QUALITY CHARACTERISTICS AND SHELF-LIFE OF 'ARMOLA' CHEESE

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ABSTRACT

QUALITY CHARACTERISTICS AND SHELF-LIFE OF 'ARMOLA' CHEESE

In this study, forty Armola cheese samples were collected from dairies located in Seferihisar, İzmir and their physicochemical, microbiological and sensorial quality characteristics were investigated. In addition, the lactic acid bacteria flora of cheese samples was identified using genotyping method (16S-rRNA gene sequencing).

The average total solid, fat, and protein contents, pH value, titratable acidity, water activity and salt content of these samples were found as 37.26, 19.52, 10.87, 4.70, 0.95, 0.91, and 2.51, respectively. The average microbial counts were found as follows: Total aerobic mesophilic bacteria, 7.82; psychrotrophic bacteria, 6.98; coliform bacteria, 4.56; lactococci, 7.55; lactobacilli, 7.87; enterococci, 6.17; yeast 7.33; mold <1.00; *Staphylococcus* spp., 5.94; and *Listeria* spp., 2.94 cfu/g. The high microbial counts showed that most of these samples were produced in very poor hygienic conditions. As a result of the descriptive sensory analysis, dominant flavor were salty and sour as basic taste; however, creamy, cooked and whey tastes were as aromatics. According to genotyping identification results, the dominant bacteria were found as *Enterococcus ratti*, *Enterococcus durans, Enterococcus hirae*, *Streptococcus lutetiensis, Streptococcus equines, Streptococcus luteciae*, *Lactobacillus paracasei subsp. tolerans, Lactobacillus casei subsp. casei, Lactobacillus zeae,* and *Lactobacillus paracasei subsp. paracasei*.

Due to the short shelf-life of Armola cheese, antimicrobials, Nisaplin®, Natamax®, and Microgard™ 100, were also used alone or in combination to extend the shelf-life of the product. Microbiological, sensory, color and pH analyses were conducted during storage. Because of the inhibition effects on yeasts, Natamax® and its combinations were the most effective on extending the shelf-life.

ÖZET

‘ARMOLA’ PEYNİRİNİN KALİTE KARAKTERİSTİKLERİ VE RAF ÖMRÜ

Bu çalışmada, Seferihisar’da bulunan mandıralardan kırk adet peynir örneği toplanmıştır ve Armola peynirlerinin kimyasal, fiziksel, mikrobiyolojik ve duyusal karakteristikleri araştırılmıştır. Ayrıca, peynirlerdeki laktik asit bakterileri izole edilmiş ve genotipik metotla (16S-rRNA gen dizileme) doğal mikroflora belirlenmiştir.

Ortalama toplam katı madde, yağ, azot ve protein içeriği, pH değeri, titre edilebilir asitlik, su aktivitesi ile tüm örneklerin tuz içeriği sırasıyla 37.26, 19.52, 10.87, 4.70, 0.95, 0.91, and 2.51 olarak bulunmuştur. Peynir örneklerinde ortalama mikrobiyal yük toplam mezofilik bakteri için 7.82; psikrofil bakteri için 6.98; koliform bakteri için 4.56; laktokoklar için 7.55; laktobasiller için 7.87; enterokoklar için 6.17; mayalar için 7.33; *Staphylococcus* spp. için 5.94; ve *Listeria* spp. için 2.94 kob/g olarak bulunmuştur. Tüm örneklerde küfe rastlanmamıştır. Mikrobiyal yükün yüksek olması üretimdeki hijyenik koşulların oldukça düşük olduğunu göstermiştir. Duyusal analiz sonuçlarına göre baskın temel tatlar tuzlu ve ekşi iken kremsi, pişmiş ve peynir altı suyu aromatik tatlar olarak bulunmuştur. Genotipik tanımlama sonuçlarına göre baskın bakteriler; *Enterococcus ratti, Enterococcus durans, Enterococcus hirae, Streptococcus lutetiensis, Streptococcus equines, Streptococcus luteciae, Lactobacillus paracasei subsp. tolerans, Lactobacillus casei subsp. casei, Lactobacillus zeae,* ve *Lactobacillus paracasei subsp. paracasei* olarak bulunmuştur.

Armola peynirinin raf ömrünün kısa olması nedeniyle Nisaplin®, Natamax®, ve Microgard™ 100 antimikrobiyalleri yalnız ve/veya kombinasyonlar halinde peynirin raf ömrünün uzatılması amacıyla kullanılmıştır. Örneklerde depolama süresince mikrobiyolojik, duyusal, renk ve pH analizleri yapılmıştır. Mayalar üzerindeki engelleyici etkisinden dolayı Natamax® ve kombinasyonları Armola peynir örneklerinin raf ömrünü uzatmada en çok etkiyi göstermiştir.

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CHAPTER 1

INTRODUCTION

Cheese evolved in the ‘‘Fertile Crescent’’ between the Tigris and Euphrates rivers, about 8000–9000 years ago and this area now forms part of Turkey. There are many varieties of traditional cheese with local names in Turkey ‘Armola’ is a type of traditional cheese produced in several dairies of Seferihisar. Its name, armola, originates from Greek and its meaning is ‘whey’ in Greek language. Today, Armola cheese is commonly produced in cauldrons by mixing white cheese, whey cheese and yoghurt which are produced by goat’s milk in certain amounts in dairy industry instead of traditional method in the past.

Armola cheese has a short shelf life. In circumstances of poor manufacturing practices and storage conditions, spoilage of the product occurs within a relatively short time. The spoilage of dairy products such as cheese is mainly due to the development of yeasts which is the Specific Spoilage Organisms (Mataragas et al., 2011). One approach for extending the microbiological shelf life of Armola cheese is to introduce antimicrobials preferably naturally occurring antimicrobials (nisin, natamycin and Microgard^{TM} 100). Nisin exhibits antimicrobial activity towards a wide range of Gram-positive bacteria but shows little or no activity against Gram-negative bacteria, yeasts, and moulds. Natamycin is a natural antimycotic with a wide range of antimicrobial spectrum against yeasts and moulds (Pintado et al., 2011). Microgard^{TM}100 demonstrate inhibits Gram-negative bacteria and certain fungi (Staszewski and Jagus, 2008).

There are no reports in literature on the quality characteristics of this product. The objective of this study was initially to determine the physicochemical, microbiological, sensory properties and to characterize the natural microflora of Armola cheese. Nothing is known about the nature and evolution of the main microbial groups. Thus, technologically important strains have to be isolated and characterized. In addition, this study also aimed to determine if the microbiological shelf life of Armola cheese could be improved by using antimicrobials alone and in combination by minimizing any undesirable changes of its sensory characteristics.

CHAPTER 2

LITERATURE REVIEW

2.1. Traditional Cheeses

Cheese making began about 8000 years ago and now there are about 1000 cheese varieties in worldwide, each unique in terms of its flavor and form. Manufacture of most cheese varieties involves combining four ingredients including milk, rennet, microorganisms and salt, which are processed through a number of common steps such as gel formation, whey expulsion, acid production and salt addition, followed by a period of ripening. Variations in ingredients and following processing have led to the evolution of all these cheese varieties. While variations in processing parameters such as temperature and curd handling techniques play a major role in determining the characteristics of each cheese type, the cheese microflora plays a critical role in the development of the unique characteristics of each cheese variety. Milk composition and the influence of ripening are also important on the quality of cheeses (Beresford et al., 2001). The comparison of the compositions of popular cheeses was given in Table 2.1.

Table 2.1. Proximate composition of popular cheeses

	Moisture (%)	Lactose (%)	Fat (%)	Protein (%)	Ash (%)	Ca (mg)	NaCl (mg)	kcal
``American'' (USA)	39.2	1.6	31.2	22.2	5.8	616	1430	375
Blu (USA)	42.4	2.3	28.7	21.4	5.1	528	1395	353
Brick	41.1	2.8	29.7	23.2	3.2	674	560	371
Brie	48.4	0.4	27.7	20.8	2.7	184	629	334
Camambert	51.8	0.5	24.3	19.8	3.7	388	842	300
Cheddar	36.8	1.3	33.1	24.9	3.9	721	620	403
Colby	38.2	2.6	32.1	23.8	3.4	685	604	394
Cream	53.8	2.7	34.9	7.6	1.2	80	296	349
Edam	41.6	1.4	27.8	25.0	4.2	731	965	357
Fetaa	55.2	4.1	21.3	14.2	5.2	492	1116	264
Gjetostb	13.4	42.6	29.5	9.6	4.8	400	600	466
Gruyere	33.2	0.4	32.3	29.8	4.3	1011	336	413
Limburger	48.4	0.5	27.2	20.0	3.8	497	800	327
Monterey	41.0	0.7	30.3	24.5	3.6	746	536	373
Mozzarella	54.1	2.2	21.6	19.4	2.6	517	373	281
Munster	41.8	1.1	30.0	23.4	3.7	717	628	368
Parmiggiano	17.7	3.7	30.0	41.6	7.0	1376	1862	456
Provolone	41.0	2.1	26.6	25.6	4.7	756	876	351
Ricottac	71.7	3.0	13.0	11.3	1.0	207	84	174
Roquefortd	39.4	2.0	30.6	21.5	6.4	662	1809	369

Turkey has many traditional foods because of its history, different cultures and climates. Cheese is an important traditional food in Turkish cuisine and there are over 110 varieties of cheese (Kamber, 2008). Each traditional cheese tells us something about the history of the people who made it and the country where it was made. A combination of historical events dictates the method of production, the type of animals, the shape and flavor of cheese (Harbutt, 1999).

Goat and sheep milk is commonly used for cheese production in rural areas of Aegaean part of Turkey. There are many cheese types produced and consumed locally in the region where industrial dairy is common. The types of these cheeses are in different shape, color, and taste. Some of these cheese types are; Aydın Kuru Çökelek cheese, Koponisti cheese, Manisa Çayır cheese, Denizli Yörük cheese, Afyon Köylü cheese, Afyon Tulum cheese, Afyon Kuru Ezme cheese, Manavgat Salamura Beyaz

Yörük cheese, Milas Kaşıklı cheese, Milas Kirk Tokmak cheese, Tire Çamur cheese, İzmir Teneke Tulum cheese, Kirlihanım cheese, Afyon Tulum cheese, Karaburun Keçi Sepet cheese, Karaburun Lorlu Keçi Tulum cheese and Armola cheese (Ünsal, 2000; Kamber, 2008).

2.2. Armola Cheese

Armola is a kind of traditional cheese produced at some homes and many dairies of Seferihisar in İzmir. Armola has limited shelf life and which is estimated as 7 days. Its name, armola, originates from Greek and its meaning is 'whey' in Greek language.

2.2.1. Composition of Armola Cheese

Armola is composed of whey cheese (Lor), white cheese and strained yoghurt which are produced from goat milk as mixture of them.

2.2.1.1. Goat Milk

Milk is a polyphasic secretion of the mammalian gland which comprises globules of milk fat suspended in an aqueous medium containing a range of proteins, lactose, and water soluble vitamins, mineral salts (Kliem and Givens, 2011).

Milk contains many components with physiological functionality and it contains high levels of immunoglobulins and other physiologically active compounds for warding off infection in the newborn. The components of milk provide critical nutritive elements, immunological protection, and biologically active substances to both neonates and adults. Milk proteins are currently the main source of a range of biologically active peptides. Concentrates of these peptides are potential health-enhancing nutraceuticals for food and pharmaceutical applications. Minor whey proteins, such as lactoferrin, lactoperoxidase, lysozyme, and immunoglobulins, are considered antimicrobial proteins. Milk also contains some natural bioactive substances. These include oligosaccharides, fucosylated oligosaccharides, hormones, growth factors, mucin,

gangliosides, and endogenous peptides, which are present in milk at secretion (Sewerin and Wenshui, 2005)

Milk composition varies according to several factors, such as animal, feed and environment. Table 2.2 shows the composition of milk from different milk types.

Table.2.2. Comparative composition of milk of different species (Source: Jandal, 1996)

Component	Goat	Sheep	Cow	Human
Fat (%)	3.80	7.62	3.67	3.67-4.70
Solid-non-fat (%)	8.68	10.33	9.02	8.90
Lactose (%)	4.08	3.7	4.78	6.92
Protein (%)	2.90	6.21	3.23	1.10
Casein (%)	2.47	5.16	2.63	0.40
Whey proteins (%)	0.43	0.81	0.60	0.70
Total ash (%)	0.79	0.90	0.73	0.31
Ca (%)	0.194	0.160	0.184	0.042
P (%)	0.270	0.145	0.235	0.06
Cl (%)	0.154	0.270	0.105	0.060
Vitamin A (IU g^{-1} fat)	39.00	25.00	21.00	32.00
Vitamin B_1 (mg per 100 ml)	68.00	7.00	45.00	17.00
Vitamin B_{12}(mg per 100 ml)	210.00	36.00	159.00	26.00
Vitamin C (mg per 100 ml)	20.00	43.00	2.00	3.60
Vitamin D (IU g^{-1} fat)	0.70	ND	0.70	0.27
Energy (Cal. per 100 ml)	70.00	ND	69.00	68.00

ND, not-detected.

There are around 480 million goats worldwide providing more than 5 million tons of milk (Jandal, 1996). The European Union accounts for some 15% of the world production of goat milk. Goat milk production has also grown significantly in India, Bangladesh and Iran in recent years, which now accounts for a third of the total world output (Boyazoğlu and Morand-Fehr, 2000).

Goats have a longer lactation (up to 300 days of milking) than sheep (up to 250 days of milking) (Boyazoğlu and Morand-Fehr, 2000). Goat milk has also a stronger flavor than sheep milk. This might be due to the release of short-chain fatty acids during rough handling, which give off a goaty smell (Jandal, 1996). Moreover, due to the widely appreciated organoleptic characteristics, the production of goat's milk cheese has

recently attracted growing interest. It has been noticed that the goat milk has more easily digestible fat and protein content than cow milk. In addition, goat milk has more vitamin A, thiamine and niacin in comparison to cow's milk (Nikolic et al., 2008).

Goat milk is very useful for people suffering from problems such as eczema, asthma, migraine, colitis, stomach ulcer, digestive disorder, liver and gallbladder diseases and stress-related symptoms such as insomnia, constipation and neurotic indigestion. These patients may in future turn more to goat milk and its products to solve their problems. Goat milk products can also provide a profitable alternative to cow and sheep milk products because of their specific flavor, texture, typicity and their healthy impression. However, there are few available data on the manufacture of fluid goat milk products such as low fat, fortified or flavored milks, cultured products such as buttermilk or yoghurt, frozen products such as ice cream, condensed milk, dried milk products and cheeses (Jandal, 1996).

2.2.1.2. White Cheese

Cheese is an important dairy product consumed In Turkey. It is consumed almost three times in a day.There are three major cheese types including brined white cheese, kashar cheese and tulum cheese produced and consumed in local regions (Turkoglu et al., 2003).

Turkish white cheese produced from goat milk is one of the components of Armola cheese and it is a kind of brined (or a pickled) cheese Although Turkish White cheese has a soft texture when fresh, after ripening for 3 months in brine, it can be classified as a semi-hard or semi-soft variety. Turkish White cheese was manufactured originally from sheep’s or goat’s milk, but cow’s milk or a combination of milks is now generally used for its production.

The production steps of commercial Turkish white cheese are shown in Figure 2.1. Raw milk (preferably from goat) is clarified and standardised with respect to the casein: fat ratio and pasteurised at 80–85 °C for 2–3 s or 63°C for 30 min or 65°C for 5 min. After cooling to 32 °C, it is transferred to cheese vats, and starter culture is added at a level of 1–2 g/100 g and $CaCl_2$ at a level of 0.2 g/L. The inoculated milk is held for 30 min, and liquid rennet is added at a level (~10 g/100 kg) sufficient to coagulate the milk in 90 min. The milk starts to form a gel after 30–45 min, and the gel is sufficiently

firm after 75–90 min. The coagulum is cut into cubes and the curds are allowed to rest the whey for 5–10 min. The curds are then transferred to stainless steel moulds, which vary in sizes and are lined with cheese cloth. The surface of the cheese is covered with cheese cloth, followed by a plate on which weights are placed to compact the curd. Pressure is applied at room temperature (21 °C) for 3–6 h or until whey drainage has stopped or decreased to a low level. The pressure is 20–40 kg weights for each 100 kg of cheesemilk. The weights are removed, the cheese cloth opened and the cheese mass cut into blocks and they are placed in brine (14–16 g/100 g NaCl) for 6–12 h at 15–16 °C. The brined blocks are then arranged on the bottom of a tinned can (18 L), the can is filled with brine (14 g/100 g NaCl) and the container is closed. The salt concentration in the brine is checked and adjusted periodically during ripening. The cheese is ripened in the cans for 30–60 days at 12–15 °C and is then ready for consumption (Hayaloglu et al., 2002).

