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. *Society of Dairy Technology* 59:216-221.

Anon. 2007a. Yogurt tarihçesi.
http://tr.wikipedia.org/wiki/Yo%C4%9Furt
(accessed May 2007)

Anon. 2007b. Butanone.
http://en.wikipedia.org/wiki/Butanone.
(accessed May 2007)

AOAC. Official Methods of Analysis of AOAC International. 1999. Maryland, USA: AOAC International.

Asimopoylos, G., S. Savvidis, N. Asimopoylos. 2004. On – line monitoring of dairy products with the use of NIR technology. *Acta Montanistica Slovaca* 9 : 36-40.

Aslım, B., Y. Beyatli, Z.N. Yuksekdag. 2006. Productions and monomer compositions of exopolysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from traditional home-made yoghurts and raw milk. *International Journal of Food Science and Technology* 41:973–979.

Ayar, A., N. Akın. 2001. Farklı bileşimlerde hazırlanan aromalı yoğurtların bazı duyusal ve kimyasal özelliklerinin belirlenmesi üzerine bir araştırma. *Dünya Gıda* 6(10):65-69.

Ayhan K., F. Durlu-Özkaya, N. Tunail. 2005. Commercially important characteristics of Turkish origin domestic strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. *International Journal of Dairy Technology* 58(3):150-157.

Birollo, G.A., J.A. Reinheimer, C.G. Vinderola. 2000. Viability of lactic acid microflora in different types of yogurt. *Food Research International* 33:799-805.

Beshkova, D., E. Simova, G. Frengova, Z. Simov. 1998. Production of flavour compounds by yoghurt starter cultures. *Journal of Industrial and Biotechnology* 20:180-186.

Bongers, R.S., M.H.N. Hoefnagel, M. Kleerebezem. 2004. High-level acetaldehyde production in *Lactococcus lactis* by metabolic engineering. *Applied Environmental Microbiology* 71(2):1109-1113.

Bouzar, F., J. Cerning, M. Desmazeaud. 1996. Exopolysaccharide production in milk by *Lactobacillus delbrueckii* spp. *bulgaricus* CNRZ 1187 and by two colonial variants. *Journal of Dairy Science* 79:205-211.

Bouzar, F., J. Cerning, M. Desmazeaud. 1997. Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yogurt production. *Journal of Dairy Science* 80:2310-2317.

Bönisch, M.P., M. Huss, S. Lauber, U. Kulozik. 2007. Yogurt gel formation by means of enzymatic protein cross-linking during microbial fermentation. *Food Hydrocolloids* 21:585-595.

Britten, M., H.J. Giroux. 2001. Acid-induced gelation of whey protein polymers: Effects of pH and calcium concentration during polymerization. *Food Hydrocolloids* 15:609-617.

Broadbent, D.J. McMahon, D.L. Welker, C.J. Oberg and S. Moineau. 2003. Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*. *Journal of Dairy Science* 86:407–423.

Burns and D.A., Ciurczak, W.E. 2001. *Handbook of near-infrared analysis*. New York: Marcel Dekker.

Cerning, J., C. Bouillanne, M. Landon, Y. Desmazeaud. 1992. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *Journal of Dairy Science* 75:692-699.

Chaves, A.C.S.D., M. Fernandez, A.L.S. Lerayer, I. Mierau, M. Kleerebezem, J. Hugenholtz. 2002. Metabolic engineering of acetaldehyde production by *Streptococcus thermophilus*. *Applied Environmental Microbiology* 68(11):5656-5662.

De Vuyst, L., F. Vanderveken, S. Van De Ven, B. Degeest. 1998. Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *Journal of Applied Microbiology* 84:1059-1068.

De Vuyst, L., B. Degeest. 1999. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews* 23(2):153-177.

Degeest, B., L. De Vuyst. 2000. Correlation of activities of the enzymes α-phosphoglucomutase, UDP-galactose 4-epimerase, and UDP-glucose pyrophosphorylase with exopolysaccharide biosynthesis by *Streptococcus thermophilus* LY03. *Applied and Environmental Microbiology* 66(8):3519–3527.