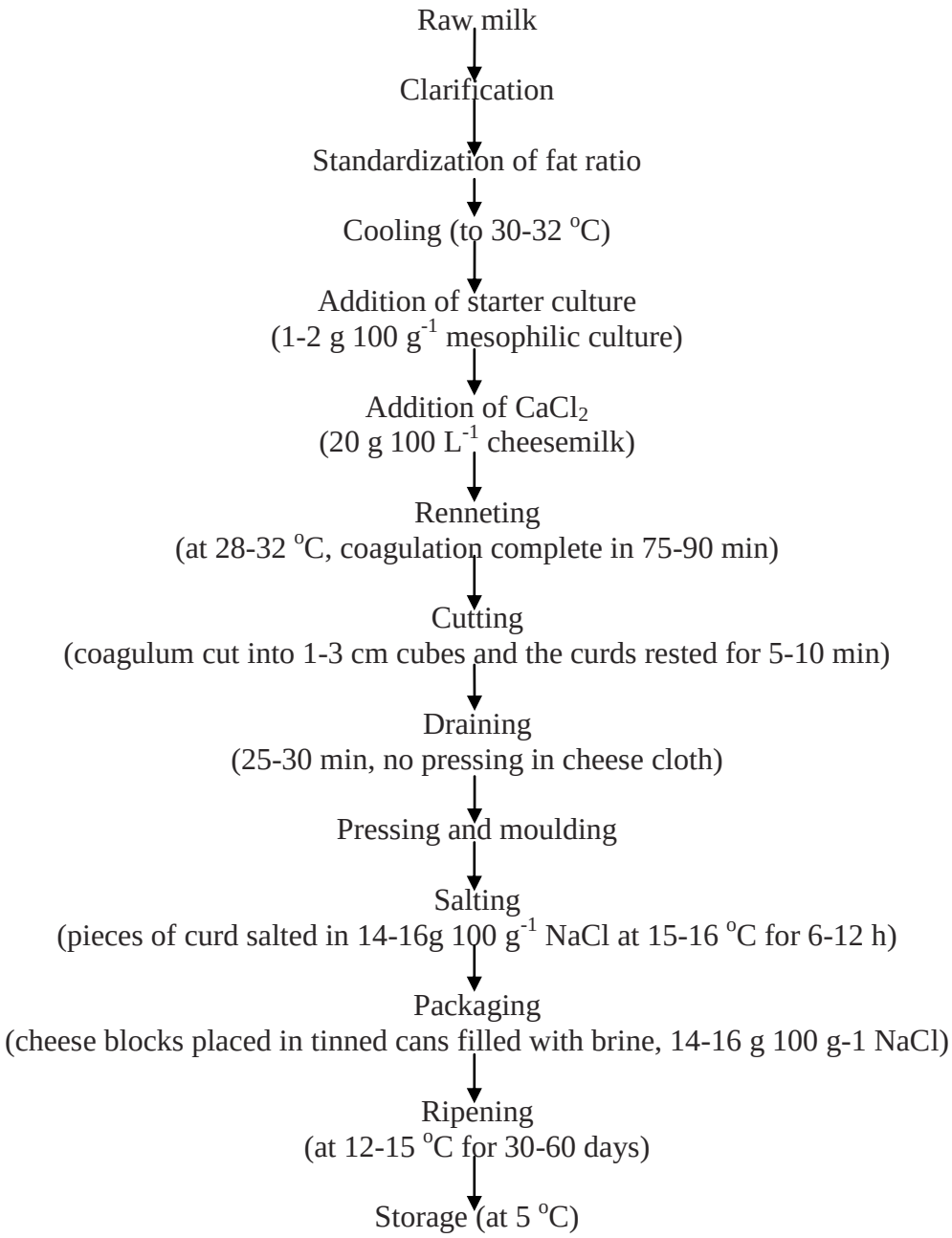

Figure 2.1. Flow diagram for manufacturing of Turkish white cheese
(Source: Üçüncü, 1999)

In the traditional or artisanal manufacture of Turkish white cheese, starter culture is not added to the cheesemilk. The milk may or may not be pasteurised and the curd is handled extensively by the cheese maker (Hayaloglu et al., 2002).

2.2.1.3. Whey (Lor) Cheese

Another component of Armola is goat whey cheese. Whey is the main product of the cheese manufacturing industry. It is obtained by coagulating milk with rennet, an edible acidic substance or heating and then draining off the liquid portion. The increased acidity causes the milk proteins (casein) to convert into solid masses, or curds. The remaining liquid, which contains only whey proteins, is the whey. Partition of milk into cheese and whey is shown in Figure 2.2. (Irkın, 2009; Temiz et al., 2009).

It has been estimated that 15 00 000 tons of whey are being produced annually in cheese manufacturing in Turkey. In the Mediterranean region, whey is used for the production of some kinds of cheeses. Whey cheeses are very popular cheeses; Ricotta (Italy), Manouri (Yugoslavia), Getost, Brunost (Norway), Ziger (Germany), Broccio (France), Anthotyro (Greece) and Requeson (Spain) of this type of cheeses. A large portion of whey is used for the production of whey cheese called Lor, in every region of Turkey. In Lor cheese production, whey from the Kashar cheese production was heated at 50–55 °C. Fat was separated from whey, then was heated to 80 °C in a boiler tank with 2% (w/w) salt added. Serum proteins began to accumulate at the surface of the whey, and the temperature increased to 90–95 °C. The coagulum's of Lor cheese was collected from the tank above the whey surface into the thin clothes and drained for 12 h at 25 °C. Although whey cheese is normally consumed without ripening, but in some areas it is left for maturation. In the past, it was usually produced to make butter and cheese to meet house servant in villages according to traditional protocol but in recent years, it begun to produced in factory (Irkın, 2009; Temiz et al., 2009).

In general, the fat content of whey cheeses is lower than that of rennet curd cheeses; also, milk serum proteins in these cheeses have high nutritional value because of containing essential amino acids in the human diet (Temiz et al., 2009).

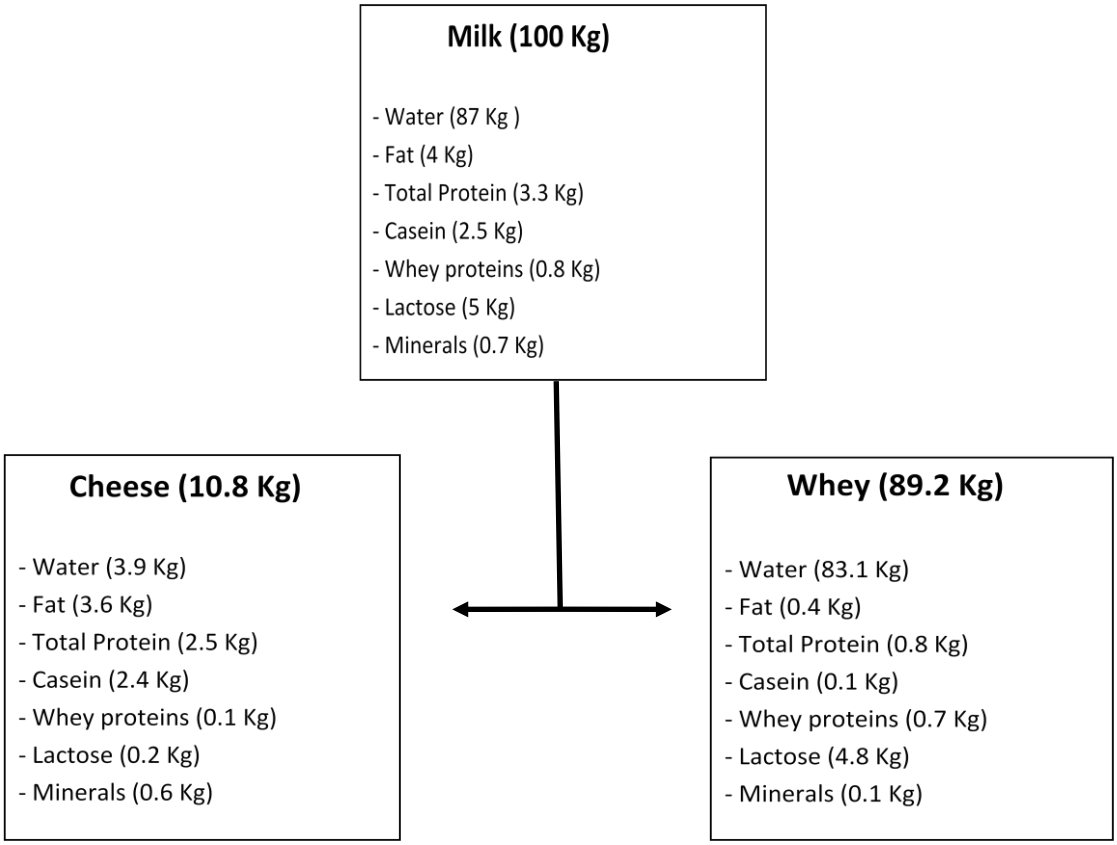

Figure 2.2. Partition of Milk into Cheese and Whey
(Source: Hutkins, 2007)

2.2.1.3. Yoghurt

Yoghurt is a highly nutritious protein-rich product obtained by fermentation of milk with *S. thermophilus* and *L. bulgaricus*. The product is highly acceptable to consumers in terms of its flavor and aroma and also attributed to acetaldehyde and its texture (Kumar and Mıshra, 2004).

Many types of concentrated fermented milk products are produced to extend shelf-life. Strained yoghurt is one of the most consumed concentrated fermented products. For maintaining quality, removing yoghurt serum is one of the most important factors. The elimination of yoghurt serum is the main stage for strained yoghurt. Some methods including ultrafiltration and centrifugation have been used in the manufacturing of strained yoghurt because of this reason. However, special cloth bag for draining of serum is widely used in the traditional method. Strained yoghurt is a traditional product indigenous to Turkey and also called “Süzme” or “Torba” yoghurt.

According to the traditional method for producing strained yoghurt, the initial yoghurt (set yoghurt) is poured into a special cloth bag and stirred; it is then left overnight to strain off the serum. This process extends the shelf-life of the product mainly due to the reduction in water activity. Strained yoghurt is unique with the exact character of the product directly affected by the level of moisture retained, the degree of acidity, the microorganisms used, as well as the type of milk used. Traditionally, cow's, ewe's, goat's and buffalo's milks have been used for the production of strained yoghurt in Turkey. Yoghurt from goat's milk differentiate from other milks due to beneficial effects on human health (Şenel et al., 2011).

2.2.3. Production of Armola Cheese

There are two types of production including dairy production and traditional production. In the dairy production of Armola cheese, whey cheese (Lor), white cheese and strained yoghurt which are produced from goat's milk are mixed together in caldron and it is consumed by adding olive oil and oregano.

In some villages, the raw milk for the traditional production of the cheese is poured directly into an animal skin or a cauldron. Then, it is salted and left to maturation. With time, the acidity increases and the coagulates. If the mile coagulates inside an animal skin, a large proportion of the whey will seep out of the hide. However, if the coagulation process occurs inside a cauldron, the cheese is packed into an animal skin after the whey has been drained off. Approximately one month later, from both methods a mature cheese with the consistency of yoghurt is obtained (Figure 2.3). In recent years, the traditional production is not in use (Kamber, 2008).

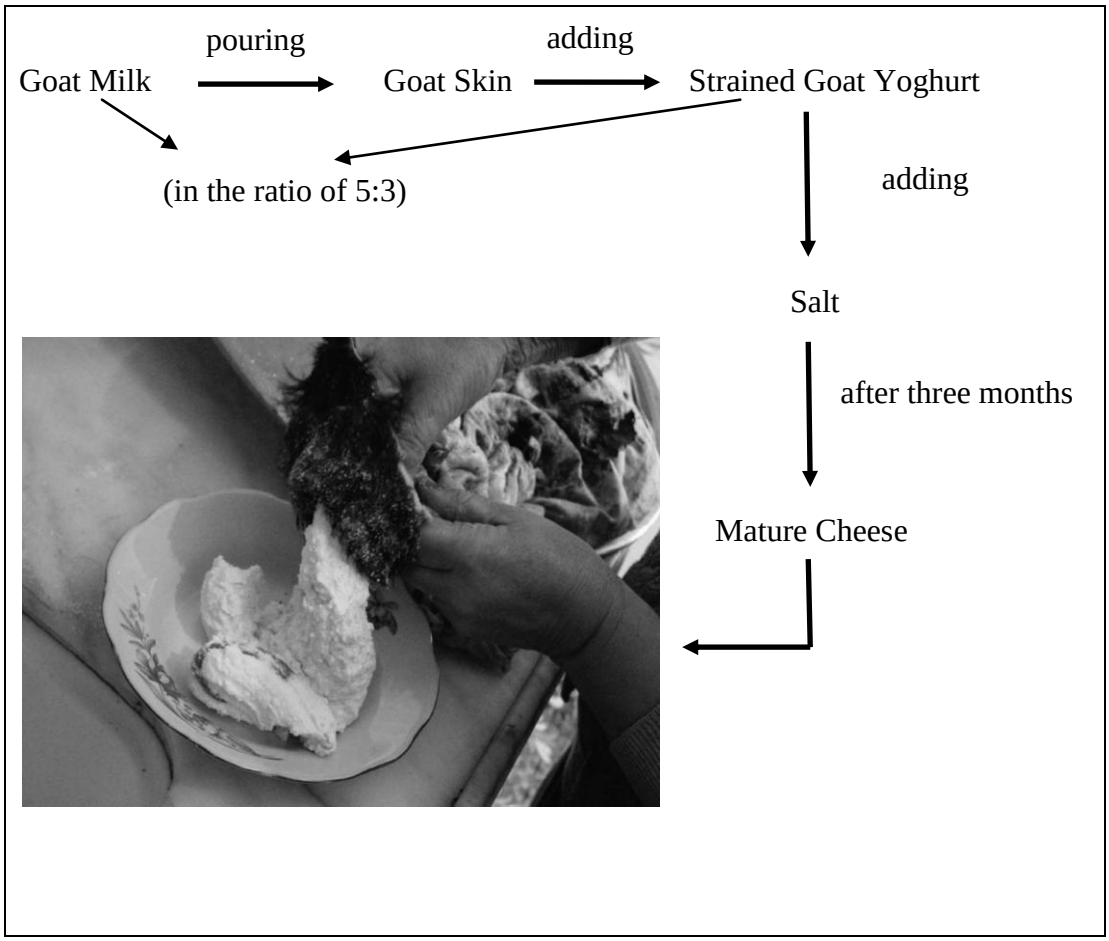

Figure 2.3. Traditional production of Armola cheese
(Source: Kamber, 2008)

2.3. Microbiology of Cheeses

Microorganisms, including bacteria, yeast and moulds, are present in cheese and they are an essential component of all natural cheese varieties. They play important roles during cheese manufacturing and ripening. Microorganisms can be divided into two main groups including starters and secondary flora. The starter flora involving *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus helveticus* and *Lactobacillus delbrueckii* used either individually or in various combinations depending on the cheese variety, in charge of acid development during cheese production. The secondary microflora may be divided into a number of primary groups including non-starter lactic acid bacteria (NSLAB) consisting of non-starter lactobacilli; *Pediococcus*, *Enterococcus* and *Leuconostoc*, propionic acid bacteria (PAB), moulds and bacteria and yeast are associated with particular cheese varieties. The secondary flora consists of

complex mixtures of bacteria, yeasts and moulds, and it is generally associated with particular cheese varieties (Beresford et al., 2001; Beresford and Williams, 2004).

The growth of LAB itself shows positive effects, because LAB produce lactic acid during sugar fermentation that inhibits the majority of unwanted microorganisms (pathogenic and spoilage) due to the acidification of the environment. Moreover, several LAB are able to release some metabolites with specific antagonistic activity and antibacterial effects such as antifungal compounds bacteriocins and antibiotics (Settanni et al., 2010). The genus Enterococcus is the most controversial group of lactic acid bacteria (Moreno et al., 2006). High levels of contaminating enterococci in some fresh or soft industrial cheeses produced with pasteurised milk and selected lactic starter culture cause deterioration of sensory properties of these products. Therefore, the prevalence of enterococci in these cheeses is undesirable. Otherwise, several studies have indicated that strains of enterococci can have a positive influence on the production and ripening of traditional cheeses. Moreover, they play an important role in improving flavor development and quality (Moreno et al., 2006). In these cheeses, enterococci generate an essential part of the fresh cheese curd microflora (levels of enterococci may range from 10^4 to 10^6 cfu/g), and also they can be the predominant microorganisms in the fully ripened product (levels of enterococci may range from 10^5 to 10^7 cfu/g). It is reviewed that *Enterococcus faecium* is capable of producing a variety of bacteriocins, called enterocins with activity against *Listeria* spp., which has an important impact on the safety of cheeses that are made from raw milk (Giraffa, 2003). Furthermore, several authors have claimed that dairy food strains of enterococci contribute to the ripening and aroma development of traditional cheeses (Ogier and Serror, 2008).

Aygun et al. (2005) studied microbiological and chemical quality of Carra cheese. In that study, 50 randomly samples were purchased from different retail markets in Antakya (Antioch) region. In these samples, the numbers of microorganisms were found as follows: total mesophilic bacteria, 1.87×10^8 cfu/g; *Staphylococcus aureus*, 2.51×10^3 cfu/g; enterococci, 4.40×10^5 cfu/g; *Enterobacteriaceae*, 5.60×10^5 cfu/g; yeast and moud, 4.80×10^7 cfu/g; coliform, 1.02×10^4 cfu/g; *Escherichia coli*, 4.27×10^3 cfu/g. *Samonella* spp. were not detected in any of the samples. Mean moisture, salt and fat contents of Carra cheese were found as 41.26%, 7.82% and 26.77%, respectively. The pH value of the samples varied between 4.53 and 6.32 with a mean of 5.24. The

microbiological findings showed the presence of high counts of microorganisms investigated and the poor hygienic quality of Carra cheese.

In conclusion, high microbial diversity have effects on quality characteristics of traditional cheeses. Therefore, characterization of the microbial communities in raw milk, cheese and follow the dynamics of the entire populations throughout the cheese making and ripening processes are important.

2.4. Molecular Identification of Microbial Flora

The main objective of the cheese microbiologist is to determine the cheese microflora and its evolution during ripening. It is important that the complete flora is monitored and that the individual components are accurately identified. A wide range of identification methods are available in order to identify and characterize the microflora of cheeses. These techniques can be divided into three groups including methods which depend on cultivation followed by phenotypic characterisation, on cultivation followed by molecular characterisation, and on molecular characterisation only. All of these methods have associated advantages and disadvantages (Beresford et al., 2001).

Although the application of phenotypic techniques has proven to be useful for certain LAB, phenotypes displayed by strains cannot always correspond to similar or even closely related genotypes. Consequently, usage of genotypic characterization methods may provide more reliable classification and differentiation (Temmerman, 2004). List of techniques used for the identification of Lactic Acid Bacteria are given in Table 2.3.

Table 2.3. List of techniques used for the identification of Lactic Acid Bacteria

Technique	Principle	Discriminatory power	Reference
Phenotypic methods Morphological analysis	Microscopic analysis	Genus level or less	Gonzalez et al. (2000)
Physiological analysis	Growth characteristics, simple tests	Genus level of less	Corsetti et al. (2001)
Biochemical characterization	Assimilation and fermentation patterns (API, BIOLOG,. . .)	Genus or species level	Muyanja et al. (2003)
Protein profiling	Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis of cellular proteins	Species level	Leisner et al. (2001)
Genotypic methods Specific primers	PCR with group-specific primers	Depending on primer	Nomura et al. (2002)
Sequencing	Determination of gene sequences (16S rDNA)	Genus to species level	Booysen et al. (2002)
RFLP	Restriction Enzyme Analysis (REA) of DNA or PCR amplicons	Species to strain level	Giraffa et al. (2002)
AFLP	Combination of REA and PCR amplification	Species to strain level	Giraffa and Neviani (2000)
RAPD-PCR	Randomly primed PCR	Species to strain level	Booysen et al. (2002)
Rep-PCR	PCR targeting repetitive interspersed sequences	Species to strain level	Gevers et al. (2001)
PFGE	REA and pulsed-field gel electrophoresis	Strain level	Ventura and Zink (2002)
Ribotyping	REA and oligonucleotideprobe detection	Species to strain level	Lyhs et al. (2002)
Hybridisation probes	DNA–DNA hybridisation using labeled probes	Genus to species level	Manero and Blanch (2002)

One of the main advantages of DNA-based identification and detection methods is their independence of variations in the growth conditions of the microorganisms. Genotypic methods show various levels of discriminatory power, from species level to differentiation of individual strains (typing). Many genotypic methods are based on the

principle of Polymerase Chain Reaction (PCR), which facilitates the selective amplification of specifically targeted DNA fragments through the use of oligonucleotide primers under controlled reaction conditions (Temmerman, 2004). The PCR has enabled the detection of unculturable microorganisms in virtually any dairy source, so it has been used extensively in the assessment of dairy microbial diversity. This technique relies on the assumption that the gene sequences present in the environment are complementary to the ‘‘universal’’ primers used in their amplification.In theory, PCR primers that amplify a 16S rDNA fragment can be designed for amplification at any taxonomic level (Table 2.4.) (Baker, 2003).

Table 2.4. Bacteria specific 16S rRNA gene sequence/PCR primers designed by Watanabe et al. (2001)

Primer name	**Sequence (5 →3)**
E8F	AGAGTTTGATCCTGGCTCAG
E9F	GAGTTTGATCCTGGCTCAG
E334F	CCAGACTCCTACGGGAGGCAGC
E341F	CCTACGGGIGGCIGCA
E786F	GATTAGATACCCTGGTAG
E533R	TIACCGIIICTICTGGCAC
E926R	CCGICIATTIITTTIAGTTT
E939R	CTTGTGCGGGCCCCCGTCAATTC
E1115R	AGGGTTGCGCTCGTTG
E1541R	AAGGAGGTGATCCANCCRCA

16S rRNA sequence analyses are powerful tools for assessing the genetic diversity of environmental samples (Baker, 2003). When unknown bacterial isolates have to be identified, a powerful tool with high discriminatory power is 16S or 23S rDNA sequencing. The obtained sequence is to be compared with DNA sequences stored in online databases of previously sequenced DNA, of which the most popular ones are the EMBL (http://www.ebi.ac.uk/embl/) and Genbank (http:// www.ncbi.nlm.nih.gov/Genbank/) databases. Searching these databases for corresponding sequences can be performed using a search algorithm, such as BLAST or FASTA (Temmerman, 2004).

2.5. Shelf-Life of Cheeses

Consumers demand products that are minimally processed, nutritious, safe, with longer shelf-life and good taste. Because of this reason, industry and researchers have studied and developed new processing and preserving technologies (Evert-Arriagada et al., 2011).

Almost all groups of microorganisms under some conditions can contribute to spoilage of foods (Gram et al., 2002). The growth of microorganisms in cheese depends on the availability of nutrients, water activity, pH, ionic strength, temperature and atmosphere composition of the headspace. Cheeses are open to microbial spoilage because of the high moisture content, low concentration of salt and pH close to natural, and consequently they have a limited shelf-life (Dermiki et al.,2008). Some microbiological spoiling observed in cheese is also a result of lipolytic and proteolytic activities of some microorganisms. Yeasts can contribute to taste, smell, and aroma formation in ripening process of cheese, otherwise, they can cause spoiling. They cause organoleptic change by hydrolysing fats when they reach 10^7–10^8 cfu/g in cheese. Another important microorganism group that is often isolated in cheese is mesophilic microorganisms. They come from different sources and contaminate the product during production and ripening of cheese (Var et al., 2006).

Microbiological method is one of the factors which is used to determine the shelf-life of the products (Arriagada et al., 2011). The extent of growth of certain classes of microorganisms and changes in sensory parameters are widely used to mark the end of the shelf-life of foods. For instance, Kykkidou et al. (2007) showed that microbiological shelf-life was defined as the period in which the yeast population exceeds 5 log cfu/g, and this limit was taken to mark the end of the shelf-life of Galotyri cheese. In addition to this, Sinigaglia et al. (2008) reviewed that the short shelf life of traditional mozzarella cheese has been attributed to microbiological spoilage. This spoilage is often caused by the growth of coliforms, *Pseudomonas* spp. and/or by psychrotrophic bacteria that grow on the cheese surface, mostly coming from water used in the manufacture. In order to prevent the growth of pathogenic and spoilage microorganism in cheeses, the effects of different technologies including lactic starter inoculation in cheese modified atmosphere or vacuum packaging, surface pasteurization and usage antimicrobial agents are applied (Evert-Arriagada et al., 2011).

Factors such as heat, oxygen, light and certain metal ions, notably iron and copper, also play a part in the occurrence of oxidation. For these reasons a high oxidative stability of lipids is very important to human health and also has economic importance. In addition, lipid oxidation leading to rancidity is often a decisive factor determining the shelf life of food products (Arslan et al., 2009).

Despite the importance of microorganisms in food spoilage, the definition and assessment of spoilage rely on sensory evaluation are the another factors for determining the shelf life of many food products (Hough et al.,1999; Gram et al., 2002). Sensory evaluation is used for measuring and quantifying the relationship between the sensory characteristics of a food and its consumer preferences. Techniques such as descriptive sensory analysis have been applied to Descriptive sensory analysis is a research tool to characterize the aromas and flavors in cheese (Zhang et al., 2011). The end of shelf life can be determined from sensory data by various graphical methods (Arslan et al., 2009). When performing shelf-life studies, the food is evaluated at different times, from fresh to deteriorated and different sensory attributes have been used including off-odor or flavor, overall acceptability, quality and deviations of typical flavor. These attributes can be subjective (Hough et al., 1999; Hough et al., 2007).

2.6. Antimicrobial Agents

Today, consumers prefer high-quality foods that are minimally processed, dainty, suitable to use, available throughout the year, nutritious and economical. Preservative-reduced or preservative-free convenience foods may have shorter shelf-lives unless formulations, processing methods and packaging are modified.

Control of microorganisms in food can be carried out by several aspects, including careful selection of raw materials, hygienic handling to prevent or minimise entry of microorganisms into food; removing or reducing the number of microorganisms through washing, centrifugation, or filtration; destroying microorganisms with heat (i.e. pasteurisation, sterilisation) or irradiation (when permitted); and inhibiting growth of microorganisms through environmental control (e.g. refrigeration, freezing, drying, packaging), adding natural or chemical antimicrobial preservatives (when permitted), or by adding desirable microorganisms in fermented foods (e.g. cheeses, fermented meats) to compete with undesirable ones.

Antimicrobial preservatives (e.g. nisin) have been used in food for many years because of the demand for preservative-reduced and preservative-free foods (Beales and Smith, 2004).The practical application of natural antimicrobial changes the sensory and textural properties of foods, when they are added. These compounds can be used in three ways involving purified or semipurified antimicrobial additives, bacteriocin-based ingredients from fermented foods, and bacteriocin-producing starter cultures. Recent strategies for controlling spoilage and pathogenic microorganisms tend to apply combining different antimicrobial agents to inhibit microbial growth and improve food safety (Sobrino-Lopez and Martin-Belloso, 2008). Synergism and antagonism have been reported between antimicrobials. For instance, Staszewski and Jagus (2008) investigated that antimicrobial activity of Microgard^{TM} individually or in combination with nisin against *Listeria innocua* in liquid cheese whey. Microgard^{TM} (300) did not reduce the initial count of *L. innocua* during storage and showed a response similar to the untreated whey. In comparison, nisin showed a bactericidal effect on growing *L. innocua.* Initially, a significant antagonistic effect was detected when Microgard^{TM} (300) was combined with nisin in all systems evaluated. However, during storage, different responses were observed. Some combinations were more effective than single treatments on control of *L. innocua.*

2.6.1. Nisin

Nisin is a peptide composed of 34 amino acid residues, with a molecular mass of 3.5 kDa, and is classified as a class-I bacteriocin or lantibiotic (Lo´pez and Belloso, 2008). It binds electrostatically to the negatively charged phospholipids and increases the permeability of the membrane by pore formation, resulting in rapid efflux of essential intracellular small molecules.The efflux of cellular constituents lead to a complete collapse of the proton motive force and subsequently in cell death (Gallo et al., 2007).

It is produced by strains of *Lactococcus lactis subsp. lactis* isolated from milk and vegetable-based products. It exhibits antimicrobial activity against a wide range of spores and Gram-positive bacteria, spoilage microorganisms, also has little effect on Gram-negative bacteria. Nisin inhibits not only closely related species but are also

effective against food-borne pathogens such as *Listeria monocytogenes.*, *Staphylococcus aureus* (O' Sullivan et al., 2002).

The nisin was accepted into the European food additive list, where it was assigned the number E234. it was also approved by the Food and Drug Administration (1988) in the USA as GRAS; to date, it is the only bacteriocin that has been approved by the World Health Organization for use as a food preservative and it is commercialized as a dried concentrated powder prepared from a skim-milk derived fermentate and is called Nisaplin® (O' Sullivan et al., 2002; Lopez and Belloso, 2008).

Kykkidou et al. (2007), investigated that the use of nisin as an antimicrobial treatment for shelf-life extension of Galotyri, a Greek soft acid-curd cheese, stored aerobically under refrigeration for a period of 42 days. Three different treatments were tested: N0, control sample with no nisin added; N1, 50 IU g^{-1} nisin; and N2, 150 IU g^{-1} nisin, the latter two treatments added post-production to the Galotyri cheese. Total mesophilic bacteria, lactobacilli, lactococci and yeasts were enumerated in all treatments. Based primarily on sensory evaluation (appearance and taste) and a microbiological acceptability limit for yeasts (5 log cfu/g), the use of nisin treatments extended the shelf-life of fresh Galotyri cheese stored at 4 °C by ca. 7 days (N1) and 21 days (N2) with cheese maintaining good sensory characteristics.

2.6.2. Natamycin

Natamycin (also known as pimaricin) is a polyene natural antimycotic. It is produced by *Streptomyces natalensis* and related species. It is commonly used as a food additive in dairy-based food products in order to prevent spoilage by fungi (Pintado et al., 2010; Hondrodimou et al., 2011). Natamycin has a wide range spectrum of activity against spoilage fungi and it is a very stable product with efficacy against *Aspergillus* flavus and aflatoxin production. It binds irreversibly to ergosterol and laeds to a loss of solutes from the cytoplasm by disrupting the fungal cell membrane. In addition, it shows certain advantages compared with sorbate, because it remains localised on the surface of the products applied. Moreover, it is not dependent on low pH for its activity and has no effect on the bacterial microbiota important in the fermentation and maturation of such products (Hondrodimou et al., 2011). Although it was proved that natamycin has no toxic effect even at the high levels of ingestion, its application as food

additive is restricted because of the possible danger of resistance formation (Hanušová et al., 2006).

E code of natamycin is E 235, and it is classified as preservative, yeast preventive, and antibiotic in food additive list. Its daily maximum allowable amount (ADI-value) is 0.3 mg/kg according to joint FAO/WHO Expert Committee on Food Additives. Based on the Directive 95/2/EC of the European Commission on food additives, the use of natamycin (E235) is allowed for surface treatment of hard, semi-hard and semi-soft cheeses, as well as in dried cured sausages (Hondrodimou et al., 2011).

2.6.3. Microgard™

Microgard™ is bacteriocin-like inhibitory product obtained by fermentation of skim milk (Microgard™ 100 and 300) or dextrose (Microgard™ 200) with *Propionibacterium shermanii* or specific *Lactococci*. Their active compounds include diacetyl as well as lactic, propionic and acetic acid and other undefined lowmolecular-mass inhibitors around 700 Da. Microgard™ products have been approved by the US Food and Drug Administration as GRAS for use in cottage cheese. They are estimated to be added to 30% of the cheese produced in the United States (Staszewski and Jagus, 2008).

Al-Zoreky et al. (1991) studied the effectiveness of Microgard™ (100) to inhibit most Gram-negative spoliage bacteria and demonstrated that Microgard™ (100) prolonged the shelf life of cottage cheese by inhibiting *Pseudomonas, Salmonella* and *Yersina* and certain fungi. However, it was ineffective against Gram-positive bacteria. Recently, Microgard™ (300) has been developed to target the Gram-positive bacteria but no published study has confirmed its effectiveness (Lemay et al., 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

The chemicals used in the study are listed in Appendix A.

3.1.2. Samples

In the first part of the study, the quality characteristics including microbiological, physicochemical and sensory analyses of forty Armola cheeses were investigated. The cheese samples were collected from six dairies in Seferihisar, İzmir, Turkey during 2010-2011. Dairy names and samples numbers are presented in Table 3.1. In the second part of the study, molecular characterization of natural microbial flora was determined by DNA isolation, PCR and 16S rRNA gene sequencing.

In the last part of the study, one of the dairies was choosen to obtain Armola cheese samples for the determination of the shelf-life of the samples containing different antimicrobials. The samples were analyzed in terms of microbiological, pH, color and sensory analysis at 0, 1, 5, 8, 11, 14, and 20 days of storage.

Table 3.1. Names of dairies and samples numbers

Dairy Names	**Sample Numbers**
Dairy A	1, 6, 11, 16, 21, 26, 31, 36
Dairy B	2, 7, 12, 17, 22, 27, 32, 37
Dairy C	3, 8, 13, 18, 23, 28, 33
Dairy D	4, 9, 14, 19, 24, 29, 34
Dairy E	5, 10, 15, 20, 25, 30
Dairy F	38, 39, 40

3.2. Methods

3.2.1. Physicochemical Analysis

3.2.1.1. Total Solid Content

Total solid content of the cheeses were determined gravimetrically by drying a sample to constant weight in an oven at 105ºC. Cheese sample (3 g) was crushed with 20 g sea sand and glass stick in predried weighing dish. The difference in weight before and after drying for 4-5 hours at 105ºC gives the results of total solid content (Metod 33.2.44; 990.20, AOAC 2006).

Total solid content (%) = [(Total solid content of cheese (g) / cheese (g))] ×100 (3.1)

3.2.1.2. Fat Content

Fat content of cheese samples were determined by Gerber method. Cheese sample (3 g) was weighed into a butyrometer vessel and then the vessel was filled with 10 ml H_2SO_4 (d: 1.22 g /ml). Plug was inserted into butyrometer and waited until the cheese was melted in 70ºC water bath. Then 1 ml amyl alcohol was added. Butyrometer vessel was completed to the level of 35% with H_2SO_4 solution. The butyrometers were placed in water bath for 5 min. After that the butyrometers were centrifuged in Gerber centrifuge for 10 min. The oil level was read as percentage oil in cheese from butyrometer vessel (IDF 1997).