Degeest, B., L. De Vuyst, F. Vaningelgem. 2001. Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. *International Dairy Journal* 11:747–757.

Degeest, B., L. De Vuyst, F. Mozzi. 2002. Effect of medium composition and temperature and pH changes on exopolysaccharide yields and stability during *Streptococcus thermophilus* LY03 fermentations. *International Journal of Food Microbiology* 79:161– 174.

Duboc, P., B. Mollet. 2001. Applications of exopolysaccharides in the dairy industry. *International Dairy Journal* 11:759–768.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28(3):350-356.

Erkuş, O. 2007. Isolation, phenotypic and genotypic characterization of yogurt starter bacteria. İzmir Institute of Technology Thesis of MS.

Escalante, A., C. Wacher-Rodarte, M. Garcia-Garibay, A. Farres. 1998. Enzymes involved in carbohydrate metabolism and their role on exopolysaccharide production in *Streptococcus thermophilus*. *Journal of Applied Microbiology* 84:108-114.

Folkenberg, D.M., P. Dejmek, A. Skriver, H.S. Guldager, R. Ipsen. 2006. Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures. *International Dairy Journal* 16:111-118.

Frengova, G.I., E.D. Simova, D.M. Beshkova, Z.I. Simov. 2000. Production and monomer composition of exopolysaccharides by yogurt starter cultures. *Canadian Journal of Microbiology 46*(12):1123–1127.

Gallardo-Escamilla, F.J., A.L. Kelly, C.M. Delahunty. 2005. Influence of starter culture on flavor and headspace volatile profiles of fermented whey and whey produced from fermented milk. *Journal of Dairy Science* 88:3745–375.

Gardini, F., R. Lanciotti, M.E. Guerzoni, S. Torriani. 1999. Evaluation of aroma production and survival of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* in fermented milks. *International Dairy Journal* 9:125-134.

Güler-Akın, M.B. 2005. The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yogurt made from ewe's milk. *Society of Dairy Technology* 58(3):174-179.

Güler-Akın, M.B., S.M. Akın. 2007. Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk. *Food Chemistry* 100:788-793.

Goh, K.K.T., R.D. Haisman, H. Singh. 2005a. Examination of exopolysaccharide produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* using confocal laser scanning and scanning microscopy techniques. *Journal of Food Science* 70(4):224-229.

Goh, K.K.T., R.D. Haisman, R.H. Archer, H. Singh. 2005b. Evaluation and modification of existing methods for the quantification of exopolysaccharides in milk-based media. *Food Research International* 38:605-613.

Hassan, A.N., J.F. Frank, K.B. Qvist. 2002. Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy. *Journal of Dairy Science* 85:1705–1708.

Hassan, A. N., R. Ipsen, T. Janzen, K.B. Qvist. 2003. Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *Journal of Dairy* Science 86:1632-1638.

He Y., D. Wu, S. Feng, X. Li. 2007. Fast measurement of sugar content of yogurt using Vis/NIR-Spectroscopy. *International Journal of Food Properties* 10(1):1-7.

Janhoj, T., C.B. Petersen, M. Frost, B.R. Ipsen. 2006. Sensory and rhelogical characterization of low-fat stirred yogurt. *Journal of Texture Studies* 37:276–299.

Jaworska, D., B. Waszkiewicz-Robak, W. Kolanowski, F. Swiderski. 2005. Relative importance of texture properties in the sensory quality and acceptance of natural yoghurts. *International Journal of Dairy Technology* 58(1):39-46.

Kalviainen N., K. Roininen, H. Tuorila. 2003. The relative importance of texture, taste and aroma on a yogurt-type snack food preference in the young and the elderly. *Food Quality and Preference* 14:177-186.

Kılıç, S. 2001. *Süt Endüstrisinde Laktik Asit Bakterilerileri*. No: 542 İzmir: Ege Üniversitesi Ziraat Fakültesi Yayınları.

Lin, T.Y., M.F. Chang Chien. 2007. Exopolysaccharides production as affected by lactic acid bacteria and fermentation time. *Food Chemistry* 100:1419-1423.