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

3.2.1.3. Protein Content

For determination of total protein, Kjeldahl method was used (IDF, 1993). Armola sample (1 g), catalyst, antifoaming agent and H_2SO_4 (15 ml) were added to Kjeldahl tubes and they were placed into digestion unit (Gerhardt Kjeldaterm, Germany). The digestion was done at 420ºC until the solution in tubes became transparent. After the solution in tubes was cool, the tubes were placed into the distillation unit (Gerhardt Vapodest 50S, Germany). One hundred ml distilled water; 25 ml H_3BO_3 (% 3) and indicator were added into distillation unit. Percent protein content was observed with distillation, the flask was titrated with 0.1 N HCl. The nitrogen values obtained were transfered to the percentage protein using the formula:

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.38 \quad (3.2)$$

Where; 6.38 conversion factor of dairy products.

All measurements were done twice and average values were reported.

3.2.1.4. The pH Value and Titratable Acidity

The pH value of the Armola cheese was determined using a pH meter (Hanna HI 221, Germany). Measurements were done twice and average values were reported.

For determination of titratable acidity, 10 g cheese was weighed and crushed with 105 ml water (40ºC) in porcelain mortar. This solution was filtered and 25 ml of filtered solution was used for titration. Three drops of phenolphthalein were added and titrated with 0.1 N NaOH until the first permanent pink color.

$$\% \text{ lactic acid} = (0.1 \text{ N NaOH amount (ml)} \times 0.009 \times 100) / \text{Cheese amount (g)} \quad (3.3)$$

3.2.1.5. Water Activity

The water activity of samples was measured with water activity meter (Hygrolab V3, Bassersdorf). Five gram sample was weighed into the sample cup and placed into Hygrolab water activity meter. Measurements were done at room temperature. When the partial pressure in the air above sample is unchanged, water activity is read from the monitor as % relative humidity of air ×100.

3.2.1.6. Salt Content

The salt content of samples was determined by Mohr method. Five g cheese sample was crushed in porcelain mortar with the help of hot distilled water and watery part was transferred into a graduated cylinder with a lid. Same process was repeated 5 times. Then water level was completed to 500 ml with distilled water at room temperature. The solution was filtered and 25 ml of this solution was transferred into the volumetric flask and neutralized with 0.1 N NaOH. Then, 0.5 ml of K_2CrO_4 (5% w/v) was added and titrated with 0.1 N $AgNO_3$ until tile red color was occurred (IDF 1988).

$$\% \text{ Salt} = ((V1-V2) \times 0.585 \times F) / P \quad (3.4)$$

V1: Used 0.1 N $AgNO_3$ amount (ml) from experiment with cheese solution
V2: Used 0.1 N $AgNO_3$ amount (ml) from experiment with deionized water
P: Cheese amount included in titration (0.25 g)
F: Factor of 0.1 N $AgNO_3$

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

3.2.3. Microbiological Analyses

3.2.3.1. Sampling Procedure

For microbiological analyses, Armola samples (25 g) were transferred into individual sterile stomacher bags, mixed with 225 ml of 0.1% buffered peptone water (BPW) and homogenized in a stomacher (Bagmixer 400, Interscience, France) for 60 s. For each sample, appropriate serial decimal dilutions were prepared in BPW and then plated by spreading or pouring methods. For total aerobic mesophilic bacteria, psychrotrophic bacteria, coliforms, *Lactobacillus* ssp., *Lactococcus* ssp., *Enterococcus* spp., pour plate method was used whereas spread plate technique was used for yeast and mold, *Staphylococcus* spp., and *Listeria* spp. Each sample was incubated at suitable incubation conditions. Two measurements were carried out and average values were represented. After the incubation, the plates with colony forming units (CFU) ranging from 30 and 300 were selected for isolation and enumeration. Microbiological counts were converted to log cfu/g and the means and standard deviations were calculated (Samelis et al., 2003).

3.2.3.2. Total Aerobic Mesophilic Bacteria Enumeration

Skim milk plate count agar was used for total aerobic mesophilic bacteria. Plates were incubated at 32°C for 72 h (Mucchetti et al., 2008).

3.2.3.3. Psychrotrophic Bacteria Enumeration

Skim milk plate count agar was used for the enumeration of psychrotrophic bacteria. Plates were incubated at 7ºC for 10 days (Al-Kadamany et al., 2003).

3.2.3.4. Coliform Bacteria Enumeration

Double layer violet red bile agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 h (Mucchetti et al., 2008).

3.2.3.5. *Lactobacillus* spp. Enumeration

MRS agar was used for the enumeration of *Lactobacillus* spp. Plates were incubated at 37°C for 48 h. in sealed jar containing anaerogen sachet (Rogga et al., 2005).

3.2.3.6. *Lactococcus* spp. Enumeration

M17 agar was used for the enumeration of *Lactococcus* spp. Plates were incubated at 37°C for 48 h (Rogga et al., 2005).

3.2.3.7. *Enterococcus* spp. Enumeration

Kanamycin esculin azide agar was used for the enumeration of *Enterococcus* spp. Plates were incubated at 37°C for 48 h (Rogga et al., 2005).

3.2.3.8. *Staphylococcus* spp. Enumeration

Baird Parker agar supplemented with egg yolk tellurite medium was used for the *Staphylococcus* spp. enumeration. Plates were incubated at 37°C for 48 h (Rogga et al., 2005).

3.2.3.9. *Listeria* spp. Enumeration

PALCAM Listeria Selective Agar with its supplement was used for *Listeria* spp. enumeration. Plates were incubated at 37°C for 48 h (Samelis et al., 2003).

3.2.3.10. Yeast and Mold Enumeration

Yeast glucose chloromophenical agar was used for yeast and mold enumeration. Plates were incubated at 25°C for 5 days (Mucchetti et al., 2008).

3.2.4. Moleculer Characterization of Microbial Flora

Samples of Armola cheeses, collected from six different dairies located in Seferihisar, were plated on MRS for lactobacilli, M17 for lactococci and kanamycin esculin azide agar for enterococci. Five to ten colonies were selected from each plate or all sampled if the plate contained less than 10 colonies, for each sample. These isolates were propagated in MRS, M17 and nutrient broth for genomic DNA isolation.

3.2.4.1. Extraction of Bacterial Genomic DNA

We applied Phenol-chloroform extraction method with some modifications (Giraffa, 2000).

Approximately 100–200 ml of each strain grown overnight in MRS, M17 and nutrient broth (~ 10^7 CFU) were pelleted by centrifugation at 14,000 × *g* for 10 min. The pellets were washed twice with sterile water, TE_1 buffer (100 mM Tris–10 mM EDTA, pH 8.0), in a clean 1.5-ml microcentrifuge tube and repelleted by centrifugation. Total DNA was extracted from washed cell pellets by a standard alkaline lysis method. Washed cell pellets were resuspended in 200 µl of TES buffer (TE_1–25 % sucrose, pH 8.0). Following addition of 30 mg/ml of lysozyme, tubes were incubated at 37 °C for 30 min. In a second step, 370 µl Proteinase K (1 mg/ml) were added and 30 µl of sodium dodecyl sulfate (10 %) were added, followed by an incubation at 37 °C for 15 min. DNA was extracted three times with equal volumes of phenol and chloroform and precipitated with cold (-20 °C) isopropanol. After centrifugation at 14,000 × *g* for 30 min, 1 ml of ETOH (70%) was added to wash DNA. After a brief spin at 14,000 × *g* for 5 min, purified DNA was dried and resuspended overnight 150 µl of TE buffer. Ribonuclease A (10 µg/ ml) was added to resuspended DNA and incubated at 37 °C for

1 h. The concentrations of DNA were measured with Nanodrop (8000-Thermo Scientific) and adjusted to 25-60 ng/µl. The DNA was stored at -20 ^{o}C.

3.2.4.2. PCR

Different primer pairs (E334F 5' -CCAGACTCCTACGGGAGGCAGC- 3' and E939R 5' -CTTGTGCGGGCCCCCGTCAATTC- 3') were used to amplify the 16S rRNA gene fragment of lactobacilli, lactococci and enterococci (Baker et al., 2003; Forney et al., 2004). While the forward primer is complementary to the 5´- end of 16S rRNA genes, the reverse is complementary to the 3´- end of 16S rRNA genes. Amplification conditions consisted of an initial denaturation step of 94 °C for 120 s, followed by 30 cycles of: 94 °C for 60 s, 69 °C for 60 s, and 72 °C for 60 s; a final elongation step of 72 °C for 10 min was performed. PCR was performed in 20 µl amplification mixtures in with 2 µl 10× Tag DNA buffer, 2 µl of dNTP mix (2 mM each), 1.2 µl of $MgCl_2$ (25 mM), 0.4 µl of each primer (10 mM), 0.5 U/µl of *Taq* DNA polymerase (5 U/µl), 5 µl of total DNA and 8.5 µl sterile distilled water. PCR profiles were visualised with electrophoresis in agarose gels (Giraffa, 2000).

3.2.4.3. Agarose Gel Electrophoresis of Amplified PCR Products

The gel was made by adding 1 g agarose to 100 ml 1×TAE buffer (0.04 M Tris-Acetate, 0.001 M EDTA [pH 8.0]) by boiling. The solution was cooled to 40-50 ^{o}C before adding 5 µl of ethidium bromide (10 mg/ml) into 50 ml 1×TAE solution. The solution was poured into a gel tray including combs. After the gel was solidified, the combs were removed. The casting tray was placed into the tank containing 1×TAE buffer. Ten µl of each PCR product mixed with 2 µl of 6× gel loading dye and 10 µl of DNA molecular weight marker were loaded into the wells. The electrophoresis was performed for approximately at 100 V for 20 min. The bands were visualized on an UV illuminator and recorded in a gel documentation system (Vilber Lourmat, France).

3.2.4.4. 16S rRNA Gene Sequencing

The PRC products were prepared to apply 16S rRNA sequencing analyses. Firstly, Sephadex and spin columns were used to purify amplified PCR products. Then, the DNA nanogram levels were measured with Nanodrop spectrophotometry (Nanodrop, 8000-Thermo Scientific), because the amplified PCR products range should be 20 ng/µl for 750 bp. After that, the isolates were sequenced by using ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit with forward primer (E 334) as one-sided sequencing. The sequences were performed in PCR machine with proper conditions: a denaturation step for 1 min at 96°C; 30 amplification cycles: denaturation 10 sec at 96°C, annealing 30 sec at 60 °C, and 4 min extension at 60°C; hold at 4°C. The PCR product applied cycle sequences were passed through Sephadex and spin column. The amplified and sequenced isolates were put into capillary genetic analyzer machine (Applied Biosystem, ABI 3130XL). The idea of sequencing is based on the fact that each fluorescence color represents each nucleotide. Therefore, the sequence of the PCR product was compared with known 16S rRNA gene sequences available in the GenBank (NCBI) database by the basic local alignment search tool (BLAST) and/or MEGA 5.05 program.

3.2.5. Sensory Analysis

Sensory Spectrum™ presents a written record of a list of product's sensory aroma and flavor attributes, chemical feeling factors, and after taste. In this sensory analysis, a roundtable discussion with a five-member panel was conducted to identify the descriptive flavor terms for the Armola cheeses collected from different dairies in Seferihisar. Panel members were selected based on willingness to participate and time available. The panelists were staff and graduate students in the Department of Food Engineering at Çanakkale Onsekiz Mart University. The panelists' ages ranged from 26 to 43. The panelists identified and defined the flavor terms from representative cheeses. During the training sessions, real food samples and chemicals were used to identify descriptors. Sensory analyses were done in duplicate. Table 3.2. showed the descriptive terms used to define Armola cheese flavor. The panelists quantified the descriptive

terms using 15-point product specific scales where zero indicates the absence of intensity, and 15 corresponds to an extreme intensity.

Table 3.2. Terms used to describe the flavor of Armola cheese using descriptive analyses methods (Source: Delahunty and Drake, 2004)

Term	Definition	Standard
Cooked, cooked milk	Aromatics associated with cooked milk	Skim milk heated to 85 ~ for 30 min
	The combination of sweet, brown flavor notes and aromatics associated with heated milk	Evaporated milk UHT milk 3.6% fat, cooked for 10 min
Whey	Aromatics associated with Cheddar cheese whey	Fresh Cheddar whey Whey powder
Creamy	Fatty, creamy tasting, of the nature of or containing cream	Mascarpone cheese, butter, Decanolactone (0.1% in PG), UHT Cream 35% fat
Sulfur	Aromatics associated with sulphurous compounds	Boiled mashed egg. H_2S bubbled through water; struck match
Free-fatty acid	Aromatics associated with short chain fatty acids	Butyric acid (20 mg/kg)
Animalic	The combination of aromatics reminiscent of farm animals and barnyards	4-Methyl-octanoic acid (2% in PG d), Na-Caseinate 1-Phenyl-2-thiourea (5000 mg/kg in PG)
Moisty cloth	Aroma associated with wet cloth	Dirty moisty cloth
Brothy	Aromatics associated with boiled meat or vegetable stock soup	Canned potatoes Low-sodium beef broth cubes Methional (20 mg/kg)
Fermented	Aromatics associated with fermented milk	Yoghurt
Yeasty	Aromatics associated with fermenting yeast	Raw yeast dough, yeast in 3% warm sucrose water, fruit
Smokey	The penetrating, dark brown, acrid aromatic of charred wood Aroma and taste of hickory-smoked ham The penetrating smoky taste and aromatics, similar to charred wood Tainted by exposure to smoke Perception of any kind of smoke odour (hickory, apple, cherry, mesquite or artificial flavoring)	Oil of cade, barbecue dressing, Hickory smoked ham, Applewood cheese, Guaiacol (0.5% in PG), Guaiacol in vaseline oil (several concentrations), Liquid smoke flavoring. 40 µl + cotton in 60-ml flas

(cont. on next page)

Table 3.2. (cont.)

Sweet	Fundamental taste sensation of which sucrose is typical Fundamental taste sensation elicited by sugars Fundamental taste sensation produced by aqueous solutions of several products such as sucrose or fructose	Sucrose (1,3, 4 or 5% in water) Condensed milk 1.2 g sucrose/100 g Quark
Salty	Fundamental taste sensation of which sodium chloride is typical Fundamental taste sensation elicited by salts Fundamental taste sensation produced by aqueous solutions of several products such as sodium chloride	Sodium chloride (0.25, 0.5, 0.75 or 1% in water)
Sour	Fundamental taste sensation elicited by acids Fundamental taste sensation of which lactic and citric acids are typical	Citric acid (0.08% in water) Lactic acid (0.05 and 0.085% in water)
Bitter	Fundamental taste sensation of which caffeine or quinine are typical A chemical-like taste	Caffeine (0.02, 0.06 or 0.08% in water) Tonic water, quinine (0.01% in water)
Umami	Chemical feeling factor elicited by certain peptides and nucleotides	Monosodium glutamate (1% in water)
Prickle/bite	Chemical feeling factor of which the sensation of carbonation on the tongue is typical	Soda water

The panelists were provided with water, unsalted bread and expectoration cups to cleanse the palate between samples. The cheeses were presented in plastic plates and coded with three-digit numbers. Panelists evaluated each cheese twice. Duplicate samples were served in different sessions. Sensory evaluation ballot is given in Appendix B.

3.2.6. Extension of Shelf-Life of Armola Cheese

Armola cheese was provided from one of the dairies which had the best hygienic conditions based on the microbiological results obtained from collected samples. Total of 16 kg cheese sample was transported to the laboratory under refrigeration condition and stored at 4 °C until used. Then, cheese was divided into eight equal portions for eight different treatments. The pH, microbiological, sensory and color analyses were

conducted. The concentrations of antimicrobials used in each treatment were determined with preliminary studies. The treatments and the content of Nisaplin® and Natamax® (Danisco, Denmark) are given in Table 3.3. and Appendix C.

Table 3.3. Treatments applied to Armola cheese

Samples	**Treatments**
Control	No Antimicrobial
Group A (GA)	Nisaplin® (0.05 %)
Group B (GB)	Natamax® (0.005 %)
Group C (GC)	Microgard™ 100 (0.5 %)
Group D (GD)	Nisaplin® (0.05 %) + Natamax® (0.005 %)
Group E (GE)	Nisaplin® (0.05 %) + Microgard™ 100 (0.5 %)
Group F (GF)	Natamax® (0.005 %) + Microgard™ 100 (0.5 %)
Group G (GG)	Nisaplin® (0.05 %) + Natamax® (0.005 %) + Microgard™ 100 (0.5 %)

Stock solutions of Nisaplin®, Natamax®, and Microgard™ 100 were prepared in sterile distilled water immediately prior to addition. Solutions were added aseptically into the cheese samples (packaged aerobically in plastic containers with lids) and the cheese samples were stored at 4 ± 0.5 °C. The samples were analyzed at day 0, 1, 5, 8, 11, 14 and 20.

3.2.6.1. The pH Value

The pH value was recorded using a pH meter (Hanna HI 221, Germany) equipped with a glass electrode that was immersed directly into the cheese sample. Each measurement was performed three times per treatment for each replicate of cheese.

3.2.6.2. Microbiological Analyses

Armola samples were assayed for different microorganisms using six different agar media. Total aerobic mesophilic bacteria, coliforms, lactobacilli, lactococci, enterococci and yeasts were determined by using standard microbiological methods previously described in 3.2.3 section. Analyses were run in duplicate for each replicate.

3.2.6.3. Sensory Analysis

Consumer acceptance test was conducted to determine consumer responses on Armola cheeses treated with different antimicrobials and without any antimicrobial. Quantification of appearance, odor, flavor, consistency and overall acceptability differences of the eight cheese samples as well as understanding their effects on consumer acceptability were determined. In this sensory analysis, 5 trained panelists (ages ranged from 24 to 42) tasted Armola samples treated with the antimicrobials. The panelists were staff and graduate students in the Department of Food Engineering at Izmir Institute of Technology. The Armola samples were presented in plastic plates with plastic spoon. Room-temperature drinking water and unsalted crackers were provided to consumers to cleanse their palate in between samples evaluation, in order to minimize sensory carryover and/or fatigue effects. The panelists were seated in a testing room with controlled lighting and evaluated the eight cheeese samples for acceptability of appearance, odor, flavor, consistency and overall acceptability using a 5-point hedonic scale, where 1 is the lowest quality and 5 is the highest quality. Sensory analyses were done in duplicate. Duplicate samples were served in different sessions. Sensory evaluation ballot is given in Appendix B.

3.2.6.4. Color Analysis

For color analysis, Minolta CR400 (Tokyo, Japan) colorimeter was used with a reading area of 8 mm. The instrument was calibrated before each analysis using a white tile standard. Cheese samples were transferred into quartz glass cell and measurements were performed. The colorimeter directly calculated three color features of CIE *L** (lightness), *a** (redness–greenness), and *b** (yellowness–blueness). Color measurement was performed in duplicate with five readings for each replicate sample.

3.2.7. Statistical Analysis

Mean values, standard deviations, maximum and minimum values were calculated for all the determined parameters using Excel (Microsoft Office 2007).

Multidimensional Scaling (MDS) method was applied in this study to provide a visual representation of the similarities or distances among samples. Since different scales were used to measure quality characteristics of Armola cheeses, values were standardized before computing proximities. SPSS (Statistical Package for the Social Sciences) (version 13; SPSS Institute Inc., Chicago, IL) was used for all statistical analyses.

Analysis of variance (ANOVA) was applied using Minitab 14 (Minitab Inc., State College, PA, USA) to determine the effects of different antimicrobial treatments and storage time on the pH, microbiological, sensory and color results of Armola cheese samples. Multiple comparisons of means were performed using Fisher's LSD (Least Significant Differences) test with a level of 95% confidence interval.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Physicochemical Characterization of Armola Cheeses

Forty cheese samples collected from different dairies in Seferihisar were analyzed to determine the quality characteristics of Armola cheeses. Total solid, fat, and protein contents, pH, titratable acidity, water activity and salt content of Armola cheese samples were determined. Results obtained from the physicochemical analyses of the cheeses are presented in Table 4.1.

The average total solid content was 37.26% changing between 54.8% and 25.25%. Fat plays important roles in cheese in terms of effecting firmness, adhesiveness, mouth-feel, and flavor (Fox et al., 2000). The average fat content of Armola cheese samples was 19.52% with changing from 12.00% to 26.75%. The average fat content in dry matter was 31.48%. Armola cheese samples had 10.87% protein content in average ranging from 7.34% to 17.07%. The protein content of Armola cheese showed similar result (11 % protein content) with Galotyri-type cheese, a traditional Greek acid/rennet-curd cheese (Katsiari, 2009).

The average titratable acidity of samples was 0.95 ranging from 0.55 to 1.29. The lactic acid bacteria not only contribute to the taste of cheese but also help cheese maintain its convenient texture and protect it against any kind of microbiological spoilage. TS 591 (2006) and TS 3001 (2006) suggest that titratable acidity should not exceed 3 % as lactic acid in cheeses. The titratable acidity values of all Armola cheeses did not exceed 3 %. The pH value of Armola cheese samples were ranged from 4.24 to 5.51. The average pH value was 4.70.

The water activity is a measure of the availability of water for biological functions and relates to water present in a food in free form. Water activity affects the microbial activity during ripening and water activity value of less than 0.92 is necessary to prevent bacterial growth (Fox et al., 2000). Armola cheeses had maximum 0.97 and minimum 0.85 water activity values. Average water activity value was 0.91.

Salt plays a major role in the texture, flavor, and microbial quality of cheese. It affects the growth of microorganisms and their enzymatic activity directly by inhibition by lowering the water activity. Armola cheeses were analyzed for salt content and it was found that average salt content of Armola cheeses was 2.51% ranging from 1.17% to 4.91%. The average salt content in dry matter was 4.10 for the samples.

Total solid content, fat content and protein content of Armola cheese showed similarities with Myzithra Kalathaki cheese which is a typical Greek whey cheese. The result of physicochemical analysis of Myzithra Kalathaki cheese were as follows; average total solid content 33.29%, fat content 18.5% and protein content 10.5%, respectively. Due to the high moisture content and low concentration of salt, this cheese was susceptible to microbial spoilage and consequently had a limited shelf-life (11 day) (Dermiki et al., 2008). In addition, the moisture content (61.63%) and protein content (14.23%) of Pichtogalo Chanion, a kind of soft Greek cheese, was found similar with Armola cheese (Litopoulou-Tzanetaki, 2011).

Table 4.1. Results of physicochemical analyses of Armola cheeses

	Total solid content (%)	Fat content (%)	Protein content (%)	pH	Titratable Acidity (%)	Water Activity	NaCl content (%)
1	33.00±1.41	19.25±2.47	17.07±0.50	4.75±0.04	1.15±0.03	0.96±0.00	2.34±0.00
2	25.50±0.71	17.00±0.00	11.48±0.00	5.51±0.01	0.55±0.03	0.92±0.05	2.11±0.33
3	32.00±4.24	26.00±1.41	11.71±0.23	4.43±0.01	0.79±0.05	0.94±0.00	1.76±0.17
4	37.83±0.71	23.00±1.41	13.11±0.41	4.87±0.01	1.04±0.08	0.91±0.01	2.34±0.66
5	35.83±1.65	17.00±0.71	15.82±1.17	4.76±0.01	1.23±0.13	0.89±0.02	4.10±0.17
6	36.10±1.56	12.00±1.41	10.72±1.53	4.47±0.01	1.25±0.00	0.94±0.02	2.46±0.17
7	54.80±3.96	14.25±1.77	12.03±0.23	5.19±0.00	0.74±0.01	0.95±0.00	2.46±0.17
8	35.87±0.09	23.75±3.18	8.77±1.04	4.24±0.01	1.07±0.04	0.87±0.02	2.22±0.33
9	37.73±0.85	19.00±2.83	11.99±0.63	4.61±0.00	0.95±0.02	0.85±0.02	2.81±0.17
10	35.93±4.81	21.50±0.71	13.27±0.27	4.53±0.01	0.62±0.08	0.90±0.00	2.22±0.33
11	38.25±2.47	23.50±0.71	8.68±0.00	4.80±0.01	1.25±0.05	0.95±0.01	3.04±0.33
12	25.25±8.84	15.50±0.71	12.19±0.45	4.73±0.01	0.74±0.03	0.96±0.00	2.34±0.33
13	45.67±3.30	15.25±0.35	16.24±1.31	4.53±0.01	1.10±0.05	0.91±0.02	2.22±0.33
14	44.67±4.71	19.50±0.71	13.14±3.16	4.53±0.01	0.91±0.11	0.92±0.00	2.81±0.17
15	52.33±4.24	17.75±1.77	8.39±0.23	4.67±0.00	1.02±0.05	0.90±0.02	2.22±0.33
16	29.00±8.96	21.00±0.00	7.66±0.27	4.94±0.01	0.70±0.03	0.94±0.00	2.75±0.25
17	36.67±1.41	20.75±0.35	11.04±0.36	4.69±0.01	0.55±0.03	0.91±0.00	2.11±0.00
18	35.33±1.89	26.75±1.06	11.68±1.08	4.46±0.01	0.89±0.03	0.91±0.00	3.16±1.82
19	37.83±0.71	19.50±0.00	8.26±1.85	4.64±0.01	0.91±0.05	0.91±0.00	2.93±0.17
20	36.17±3.06	17.50±3.83	12.15±2.93	4.54±0.01	0.98±0.05	0.90±0.00	3.86±0.17
21	41.67±3.77	23.50±2.12	9.98±2.30	4.63±0.00	0.83±0.05	0.87±0.01	2.57±0.33
22	51.00±6.13	17.75±1.06	11.23±0.09	4.67±0.01	0.74±0.03	0.90±0.01	2.57±0.33
23	45.67±3.30	22.75±1.06	7.85±0.81	4.35±0.01	1.10±0.05	0.89±0.00	2.11±0.33
24	44.67±4.71	20.25±1.06	12.03±1.49	4.63±0.03	1.02±0.05	0.87±0.02	2.93±0.17
25	52.33±4.24	20.00±0.71	12.79±0.86	4.52±0.01	1.02±0.05	0.89±0.01	4.91±0.33
26	34.67±0.00	21.00±0.00	10.50±0.77	4.83±0.02	0.96±0.03	0.93±0.01	2.47±0.01
27	25.67±9.90	16.00±0.00	10.18±1.49	4.87±0.03	1.12±0.03	0.89±0.02	2.28±0.03
28	32.33±0.47	22.25±1.77	8.96±0.95	4.41±0.01	1.29±0.00	0.93±0.02	2.22±0.33
29	33.50±0.24	20.25±3.89	8.04±0.63	4.84±0.02	0.89±0.03	0.91±0.02	2.81±0.17
30	36.33±0.94	18.75±0.35	13.14±2.53	4.53±0.04	1.21±0.11	0.88±0.04	2.47±0.01
31	34.50±1.65	21.75±0.35	11.29±1.35	5.02±0.04	0.91±0.00	0.89±0.01	1.64±0.33
32	37.67±0.47	17.00±0.00	7.34±0.00	5.18±0.00	0.76±0.11	0.92±0.01	2.11±0.33
33	34.00±0.00	13.50±0.71	10.94±2.03	4.35±0.01	1.23±0.13	0.91±0.02	1.87±0.33
34	34.83±2.12	20.25±1.06	11.07±0.23	4.66±0.00	0.91±0.21	0.92±0.00	3.04±0.00
35	41.49±0.69	18.75±0.35	10.21±0.54	4.58±0.00	1.01±0.02	0.97±0.00	3.16±0.00
36	32.03±0.98	20.25±0.35	9.16±0.32	5.51±0.00	0.98±0.03	0.96±0.00	2.43±0.06
37	37.64±0.90	17.00±0.00	7.53±0.18	4.43±0.00	0.77±0.01	0.95±0.00	2.23±0.00
38	35.67±3.30	20.25±1.06	9.86±0.59	4.70±0.01	0.95±0.27	0.87±0.03	1.64±0.33
39	26.83±1.18	19.75±4.60	8.80±0.81	4.44±0.01	1.10±0.05	0.93±0.01	1.17±0.00
40	32.33±0.47	20.00±2.83	8.39±0.50	4.96±0.00	0.95±0.27	0.90±0.03	1.40±0.17
Ave.	37.26	19.52	10.87	4.70	0.95	0.91	2.51
Min.	25.25	12	7.34	4.24	0.55	0.85	1.17
Max.	54.80	26.75	17.07	5.51	1.29	0.97	4.91

Mean value ± standard deviation; Ave.: Average value; Min.: Minimum value; Max.: Maximum value

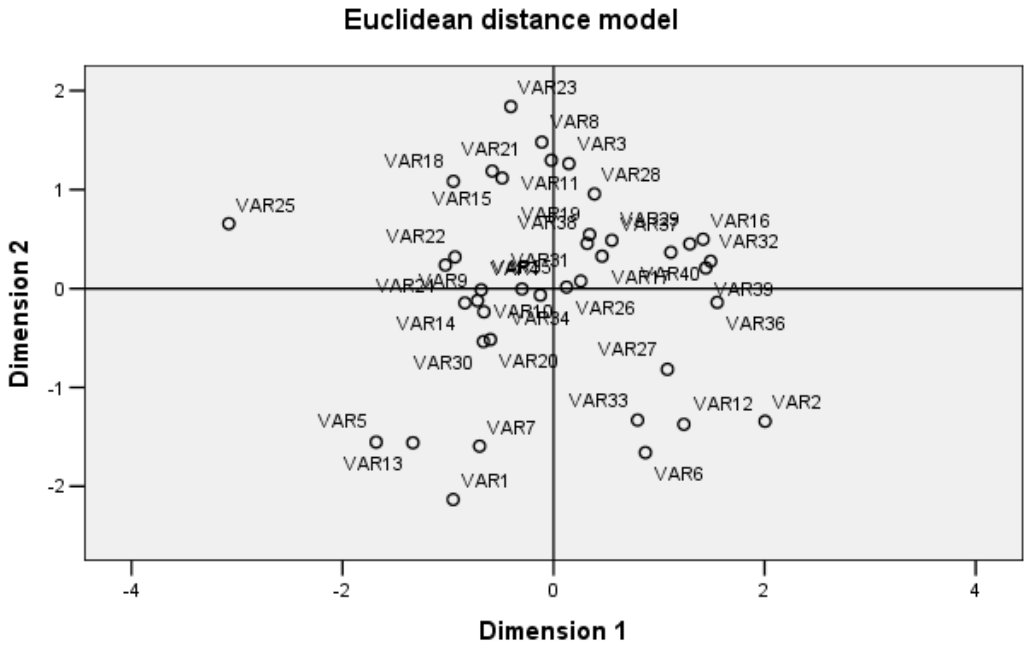

Figure 4.1. Geometrical representation of Armola cheeses in terms of physicochemical characteristics by multidimensional scaling (Each var represents the cheeses in order) (Stress= 0.22, R^2= 0.76)

Figure 4.1. shows relations between physicochemical characteristics. Sample 25 was different from other samples because of high salt content. In addition, samples 1, 3, 5, and 7 differed from other samples in terms of high protein contents. Moreover, samples 2, 12, 27, and 33 were different due to the fact that they had lower total solid contents when compared to other samples. The differences of physicochemical composition between samples are due to the fact that there is no standard method for manufacturing of Armola cheese in dairies. Therefore, it is necessary that production needs to be standardized.

4.2. Microbial Characterization of Armola Cheeses

In microbiological analyses of Armola cheese samples, minimum total aerobic mesophilic bacteria count was 6.15 log cfu/g and maximum value was found to be 8.87 log cfu/g. Maximum microbial counts for psychrotrophic bacteria were 8.08 log cfu/g and minimum value was 5.70 log cfu/g. Maximum microbial counts for coliforms was 6.14 log cfu/g and minimum value was 3.06 log cfu/g. The results of *Lactobacillus* spp. enumeration were minimum 6.62 log cfu/g and maximum 8.67 log cfu/g. Minimum and

maximum *Lactococcus spp.* counts were 6.54 log cfu/g and 8.72 log cfu/g, respectively. *Enterococcus* spp. counts were found in between 4.66 log cfu/g and 7.24 log cfu/g. Minimum and maximum yeast counts were 5.18 log cfu/g and 8.33 log cfu/g, respectively. None of Armola cheese samples contain mold (<1.00 log cfu/g) (data not shown). The results of *Staphylococcus* spp. enumeration were minimum 4.00 log cfu/g and maximum 7.60 log cfu/g. Maximum *Listeria* spp. counts were 3.91 log cfu/g and some of Armola cheese samples did not contain *Listeria* spp. (<1 log cfu/g). The average total aerobic mesophilic bacteria, psychrotrophic bacteria, coliform, lactobacilli, lactococci, enterococci, yeast, *Staphylococcus* spp., *and Listeria* spp. microbial counts were 7.82, 6.98, 4.56, 7.87, 7.55, 6.17, 7.33, 5.94, and 2.94 log cfu/g, respectively in Armola cheese samples.

Similarly, Kykkidou et al. (2007) reported high initial counts for total aerobic mesophilic bacteria, lactobacilli and lactococci in Galotyri cheese, a traditional Greek Protected Designation of Origin (PDO) soft acid-curd cheese, stored aerobically at 4 $^{\circ}$C. The microbial counts for all Armola cheese samples are given in Table 4.2.