Looijesteijn, P.J., W.H.M. Van Casteren, R. Tuinier, C.H.L. Doeswijk-Voragen, J. Hugenholtz. 2000. Influence of different substrate limitations on the yield, composition and molecular mass of exopolysaccharides produced by *Lactococcus lactis* subsp. *cremoris* in continuous cultures. *Journal of Applied Microbiology* 89:116-122.

McKinley, M.C. 2005. The nutrition and health benefits of yoghurt. *Society of Dairy Technology* 58(1):1-12.

Marshall, V.M., H.L. Rawson. 1997. Effect of 'ropy' strains of *Lactobacillus delbruecci* spp. *bulgaricus* and *Streptococcus thermophilus* on rheolgy of stirred yoghurt. *International Journal of Food Science and Technology* 32:213-220.

Marshall, V.M., H.L. Rawson. 1999. Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. *International Journal of Food Science and Technology* 34:137-143.

Marshall, V.M., A.P. Laws, Y. Gu, F. Levander, P. Radström, L. De Vuyst, B. Degeest, F. Vaningelgem, H. Dunn, M. Elvin. 2001. Exopolysaccharide-producing strains of thermophilic lactic acid bacteria cluster into groups according to their EPS structure. *Letters in Applied Microbiology* 32:433-437.

Marsili, R. 2002. Flavours and off-flavours in dairy foods. Dean Foods Technical Center, USA. Elsevier Science Ltd. 1069-1081.

Meilgaard, M., Civille, V.G., Carr, B.T., eds. 1999. *Sensory Evaluation Techniques (3rd Editition)*. New York: CRC Press.

Miyake T., T. Shibamoto 1993. Quantitative analysis of acetaldehyde in foods and beverages. *Journal of Agricultural and Food Chemistry* 41:1968-1970.

Mozzi, F., F. Vaningelgem, E.M. Hébert, R. Van der Meulen, M.R. Foulquié Moreno, G. Font de Valdez, Luc De Vuyst. 2006. Diversity of heteropolysaccharide-producing lactic acid bacterium strains and their biopolymers. *Applied and Environmental Microbiology* 72(6):4431–4435.

Ott, A., J.E. Germond, L.B. Fay, A. Chaintreau. 1997. Determination and origin of the aroma compounds of yogurt flavor. *Journal of Agricultural and Food Chemistry* 45:850-858.

Ott, A., J.E. Germond, M. Baumgartner, A. Chaintreau. 1999. Aroma comparisons of traditional and mild yogurts: Headspace gas chromatography quantification of volatiles and origin of α-diketones. *Journal of Agricultural and Food Chemistry* 47:2379-2385.

Ott, A., J.E. Germond, M. Baumgartner, A. Chaintreau. 2000. Sensory investigation of yoghurt flavour perception: Mutual influence of volatiles and acidity. *Journal of Agricultural and Food Chemistry* 48:441-450.

Özer, B., F. Atasoy. 2002. Effect of addition of amino acids, treatment with β-galactosidase and use of heat shocked cultures on the acetaldehyde level in yoghurt. *Society of Dairy Technology* 55(4):166-170.

Özdemir, D., B. Öztürk. 2006. Genetic multivare calibration for near infrared spectroscopic determination of protein, moisture, dry mass, hardness and other residues of wheat. *International Journal of Food Science and Technology* 41:12-21.

Özdemir, D. 2007. Near Infrared Spectroscopic Determination of Olive Oil Adulteration with Sunflower and Corn Oil. *Journal of Food and Drug Analysis* 15:40-47.

Petry, S., S. Furlan, E. Waghorne, L. Saulnier, J. Cerning, E. Maguin. 2003. Comparison of the thickening properties of four *Lactobacillus delbrueckii* subsp. *bulgaricus* strains and physicochemical characterization of their exopolysaccharides. *FEMS Microbiology Letters* 221:285-291.

Reeves, J. B., J. S. Van Kessel. 2000. Near-Infrared Spectroscopic Determination of Carbon, Total Nitrogen, and Ammonium-N in Dairy Manures. *Journal of Dairy Science* 83:1829–1836.