Table.4.2. Microbial counts of Armola cheeses (log cfu/g)

Sample Number	Total aerobic mesophilic bacteria	Psychrotrophic bacteria	Coliform	Lactobacillus spp.	Lactococcus spp.	Enterococcus spp.	Yeast	Staphylococcus spp.	Listeria spp.
1	6.32±0.22	6.72±0.31	3.10±0.01	8.07±0.09	7.02±0.20	5.43±0.06	7.11±0.05	5.27±0.04	2.24±0.34
2	8.13±0.01	7.67±0.04	4.17±0.01	8.03±0.06	7.38±0.02	5.78±0.31	7.43±0.01	5.20±0.36	3.43±0.22
3	8.15±0.08	7.17±0.15	4.05±0.04	7.78±0.03	7.14±0.06	6.14±0.06	6.85±0.12	4.72±0.18	<1
4	6.87±0.10	6.07±0.07	3.06±0.09	8.17±0.04	7.81±0.04	5.96±0.02	6.89±0.05	6.42±0.07	3.36±0.19
5	7.00±0.05	6.91±0.02	4.63±0.04	7.97±0.05	7.62±0.09	5.94±0.01	8.11±0.05	6.44±0.02	3.91±0.03
6	6.90±0.00	6.69±0.07	4.15±0.17	7.76±0.04	6.54±0.09	5.90±0.07	6.96±0.04	6.41±0.05	2.97±0.16
7	8.21±0.09	8.08±0.07	5.43±0.15	7.36±0.02	7.47±0.01	5.67±0.28	6.94±0.02	6.41±0.12	3.62±0.16
8	6.50±0.01	6.91±0.01	4.37±0.02	8.13±0.08	7.83±0.03	7.03±0.04	6.85±0.03	6.20±0.08	3.50±0.01
9	6.49±0.01	6.29±0.13	4.03±0.04	7.95±0.13	7.72±0.34	7.04±0.04	7.41±0.01	5.18±0.06	3.27±0.11
10	6.23±0.13	6.12±0.00	4.50±0.20	8.03±0.06	6.75±0.08	7.24±0.03	8.08±0.03	5.90±0.13	3.36±0.06
11	7.48±0.00	5.70±0.00	4.14±0.20	8.48±0.00	6.92±0.11	5.34±0.03	7.19±0.01	5.75±0.06	2.66±0.26
12	6.47±0.01	6.47±0.10	4.74±0.08	8.39±0.04	7.83±0.15	5.74±0.05	7.38±0.04	4.85±0.10	2.88±0.26
13	6.15±0.12	5.92±0.16	3.32±0.01	7.28±0.08	7.84±0.15	6.58±0.01	7.21±0.08	5.22±0.29	3.35±0.12
14	7.83±0.04	7.74±0.05	4.43±0.10	7.79±0.12	7.74±0.02	7.14±0.01	7.40±0.56	5.76±0.08	2.78±0.11
15	7.84±0.11	6.88±0.03	4.41±0.18	8.06±0.07	7.82±0.03	6.36±0.15	8.11±0.02	6.07±0.08	2.25±0.35
16	7.10±0.02	6.73±0.31	5.12±0.02	8.04±0.07	8.54±0.02	6.78±0.10	6.95±0.06	6.38±0.09	3.40±0.14
17	7.70±0.02	7.67±0.04	5.66±0.01	7.86±0.21	7.23±0.02	5.17±0.02	6.97±0.11	5.94±0.05	2.75±0.06
18	8.10±0.50	7.17±0.16	3.75±0.02	7.57±0.04	8.00±0.00	6.78±0.11	6.87±0.12	7.23±0.04	2.74±0.06
19	8.20±0.02	6.04±0.08	4.74±0.03	7.83±0.21	7.87±0.06	7.02±0.03	8.14±0.03	7.60±0.03	2.57±0.16
20	8.26±0.01	6.93±0.02	5.64±0.00	7.61±0.02	7.83±0.01	6.71±0.23	7.99±0.03	6.43±0.00	2.68±0.26
21	8.00±0.03	7.30±0.02	4.39±0.00	8.14±0.03	8.26±0.07	5.81±0.14	8.14±0.11	7.04±0.11	3.02±0.23
22	8.29±0.02	7.00±0.14	3.94±0.09	8.12±0.04	7.57±0.06	5.86±0.05	7.62±0.10	5.33±0.03	<1
23	8.04±0.01	7.05±0.01	3.88±0.11	8.15±0.00	7.65±0.09	6.07±0.07	7.90±0.09	6.48±0.11	2.46±0.21
24	8.08±0.03	7.44±0.03	5.06±0.07	7.57±0.01	7.75±0.11	6.09±0.05	7.80±0.01	6.80±0.12	2.17±0.21
25	8.07±0.07	7.80±0.00	4.65±0.26	7.63±0.06	7.64±0.23	6.70±0.03	7.77±0.03	6.30±0.03	2.37±0.49
26	8.87±0.02	7.18±0.01	4.77±0.06	7.66±0.07	6.75±0.06	6.90±0.02	7.94±0.01	5.86±0.04	3.29±0.24
27	8.41±0.01	6.93±0.04	3.62±0.02	7.75±0.04	7.10±0.02	5.49±0.13	5.97±0.10	4.00±0.00	2.74±0.06
28	8.34±0.01	6.80±0.00	3.48±0.00	8.02±0.03	7.34±0.01	7.13±0.05	7.24±0.12	5.28±0.28	3.45±0.03
29	8.38±0.03	7.61±0.07	5.55±0.21	8.11±0.05	6.95±0.07	5.95±0.07	7.80±0.04	6.07±0.10	2.90±0.08
30	8.38±0.03	7.84±0.00	4.78±0.01	8.08±0.00	7.67±0.10	4.98±0.19	7.00±0.08	6.12±0.12	3.16±0.06
31	8.52±0.02	7.40±0.06	5.90±0.04	7.60±0.05	7.20±0.08	6.90±0.12	7.94±0.06	6.45±0.21	<1
32	8.38±0.03	7.20±0.05	5.77±0.05	8.48±0.01	7.54±0.09	5.06±0.08	7.48±0.07	6.34±0.06	<1
33	8.56±0.02	7.49±0.03	4.63±0.05	8.67±0.00	7.21±0.08	6.82±0.03	8.33±0.09	5.63±0.05	2.74±0.17
34	8.78±0.00	7.48±0.04	5.14±0.01	8.61±0.00	7.75±0.04	6.97±0.03	7.98±0.02	6.14±0.01	3.15±0.21
35	8.29±0.04	7.37±0.09	5.24±0.00	7.89±0.04	8.01±0.24	7.21±0.07	8.17±0.13	5.98±0.08	3.58±0.00
36	8.22±0.02	7.01±0.12	5.75±0.16	7.93±0.09	6.73±0.11	5.53±0.02	6.81±0.05	5.81±0.14	<1
37	7.70±0.01	6.58±0.11	6.14±0.04	7.23±0.10	7.22±0.19	6.05±0.07	7.95±0.09	7.41±0.01	3.07±0.21
38	8.55±0.06	6.34±0.01	4.94±0.05	7.67±0.11	8.45±0.03	4.94±0.14	5.18±0.00	5.21±0.04	<1
39	8.39±0.09	7.23±0.01	3.77±0.08	6.70±0.01	7.68±0.14	4.66±0.07	5.73±0.25	4.98±0.04	2.06±0.14
40	8.78±0.07	6.39±0.14	3.47±0.18	6.62±0.01	8.72±0.00	5.84±0.06	5.64±0.44	5.10±0.02	2.20±0.09

(cont. on next page)

Table 4.2. (cont.)

Ave.	7.82	6.98	4.56	7.87	7.55	6.17	7.33	5.94	2.94
Min.	6.15	5.70	3.06	6.62	6.54	4.66	5.18	4.00	<1
Max	8.87	8.08	6.14	8.67	8.72	7.24	8.33	7.60	3.91

Mean value ± standard deviation
Ave.: Average count; Min.: Minimum count; Max.: Maximum count

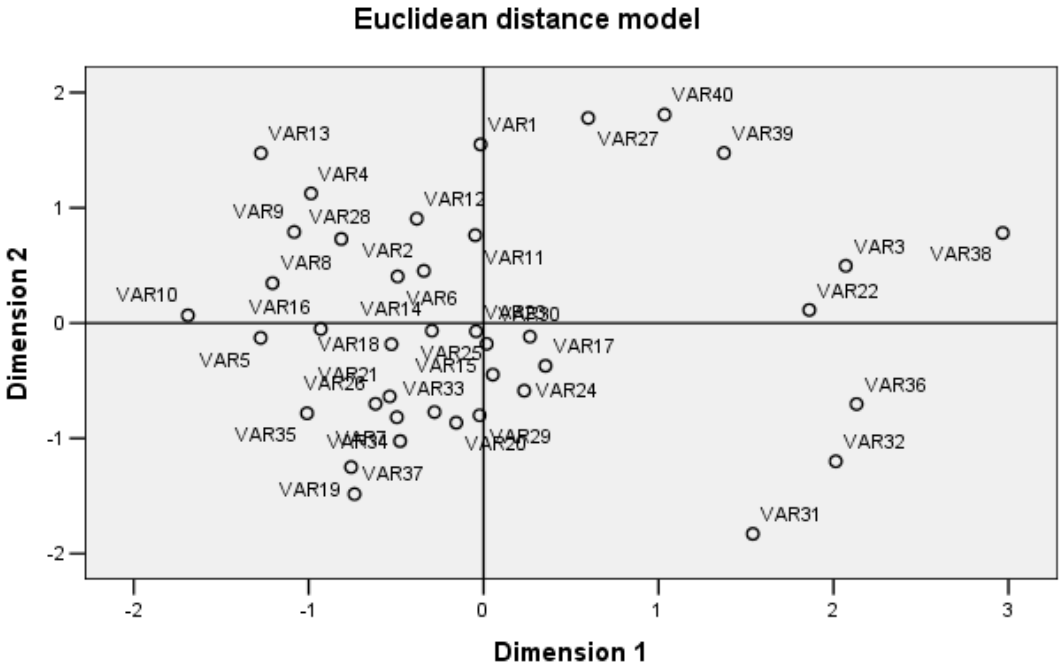

Figure 4.2. Geometrical representation of Armola cheeses in terms of microbiological characteristics by multidimensional scaling (Each var represents the cheeses in order), (Stress= 0.16, R2= 0.89)

Figure 4.2. shows relations between microbiological characteristics. Samples 27, 39 and 40 were different from other samples because of low yeast counts. In addition, samples 3, 22 and 38 differed from other samples in terms of low *Staphylococcus* spp. counts. Moreover, samples 31, 32 and 36 were different due to the fact that they had lower *Listeria* spp. when compared to other samples. The microbial results of Armola cheeses obtained from different dairies in Seferihisar indicate that manufacturing, storage, and marketing of Armola cheese were done in poor hygienic conditions. The marked differences in microbial load of samples result from different processing methods and insufficient sanitary conditions during manufacturing. For improving the

microbial quality of Armola cheese, it should be manufactured under good hygienic conditions and stored at refrigeration temperatures until consumption.

4.3. Molecular Identification of Microbial Flora

Lactic acid bacteria (LAB) were the major microbial group in Armola cheese. From the MRS, M17 and Kanamycin esculin azide agar plates, a total of 87 colonies representative of all different morphologies were chosen randomly from each plate with 5-10 colonies and transferred to broth media for further identification by DNA isolation, PCR and 16S rRNA sequencing.

4.3.1.Extraction of Bacterial Genomic DNA

Phenol-chloroform extraction method (Giraffa, 2000) was used to extract DNA from the 87 isolates obtained in this study. The DNA quality and purity were determined based on the A_{260}/A_{280} ratios (the absorbance of nucleic acids to absorbance of amino acids) measured by Nanodrop (8000-Thermo Scientific). The levels between 1.80-2.00 levels were chosen to be used in molecular studies.

4.3.2. 16S rRNA Analysis

The molecular classification of the isolated LAB strains was detected by the 16S rRNA sequence analyses. The 16S rRNA sequence coding region was amplified by polymerase chain reaction (PCR). E334F and E939R primers were used to amplify 750 bp fragment of the 16S rRNA gene. Upon PCR amplification, 87 isolates yielding a 750 bp product were assigned at the species level by sequence analyses. 16S rRNA sequence similarity was performed at GenBank data library by using the BLAST program.

The 87 selected strains exhibited similarity of 16S rDNA sequence of over 93-99% to *Enterococcus ratti, Enterococcus durans, Enterococcus hirae* (15); *Enterococcus faecalis* (7); *Streptococcus lutetiensis, Streptococcus equines,*

Streptococcus luteciae (18); *Streptococcus pasteurianus, Streptococcus alactolyticus, Streptococcus macedonicus* (4); *Streptococcus thermophilus* (12); *Lactobacillus paracasei subsp. tolerans, Lactobacillus casei subsp. casei, Lactobacillus zeae, Lactobacillus paracasei subsp. paracasei* (12); *Lactobacillus coryniformis subsp. torquens* (4); *Lactobacillus delbrueckii subsp. lactis* (9); *Lactobacillus delbrueckii subsp. indicus* (5); and *Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus pentosus* (1). 16 rRNA sequence results of these bacteria are given in Appendix D.

This diversity of LAB isolated from Armola cheese could be linked to the different varities of milk, cheese and yoghurt used in production of Armola cheese. Moreover, the differences observed from one lactic acid bacteria species to another were explained by De Roissart (1986). In fact, the acidifying activity of each strain is related to its specific capacity to break down the substances in the medium. The differences are also due to the presence or absence of nutrient transport systems (Badis et al., 2004).

4.4. Sensory Analysis of Armola Cheeses

Total of eighteen samples were collected from different dairies and analyzed using Sensory Spectrum™. As a result of sensory analyses of Armola cheeses, predominant basic taste was salty for all Armola cheese samples. Other basic tastes detected by panelists were sour, sweet, bite, umami and bitter, in decreasing order. Cooked, creamy, whey, fermented, free fatty acid, brothy, animalic, yeasty, fermented, sulfurous, moisty cloth aromatic terms were also found as aromatics for Armola cheeses in decreasing order.

The average results of sensory flovar profiles of the Armola cheeses are shown in Figure 4.3 and Table 4.3. The Scores given by panel members for each descriptive term are presented in Appendix E.

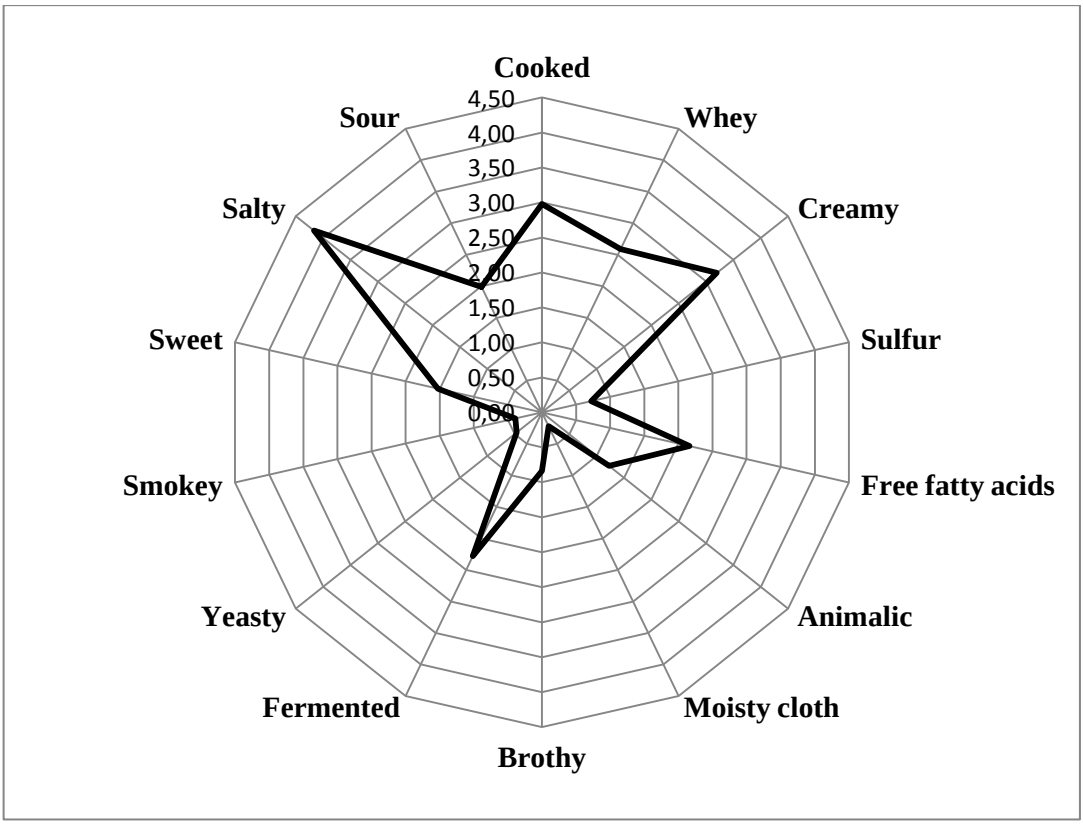

Figure 4.3. Typical flavor profile diagram of Armola cheese

Table 4.3. Descriptive sensory analyses results

	Average
AROMATICS	
Cooked	2.98±0.49
Whey	2.59±0.68
creamy	3.20±0.48
sulfur	0.72±0.56
free fatty acids	2.16±0.97
Animalic	1.23±0.57
Moisty cloth	0.22±0.16
Brothy	0.84±0.38
Fermented	2.28±0.34
Yeasty	0.46±0.51
Smokey	0.39±0.80
BASIC TASTES	
Sweet	1.52±0.55
Salty	4.17±1.43
Sour	1.99±0.74
Bitter	0.28±0.28
Umami	0.40±0.27
Prickle/bite	0.66±0.47

4.5. Shelf -Life Extension of Armola Cheese

4.5.1.The pH Value

The initial pH value of Armola cheese was 4.96 for all treatments and at the end of 20 days of storage, the pH value dropped to 4.80 for the control sample. The pH value of GB (treated with Natamax®) and GD (treated with Nisaplin® and Natamax®) cheese samples were not statistically significant than the control sample ($P>0.05$). The GF (treated with Natamax® and Microgard™ 100) sample showed the most significant reducing effect on pH value ($P<0.05$). Due to the antimycotic effect of Natamax® on

yeast population which metabolized lactic acid and caused a pH raise in the microenvironment of cheese, the pH value decreased (Torkar and Vengust, 2008). On the other hand, during storage, the pH values of GA (treated with Nisaplin®), GC (treated with Microgard™ 100), GE (treated with Nisaplin® and Microgard™ 100) and GG (treated with Nisaplin®, Natamax® and Microgard™ 100) samples significantly increased ($P<0.05$) to reach 5.32, 5.36, 5.31, and 5.02, respectively due to the bactericidal activitiy of Nisaplin® and Microgard™ 100 on lactic acid and psychrotrophic bacteria. The pH values of the samples treated with antimicrobials during storage are shown in Table 4.4. and Figure 4.4.

Table 4.4. The pH values of Armola cheese samples treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	pH						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.96±0.00 a,A	4.92±0.00 ab,ABC	4.91±0.03 bc,ABC	4.89±0.01 bc,ABC	4.93±0.00 c,AB	4.88±0.00 b,C	4.80±0.00 b,D
GA	4.96±0.00 a,A	4.92±0.00 ac,A	4.96±0.02 c,A	4.95±0.00 d,A	5.01±0.02 d,A	5.24±0.07 ef,B	5.32±0.00 d,B
GB	4.96±0.00 a,A	4.89±0.01 a,AB	4.90±0.00 b,AB	4.86±0.00 b,B	4.86±0.01 b,B	4.89±0.04 b,AB	4.74±0.00 ab,C
GC	4.96±0.00 a,A	4.95±0.00 bcd,A	4.93±0.01 bc,A	4.92±0.03 cd,A	4.92±0.00 c,A	5.12±0.05 de,B	5.36±0.07 d,C
GD	4.96±0.00 a,A	4.89±0.01 a,AB	4.93±0.00 bc,A	4.94±0.00 d,A	4.92±0.00 c,AB	4.90±0.00 bc,AB	4.81±0.08 b,B
GE	4.96±0.00 a,A	4.99±0.00 d,A	5.00±0.01 d,A	5.08±0.00 f,B	4.96±0.00 cd,A	5.35±0.02 f,C	5.31±0.03 d,C
GF	4.96±0.00 a,A	4.96±0.00 cd,A	4.81±0.00 a,B	4.76±0.00 a,AB	4.70±0.03 a,A	4.59±0.02 a,B	4.61±0.00 a,B
GG	4.96±0.00 a,A	4.98±0.02 d,AB	5.03±0.00 d,BC	5.03±0.00 e,BC	5.06±0.02 e,C	5.04±0.01 cd,BC	5.02±0.00 c,B

a-f: Values within each storage time followed by the same letter are not significantly different ($P > 0.05$).
A-D: Values within each treatment followed by the same letter are not significantly different ($P > 0.05$).
Data are means values ± one standard deviation (n=2).

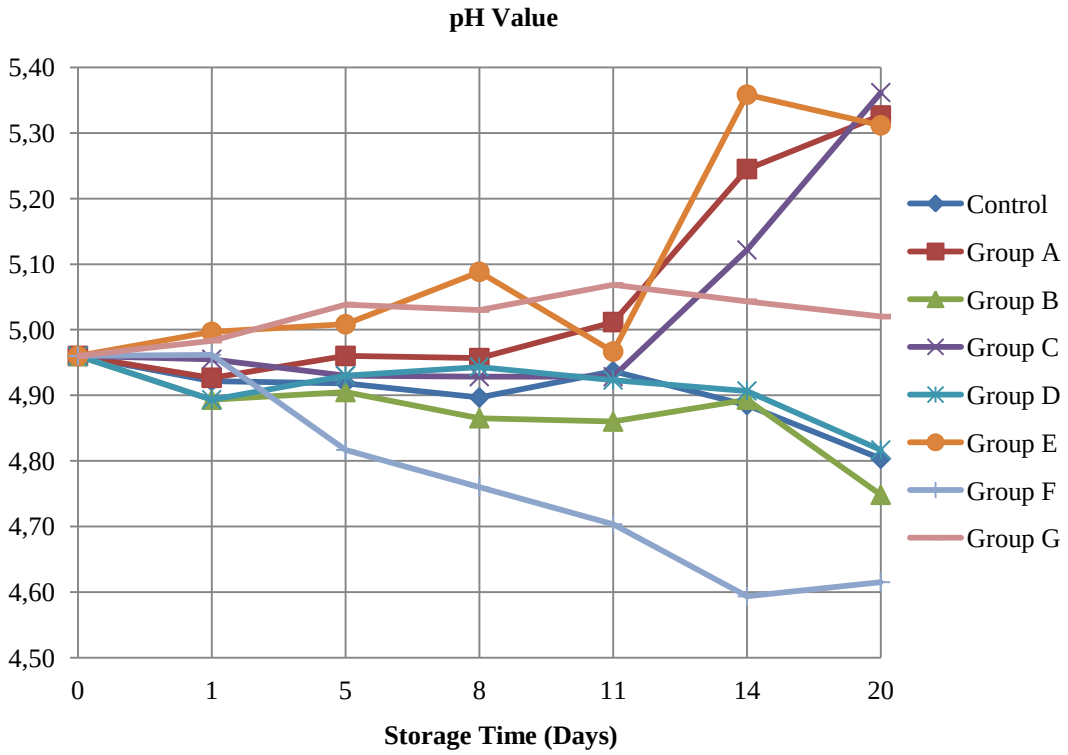

Figure 4.4. Changes in pH values of Armola cheese samples treated with different antimicrobials during storage of 20 days at 4 °C

4.5.2. Microbiological Analyses

The effects of antimicrobials (Nisaplin®, Natamax®, and Microgard™ 100), alone and their combinations on the microbiological quality (total aerobic mesophilic bacteria, coliforms, *Lactobacillus* ssp., *Lactococcus* ssp., *Enterococcus* spp., and yeast) of Armola cheeses stored at 4 °C during 20 days of storage were investigated.

4.5.2.1. Effects of Antimicrobials on Total Aerobic Mesophilic Bacteria Counts

The total aerobic mesophilic bacteria counts for control and treated Armola cheeses during storage are shown in Table 4.5. The initial concentration of total aerobic mesophilic bacteria count was 8.77 log cfu/g for Armola cheeses. The GE (Nisaplin® and Microgard™ 100) treatment showed a significant reducing effect ($P < 0.05$) on total aerobic mesophilic bacteria when compared with control sample at the end of storage. The treatment with Nisaplin®, Natamax® and Microgard™ 100 (GG) had significant

inhibitory effect on day 11; thereafter, counts increased on day 20 to reach final populations of ca. 8.82 log cfu/g. In addition, all treatments except GA (Nisaplin®), GB (Natamax®), and GD (Nisaplin® and Natamax®) showed bacteriocidal effect on day 8 compared to control sample, then counts increased during the rest of storage. As a result, the most effective treatment was the treatment with Nisaplin® and Microgard™ 100 (GE) on total aerobic mesophilic bacteria count in Armola cheese.

Table 4.5. Total aerobic mesophilic bacteria counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Total Aerobic Mesophilic Bacteria Counts (log cfu/g)						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	8.77±0.07 a,B	9.30±0.00 a,A	9.36±0.17 a,A	7.74±0.13 b,C	8.82±0.13 a,B	8.78±0.06 a,B	8.80±0.02 ab,B
GA	8.77±0.07 a,A	8.48±0.09 b,B	8.68±0.08 cd,AB	7.82±0.02 b,C	8.69±0.06 ab,AB	8.60±0.06 a,AB	8.71±0.04 abc,A
GB	8.77±0.07 a,A	8.63±0.04 b,A	8.92±0.06 bc,A	6.76±0.17 a,B	8.59±0.13 ab,A	8.71±0.01 a,A	8.71±0.21 bc,A
GC	8.77±0.07 a,B	8.59±0.08 b,BC	9.09±0.00 ab,A	7.82±0.03 a,D	8.59±0.10 ab,BC	8.68±0.00 a,B	8.44±0.00 bc,C
GD	8.77±0.07 a,AB	8.55±0.0 b,B	8.63±0.06 d,AB	7.16±0.16 ab,C	8.39±0.01 bc,B	8.75±0.00 a,AB	8.95±0.18 a,A
GE	8.77±0.07 a,A	8.52±0.16 b,AB	8.66±0.04 cd,AB	6.49±0.18 a,C	8.50±0.01 abc,AB	8.65±0.00 a,AB	8.32±0.04 c,B
GF	8.77±0.07 a,A	8.63±0.13 b,A	8.60±0.07 d,A	6.78±0.51 a,B	8.71±0.12 ab,A	8.71±0.20 a,A	8.44±0.00 bc,A
GG	8.77±0.07 a,A	8.53±0.06 b,AB	8.52±0.02 d,AB	6.52±0.29 a,C	8.20±0.00 c,B	8.68±0.09 a,AB	8.82±0.15 ab,A

a-d: Values within each storage time followed by the same letter are not significantly different ($P > 0.05$).
A-D: Values within each treatment followed by the same letter are not significantly different ($P > 0.05$).
Data are means values ± one standard deviation (n=2).

Samelis et al. (2003) investigated nisin treatments to control *Listeria monocytogenes* post-processing contamination on Anthotyros, a traditional Greek whey cheese, stored at 4 °C in vacuum packages. Nisin was added in cheese as well as in whey during production. They reported that Anthotyros cheese without nisin in the whey contained 3.67±0.3 log cfu/g of total microbial flora after preparation. Otherwise, total microbial flora of Anthotyros samples with 100 or 500 IU nisin g^{-1} of whey were

<2.0 log cfu/g. During storage at 4°C, the total microbial flora increased in all treatments, and eventually exceeded 8 log cfu g^{-1}, except in cheese samples with 500 IU nisin g^{-1} of whey. They stated that the observed effect of nisin was based on the application method and concentration of nisin.

4.5.2.2. Effects of Antimicrobials on Coliform Counts

The coliform counts for control and treated Armola cheeses during storage are shown in Table 4.6. The treatments had no significant effect (P > 0.05) on the growth of coliform bacteria in cheese samples when compared to control sample during storage. Otherwise, the best effective treatment was the treatment with Microgard^{TM} 100 (GC) resulting with 1 log cycle reduction when compared to other treatments on day 11.

Table 4.6. Coliform counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Coliform Counts (log cfu/g)						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	3.45±0.1 a,A	3.45±0.39 a,A	3.19±0.09 ab,A	3.21±0.14 a,A	3.08±0.14 a,A	3.18 ±0.07 a,A	2.85±0.01 ab,A
GA	3.45±0.18 a,A	3.32±0.28 a,AB	3.13±0.15 ab,AB	2.83±0.08 a,B	2.80±0.25 a,B	3.19±0.21 a,AB	2.85±0.01 ab,AB
GB	3.45±0.18 a,A	3.00±0.09 a,B	2.92±0.02 a,B	3.07±0.00 a,B	2.87±0.24 a,B	2.86±0.03 a,B	2.68±0.12 a,B
GC	3.45±0.18 a,B	3.41±0.29 a,A	3.00±0.14 a,BC	2.93±0.17 a,CD	2.47±0.23 a,D	3.01±0.13 a,BC	2.77±0.04 ab,CD
GD	3.45±0.18 a,A	3.39±0.28 a,A	3.49±0.10 b,A	3.07±0.05 a,AB	2.74±0.04 a,B	2.92±0.07 a,AB	2.81±0.04 a,B
GE	3.45±0.18 a,A	3.24±0.02 a,AB	3.44±0.17 b,A	3.11±0.19 a,AB	2.71±0.28 a,A	2.87±0.06 a,AB	2.89±0.04 b,AB
GF	3.45±0.18 a,A	3.11±0.23 a,AB	3.53±0.10 b,A	3.15±0.05 a,AB	2.78±0.14 a,B	2.84±0.06 a,B	2.84±0.07 ab,B
GG	3.45±0.18 a,A	3.22±0.18 a,A	3.21±0.16 ab,A	3.10±0.06 a,A	3.02±0.19 a,A	3.07±0.01 a,A	2.94±0.01 b,A

a-b: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-D: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

4.5.2.3. Effects of Antimicrobials on *Lactobacillus* spp. Counts

The *Lactobacillus* spp. counts for control and treated Armola cheeses during storage are shown in Table 4.7. and Fig 4.5. The counts of *Lactobacillus* spp. remained the same when treated with Microgard™ 100 (GC), Nisaplin® and Natamax® (GD) and Natamax® and Microgard™ 100 (GF) at end of storage. During storage, the decrease in *Lactobacillus* spp. counts was significantly greater for GG (treated with Nisaplin®, Natamax® and Microgard™ 100) samples compared to other treatment. When compared with control sample, the decline in lactobacilli populations for GA (treated with Nisaplin®), GB (treated with Natamax®), GE (treated with Nisaplin® and Microgard™ 100), and GG (treated with Nisaplin®, Natamax® and Microgard™ 100) cheese samples could be attributed to the effect of nisin on these Gram-positive bacterial species. At the end of storage, the treatments with Nisaplin® and Microgard™ 100 (GE) and Nisaplin®, Natamax® and Microgard™ 100 (GG) significantly showed the most inhibitory effect on *Lactobacillus* spp. and bacterial level was 1 log cycle lower than of the control sample ($P<0.05$).

Table 4.7. *Lactobacillus* spp. counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	***Lactobacillus* spp. Counts (log cfu/g)**						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	6.61±0.01 a,A	6.46±0.46 abc,A	6.95±0.04 a,AB	7.36±0.10 a,BC	7.57±0.23 a,BC	7.71±0.02 a,C	7.73±0.01 ab,C
GA	6.61±0.01 a,B	5.97±0.01 bc,A	6.14±0.11 c,A	6.71±0.01 c,B	6.94±0.00 cde,C	7.43±0.01 bc,D	7.38±0.02 c,D
GB	6.61±0.01 a,A	6.64±0.00 a,A	6.95±0.05 a,B	7.31±0.00 a,C	7.37±0.09 ab,C	7.39±0.00 c,C	7.44±0.01 c,C
GC	6.61±0.01 a,A	6.58±0.03 a,A	7.06±0.00 a,B	7.15±0.00 ab,B	7.32±0.01 abc,C	7.46±0.04 abc,D	7.52±0.02 bc,D
GD	6.61±0.01 a,B	5.82±0.04 c,A	6.62±0.18 b,B	7.03±0.11 b,C	7.05±0.02 bcd,C	7.64±0.05 ab,D	7.88±0.13 a,D
GE	6.61±0.01 a,B	5.97±0.10 bc,A	6.54±0.00 b,B	6.78±0.04 c,BC	6.93±0.17 de,CD	7.09±0.04 d,D	6.97±0.02 d,CD
GF	6.61±0.01 a,A	6.79±0.10 a,AB	7.06±0.07 a,BC	7.35±0.09 a,CD	7.41±0.02 ab,DE	7.34±0.13 c,CD	7.72±0.11 ab,E
GG	6.61±0.01 a,B	5.91±0.02 c,A	6.18±0.06 c,A	6.64±0.03 c,B	6.64±0.08 e,B	6.84±0.12 d,BC	6.98±0.11 d,C

a-e: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-E: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

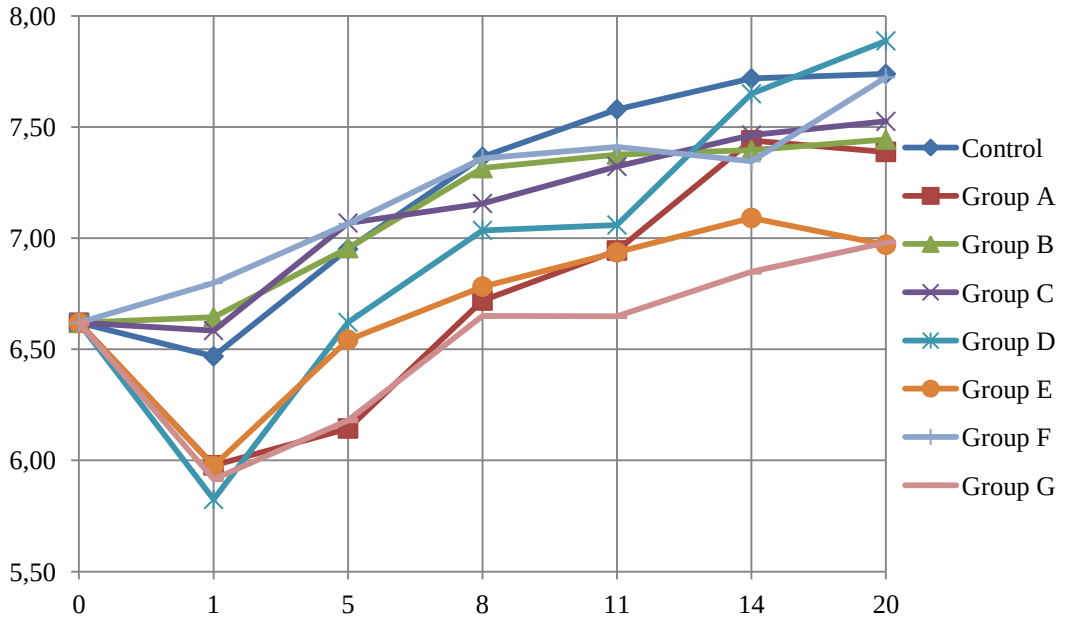

Figure 4.5. Changes in *Lactobacillus* spp. counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Kykkidou et al. (2007) reported the effects of treatment with nisin on lactobacilli counts of a Greek soft acid-curd cheese stored aerobically at 4 °C. Lactobacilli counts varied within 0.5 log range during the first 2 weeks of storage in control N0 (no nisin added) and N1 (nisin 50 IU g^{-1}) and N2 (nisin 150 IU g^{-1})-treated Galotyri cheese samples. Populations of lactobacilli (ca. 8.0–8.4 log cfu/g) declined after day 4 of storage, in all three Galotyri cheeses up to day 21 of storage, and thereafter increased to reach final populations of ca. 6.7, 5.8 and 5.0 log cfu/g, respectively. The nisin treatments had a significant effect ($P<0.05$) on lactobacilli counts for N1 and N2 cheese samples after day 14 and up to the final day 42 of storage. During this period the reduction in their population was greater for the N2 treatment. The decline in lactobacilli populations for N1 and N2 cheese samples could be attributed to the effect of nisin on these Gram-positive bacterial species.