Robinson, R.K. 1999. "*Fermented Milks: Yoghurt*" *"* in Encyclopedia of Dairy Science, edited by H. Roginski, J. Fuquay, P. Fox (Academic Press, United Kingdom).

Robinson, R.K. 2002. *"Fermented Milks: Yoghurt, Role of Starter Cultures"* in Encyclopedia of Dairy Science, edited by H. Roginski, J. Fuquay, P. Fox (Academic Press, United Kingdom).

Rodarte, C.W., M.V Galvan, A. Farres, F. Gallardo, V.E. Marshall, M.G. Garibay. 1993. Yogurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. *Journal of Dairy Research* 60:247-254.

Ruas-Madiedo, P.R., J. Hugenholtz, P. Zoon. 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal* 12:163–171.

Ruas-Madiedo, P. R., C.G. De Los Reyes-Gavilan. 2005. Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria *Journal of Dairy Science*. 88:843–856.

Salminen, S., A.V. Wright, A. Ouwehand, eds. 2004. *Lactic Acid Bacteria: Microbiological and Functional Aspects*. New York: Marcel Dekker.

Shao, Y., Y. He., S. Feng. 2007. Measurement of yogurt internal quality through using Vis/NIR spectroscopy. *Food Research International* 40:835–841.

Shihata, A., N.P. Shah. 2002. Influence of addition of proteolytic strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* to commercial ABT starter cultures on texture of yoghurt, exopolysaccharide production and survival of bacteria. *International Dairy* Journal 12:765–772.

Sodini, I., J. Montella, P. S. Tong. 2005. Physical properties of yogurt fortified with various commercial whey protein concentrates. *Journal of the Science of Food and Agriculture* 85:853–859.

Smit, G., B.A. Smit, W.J.M. Engels. 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews* 29(3):591–610.

Stingele, F., J.R. Neeser, B. Mollet. 1996. Identification and characterization of the *eps* (Exopolysaccharide) gene cluster from *Streptococcus thermophilus* Sfi6. *Journal of Bacteriology* 178(6):1680–1690.

Sultaneh, A., H. Rohm. 2007. Using near infrared spectroscopy for the determination of total solids and protein content in cheese curd. *International Journal of Dairy Technology* 60(4):241-244.

Tamime, A.Y. and H.C. Deeth. 1980. Yogurt: technology and biochemistry. *Journal of Food Protection* 43:939-997.

Tamime, A.Y. and Robinson, R.K. 1985. *Yoghurt Science and Technology*. UK: Pergamon Press.

Tamime, A.Y., Marshall, V.M.E., eds. 1997. *Microbiology and Biochemistry of Cheeses and Fermented Milks*. London:Blackie Academic.

Tekinşen, C. 2000. *Süt Ürünleri Teknolojisi*. Konya: Selçuk Üniversitesi Basımevi.

Telefoncu, A.1992. *Tıp ve Fen Bilimleri İçin Biyokimya*. Kırklareli:Sermet Matbaası.

The Dairy Council. 2007. Minerals in yogurt.
http://www.milk.co.uk/page.aspx?intPageID=84
(accessed June 2007)

Thermo Scientific. 2007. Algorithms-The Beer Lambert Law, Classical Least Squares (CLS).
http://www.thermo.com/com/cda/resources/resources_detail/1,2166,13320,00.html.
(accessed December 2007)

Tsenkova. R., S. Atanassova, K. Toyoda, Y. Ozaki, K. Itoh, T. Fearn. 1999. Near-Infrared Spectroscopy for Dairy Management of Unhomogenized Milk Composition *Journal of Dairy Science* 82:2344-2351.

Turkish Standards Institute. Turkish Standards Institute, Ankara, Turkey.1989. *Yoghurt Standard (TS 1330)*.

Ünal, B., S. Metin, D.N. Işıklı. 2003. Use of response surface methodology to describe the combined effect of storage time, locust bean gum and dry matter of milk on the physical properties of low-fat set yogurt. *International Dairy Journal* 13:909-916.

Vaningelgem, C.F, M. Zamfir, F. Mozzi, T. Adriany, M. Vancanneyt, J. Swings, L. De Vuyst. 2003. Biodiversity of exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and functional characteristics. *Applied and Environmental Microbiology* 70:900-912.

Vaningelgem, F., R. Van der Meulen, M. Zamfir, T. Adriany, A.P. Laws, L. De Vuyst 2004a. *Streptococcus thermophilus* ST 111 produces a stable high-molecular-mass exopolysaccharide in milk-based medium. *International Dairy Journal* 14:857-864.

Vaningelgem, F., M. Zamfir, T. Adriany, L. De Vuyst. 2004b. Fermentation conditions affecting the bacterial growth and exopolysaccharide production by *Streptococcus thermophilus* ST 111 in milk-based medium. *Journal of Applied Microbiology* 97:1257-1273.

Yang, Z., E. Huttunen, M. Staaf, G. Widmalm, H. Tenhu. 1999. Separation, purification and characterisation of extracellular polysaccharides produced by slime-forming *Lactococcus lactis* ssp. *cremoris* strains. I*nternational Dairy Journal* 9:631-638.

Welman, A.D., I.S. Maddox, 2003. Exopolysaccharides from lactic acid bacteria: Perspectives and challenges. *Trends in Biotechnology* 2(6):269-273.

Wittrup C., L. Norgaard. 1998. Rapid near infrared spectroscopic screening of chemical parameters in semi-hard cheese using chemometrics. *Journal of Dairy Science* 81:1803-1809.

Yaygın, H. 1999. *Yoğurt Teknolojisi*. Yayın No: 75. Antalya: Akdeniz Üniversitesi Basımevi.

APPENDIX A

CHEMICALS USED IN EXPERIMENTS

Table A.1. Chemicals Used in Experiments

No	Chemical	Code
1	MRS Broth	Merck 1.10661
2	M17 Broth	Merck 1.15029
3	MRS Agar	Merck 1.10660
4	M17 Agar	Merck 1.15108
5	Bacteriological Pepton	Oxoid LP037
6	Yeast extract	Merck A 1.03753
7	Lablemco Meat Extract	Oxoid LP029
8	Sodium Acetate	Sigma S2889
9	Agar	AppliChem A0949
10	D(+) Glucose	AppliChem A3666
11	D(+) Lactose	Sigma L3750
12	Triammonium citrate	Sigma A1332
13	$MgSO_4.7H_2O$	Merck 1.05886
14	$MnSO_4.4H_2O$	Merck 1.02786
15	K_2HPO_4	Sigma P8281
16	Tween 80	AppliChem A1390

(cont. on next page)

Table A.1 (cont.) Chemicals Used in Experiments

1	n-Amyl alcohol (for synthesis)	Merck 8.07500
2	Sulfuric acid 95-97%	Fluka
3	Sodium hydroxide	Merck 1.06498
4	Protease from *Aspergillus oryzae*	Sigma P6110
5	Ethanol	Merck 1.00983
6	Sodium acetate	Sigma S-2889
7	Boric Acid	AppliChem A2940
8	Phenol crystalline	AppliChem A1594
9	Isopropanol	AppliChem A3928
10	Ethanol (Molecular Biology Grade)	AppliChem A1151
11	Glycerol	AppliChem A2926
12	Sodium hydroxide	Merck 1.06498
13	Hydrochloric Acid	Merck 1.00317
14	Glycerol	AppliChem A2926
15	Anaerogen	Oxoid AN0025A
16	Acetaldehyde Enzymatic BioAnalysis Kit	Roche 10668613

APPENDIX B

PROCEDURES FOR ACETALDEHYDE AND EXOPOLYSACCHARIDES

B.1. Acetaldehyde Determination Procedures

The main steps in acetaldehyde determination procedures were as follows:

- 40 mg yogurt were weighted accurately into a falcon tube
- 4 ml citric acid (20% w/v) was added
- Mixture was stirred slightly and transferred into a 50 ml falcon tube and subsequently filled up to the 50 ml mark with distilled water
- Each mixture was filtered through a fluted filter paper. Clear filtered solution was used for the enzymatic analysis
- 200 µl clear yogurt solution was firstly mixed with the reaction mixture which did not contain enzyme and measured spectrophotometrically at 340 nm.
- This solution was mixed with the enzyme solution and final absorbance was read at 340 nm.
- The last two steps were done for the blank solution which did not contain yogurt solution.
- Lastly, the amount of acetaldehyde in the yogurt sample was determined by the equation given below

$$\Delta A = (A2\text{-}A1)_{sample} - (A2\text{-}A1)_{blank} \qquad \text{(Eq. 1)}$$

ΔA = Absorbance difference of yogurt sample and blank solution

$A2_{sample}$ = Absorbance of yogurt sample with enzyme

$A1_{sample}$ = Absorbance of yogurt sample without enzyme

$A2_{blank}$ = Absorbance of blank solution with enzyme

$A1_{blank}$ = Absorbance of blank solution without enzyme

The general equation for calculating the concentration of acetaldehyde:

$$C = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ (g/l)} \qquad \text{(Eq. 2)}$$

V = final volume (ml)
v = sample volume (ml)
MW = molecular weight of the substance to be assayed (g/mol)
d = light path (cm)
ε = extinction coefficient of NADH at:
340 nm = 6.3 ($l \times mmol^{-1} \times cm^{-1}$)
Hg 365 nm = 3.4 ($l \times mmol^{-1} \times cm^{-1}$)
Hg 334 nm = 6.18 ($l \times mmol^{-1} \times cm^{-1}$)

It follows for acetaldehyde:

$$C = \frac{3.250 \times 44.05}{\varepsilon \times 1.00 \times 0.200 \times 1000} \times \Delta A = \frac{0.7158}{\varepsilon} \times \Delta A \text{ (g acetaldehyde/l sample soln.)}$$

$$\text{Content}_{\text{acetaldehyde}} = \frac{C_{\text{acetaldehyde}} \text{ (g/l sample soln.)}}{\text{Weight}_{\text{sample}} \text{ in g/l sample soln.}} \times 100 \text{ (g/100g)} \qquad \text{(Eq. 3)}$$

B.2. EPS Determination Procedures

The revised protocol for EPS assay was carried out as follows:

- Swirl to mix culture medium in bottle to ensure homogeneity
- Adjust the pH of the sample to pH 7 with NaOH
- Add 100 µL of filter-sterilized Protease(10% w/w) to 10mL of sample
- Incubate the sample at 50 °C in a shaker (Forma Orbital Shaker, Thermo Electron Corporation, USA) for 4 h.
- Vortex the sample for approximately 15s
- Pipette 2.9 ml of distilled water and 7 ml of chilled absolute ethanol into the falcon tube
- Pipette 100 µL of culture medium into the falcon tube
- Leave the sample overnight at 4°C

- Centrifuge samples (maximum of 8 tubes at a time) at 27,000g, 4°C for 40 min (J-A 20 Fixed Angle Rotor, Beckman Coulter Avanti, USA)
- After centrifugation, carefully decant supernatant (separate it from pellet)
- Invert the tubes on a piece of paper towel for approximately 10 min
- Pipette 3 ml of distilled water to re-suspend the pellet in the centrifuge tube
- Pipette 7 ml of chilled 99.7% ethanol into the centrifuge tube
- Repeat step 8-10
- Re-suspend pellet in 1 ml of distilled water
- Transfer the sample to an eppendorf tube
- Prepare a blank sample using distilled water (1 ml)
- Add 1 ml of 5% (w/v) phenol solution to the sample and mix using a vortex for 15 s
- Add 5 ml of concentrated sulphuric acid directly to sample
- Mix the sample thoroughly using a vortex
- Leave the sample to stand for 30 min
- Read absorbance at 485 nm (Shimadzu-UV-Visible Spectrophotometer, Japan). Use the blank as the reference sample
- Obtain the amount of EPS from the glucose standard curve
- Amount of EPS is multiplied by 10 to account for the dilution factor
- Amount of EPS = EPS of the test sample – EPS of control sample