4.5.2.4. Effects of Antimicrobials on *Lactococcus* spp. Counts

The *Lactococcus* spp. counts for control and treated Armola cheeses during storage are shown in Table 4.8. The *Lactococcus* spp. counts showed a similar trend for GE (treated with Nisaplin® and Microgard™ 100) and GG (treated with Nisaplin®,

Natamax® and Microgard™ 100) samples during storage. Both treatments significantly showed inhibitory effect on *Lactococcus* spp. at the end storage. Initial lactococci counts at day 0 were slightly higher compared to lactobacilli populations by ca. 0.52 and 0.41 cfu/g. The lower numbers of lactobacilli populations compared to lactococci throughout the storage may also be due to the anaerobic incubation of lactobacilli (Kykkidou et al., 2007). The growth of *Lactococcus* spp. delayed when treated with Nisaplin® (GA), Natamax® (GB) and Nisaplin® and Natamax® (GD) up to day 14, 11, and 5; thereafter, counts increased to reach populations of ca. 7.38, 7.88, and 6.98 log cfu/g at the end of storage. In addition, there was no inhibitory effect on GC (treated with Microgard™ 100) and GF (treated with Nisaplin®, Natamax® and Microgard™ 100) cheese samples in terms of reducing *Lactococcus* spp. counts during storage. In conclusion, the combination of Nisaplin® with other antimicrobials was more effective than Nisaplin® alone.

Table 4.8. *Lactococcus* spp. counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	***Lactococcus* spp. Counts (log cfu/g)**						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	8.71±0.00 a,A	8.63±0.03 a,AB	8.35±0.06 a,B	8.36±0.02 a,B	8.51±0.15 a,AB	8.36±0.04 ab,B	8.56±0.16 ab,AB
GA	8.71±0.00 a,A	8.43±0.06 bc,AB	8.01±0.00 b,CD	7.73±0.05 c,DE	8.40±0.17 c,BCD	7.41±0.09 c,E	8.37±0.02 bc,ABC
GB	8.71±0.00 a,A	8.57±0.01 ab,AB	8.32±0.05 a,BC	8.18±0.02 ab,C	7.57±0.14 bc,D	8.43±0.02 ab,ABC	8.67±0.12 a,A
GC	8.71±0.00 a,A	8.41±0.04 bc,AB	8.46±0.14 a,AB	8.30±0.12 ab,B	8.28±0.11 ab,B	8.44±0.08 ab,AB	8.42±0.03 ab,AB
GD	8.71±0.00 a,A	8.38±0.01 c,AB	7.83±0.03 bc,B	8.13±0.30 abc,AB	7.76±0.37 abc,B	8.51±0.05 a,A	8.33±0.01 bc,AB
GE	8.71±0.00 a,A	8.27±0.00 c,B	7.93±0.04 bc,D	8.16±0.08 abc,BC	8.09±0.07 abc,CD	8.55±0.03 a,A	8.15±0.00 cd,BC
GF	8.71±0.00 a,A	8.59±0.04 a,BC	8.04±0.09 b,C	8.32±0.06 a,BC	8.34±0.19 ab,BC	8.50±0.06 a,AB	8.32±0.04 bc,BC
GG	8.71±0.00 a,A	8.29±0.08 c,AB	7.76±0.12 c,BC	7.90±0.01 bc,BC	7.37±0.38 c,C	8.22±0.09 b,AB	8.04±0.05 d,B

a-d: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-E: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

Kykkidou et al. (2007) also reported the effects of treatment with nisin on lactococci counts of a Greek soft acid-curd cheese stored aerobically at 4 °C. Initial (day 0) lactococci counts were 8.5 log cfu/g and they were slightly higher compared to respective lactobacilli populations by ca. 0.5 cfu/g during 35 days. The lactococci numbers significantly decreased ($P<0.05$) beyond day 21 and up to the final storage day (day 42) and counts were significantly lower ($P<0.05$) for N1(nisin 50 IU g^{-1}) and N2 (nisin 150 IU g^{-1}) treatments compared to the control samples. The decline in lactococci populations for N2 treatment was greater than N1.

4.5.2.5. Effects of Antimicrobials on *Enterococcus* spp. Counts

The *Enterococcus* spp. counts for control and treated Armola cheeses during storage are shown in Table 4.9. Initial *Enterococcus* spp. counts of Armola cheese were ca. 5.81 log cfu/g for all treatments. At the end of storage *Enterococcus* spp. did not show any significant growth in all treated Armola samples when compared to control sample. Hovewer, in GG samples treatment with Nisaplin®, Natamax®, and Microgard™ 100 significantly showed inhibitory effect on day 14. In control treatment, bacterial level was 0.34 log cycles lower compared to the initial values. The counts were equal to those initially enumerated for treatments, probably due to low acid pH values of the cheese samples. The remain unchanged of *Enterococcus* spp. is important, because dairy food strains of enterococci contribute to the ripening and aroma development of traditional cheeses (Ogier and Serror, 2008). In conclusion, the antimicrobials and their combinations had an immediate bacteriocidal effect ($P > 0.05$) on inhibiting enterococci growth compared to control sample.

Table 4.9. *Enterococcus* spp. counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	***Enterococcus* spp. Counts (log cfu/g)**						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	5.81±0.08 a,A	6.35±0.06 a,B	6.74±0.05 a,C	6.62±0.05 a,C	6.17±0.06 a,B	5.76±0.01 abc,A	6.15±0.06 a,B
GA	5.81±0.08 a,A	5.92±0.15 bcd,A	6.34±0.11 ab,B	5.77±0.09 e,A	5.78±0.06 a,A	5.97±0.13 ab,A	5.71±0.04 a,A
GB	5.81±0.08 a,A	6.09±0.13 abc,A	6.60±0.30 ab,A	6.36±0.04 bc,A	6.23±0.21 a,A	6.04±0.31 a,A	6.15±0.09 a,A
GC	5.81±0.08 a,AB	6.30±0.07 a,B	6.65±0.00 ab,B	6.31±0.03 c,B	5.98±0.43 a,AB	5.55±0.03 abcd,A	6.02±0.09 a,AB
GD	5.81±0.08 a,B	5.84±0.00 bcd,B	6.45±0.06 ab,A	6.16±0.11 cd,AB	5.89±0.25 a,B	5.40±0.03 bcd,C	5.95±0.02 a,B
GE	5.81±0.08 a,B	5.80±0.18 cd,B	6.48±0.00 ab,A	5.91±0.12 de,B	6.09±0.19 a,AB	5.14±0.11 cd,C	5.84±0.02 a,B
GF	5.81±0.08 a,A	6.18±0.05 ab,A	6.58±0.06 ab,A	6.60±0.03 ab,A	6.29±0.00 a,A	5.63±0.13 abcd,A	6.16±0.71 a,A
GG	5.81±0.08 a,AB	5.73±0.03 d,AB	6.24±0.05 b,A	5.71±0.01 e,AB	5.67±0.09 a,B	4.97±0.34 d,C	5.83±0.10 a,AB

a-d: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-C: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

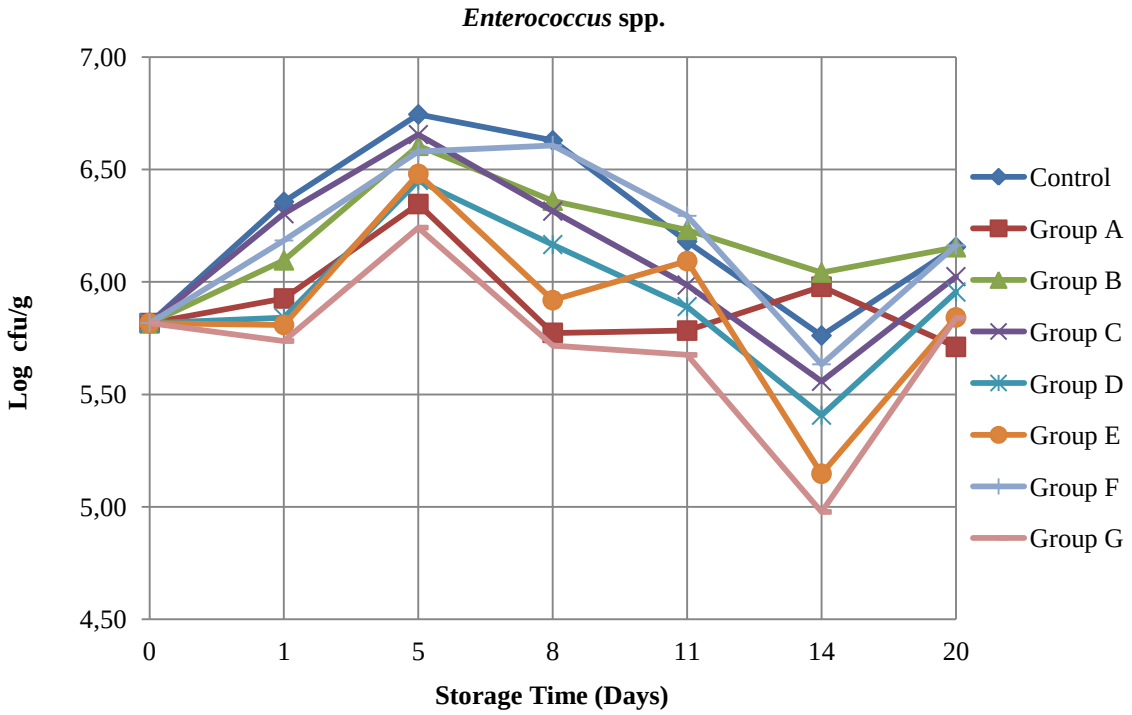

Figure 4.6. Changes in *Enterococcus* spp. counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

4.5.2.6. Effects of Antimicrobials on Yeast Counts

The yeast counts for control and treated Armola cheeses during storage are given in Table 4.5. and Figure4.7. At 20 days of storage, a significant increase was observed for control, GA (treated with Nisaplin®), GC (treated with Microgard™ 100) and GE (treated with Nisaplin® and Microgard™ 100) samples for yeast counts. On the other hand, yeast counts in the cheese samples treated with Natamax® showed a completely different pattern of growth at the end of storage. Yeast populations decreased significantly ($P<0.05$) for the GB (treated with Natamax®), GD (treated with Nisaplin® and Natamax®), GF (treated with Natamax® and Microgard™ 100) and GG (treated with Nisaplin®, Natamax® and Microgard™ 100) cheese samples which were treated with Natamax® alone or in combination to reach final yeast counts of 4.60, 4.41, 4.46, and 4.46 log cfu/g, respectively. Otherwise, the highest level was found in GA (treated with Nisaplin®) samples. As a result, treatment with Nisaplin® and Microgard™ 100 did not show significant inhibitory effect on yeast population compared to other treatments.

Table 4.5. Yeast counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Yeast Counts (log cfu/g)						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	5.60±0.47 a,A	6.28±0.01 a,AB	7.10±0.03 a,C	7.06±0.21 a,C	7.01±0.18 b,BC	6.98±0.06 b,BC	7.09±0.04 a,C
GA	5.60±0.47 a,A	6.29±0.02 a,A	7.16±0.22 a,B	7.25±0.13 a,B	7.48±0.14 a,B	7.44±0.13 a,B	7.42±0.02 a,B
GB	5.60±0.47 a,A	5.63±0.24 b,A	5.28±0.02 b,AB	5.31±0.00 cd,AB	4.64±0.04 d,BC	4.57±0.03 de,C	4.60±0.13 b,BC
GC	5.60±0.47 a,AB	6.09±0.05 a,A	6.86±0.14 a,BC	6.57±0.17 b,BC	6.58±0.13 b,BC	6.52±0.19 c,BC	6.97±0.04 a,C
GD	5.60±0.47 a,A	5.60±0.10 b,A	5.24±0.13 b,AB	5.62±0.01 c,A	4.82±0.01 d,BC	4.71±0.07 d,BC	4.41±0.07 b,C
GE	5.60±0.47 a,A	6.30±0.06 a,ABC	6.82±0.01 a,BCD	7.39±0.05 a,D	6.14±0.07 c,AB	6.89±0.09 bc,CD	7.14±0.08 a,D
GF	5.60±0.47 a,A	5.50±0.05 b,A	5.09±0.10 b,AB	4.99±0.01 d,AB	4.49±0.03 d,B	4.33±0.03 e,B	4.46±0.30 b,B
GG	5.60±0.47 a,A	5.50±0.05 b,AB	4.84±0.03 b,BC	5.02±0.01 a,BC	4.83±0.15 d,BC	4.65±0.07 de,C	4.46±0.16 b,C

a-d Values within each storage time followed by the same letter are not significantly different ($P > 0.05$). A-D Values within each treatment followed by the same letter are not significantly different ($P > 0.05$). Data are means values ± one standard deviation (n=2).

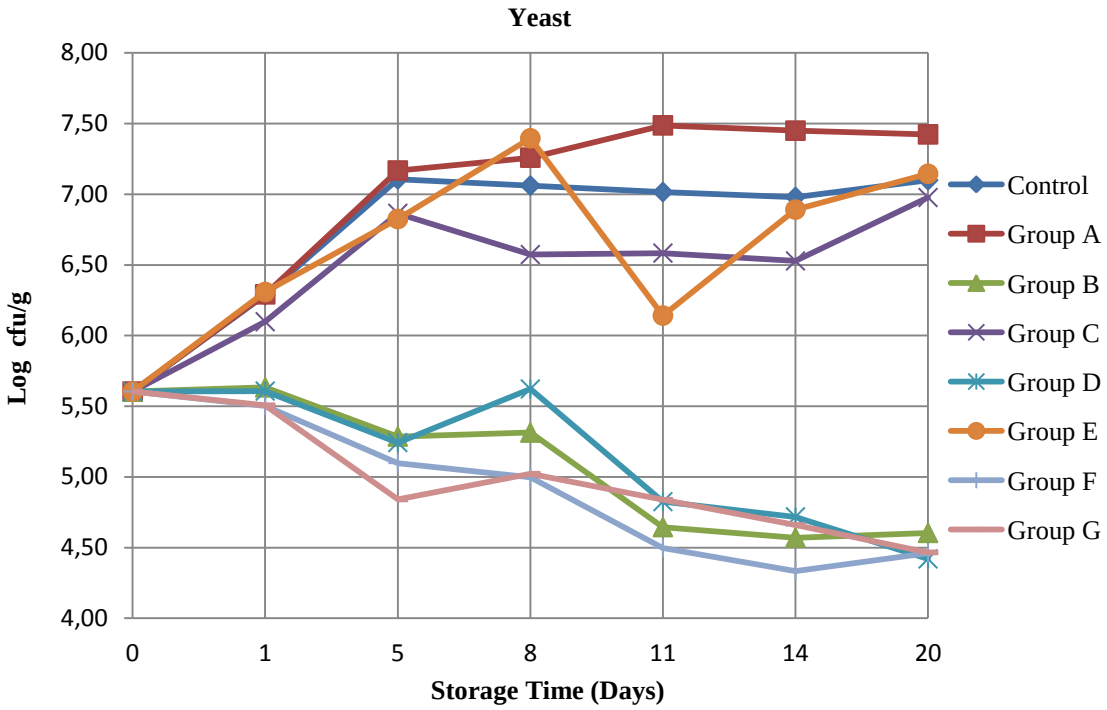

Figure 4.7. Changes in yeast counts of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

The yeast populations in the control and treated with nisin 50 IU g^{-1} (N1) and nisin 150 IU g^{-1} (N2) Galotyri cheese samples stored aerobically at 4 °C were investigated. Analysis of cheese samples showed that the mean initial (day 0) population of yeasts was low (<2 cfu/g) and stayed constant up to days 14 and 28 of storage for the control and treated Galotyri cheese samples, thereafter increasing to reach final counts of ca. 6.8, 5.8 and 5.0 cfu/g, respectively. After day 14 and up to the final day 42 of storage, yeast populations were significantly lower ($P<0.05$) for the N1 and N2-treated Galotyri cheese samples compared to the control samples (Kykkidou et al., 2007).

Var et al. (2006) studied that the effects of antimicrobial agent (natamycin) and packaging materials on the microbiological properties of Kashar cheese during ripening period. Natamycin and packaging materials had no effect on the total aerobic mesophilic bacteria, yeast, and lipolytic microorganism counts. However, natamycin had showed inhibitory effect on yeast growth alone and in combination with packaging material. At the end of the 150th day, the lowest level of yeast number was found in cheese which applied natamycin and packaging material.

4.5.3. Sensory Analysis

The sensory quality of the control and treated Armola cheese samples were investigated during 20 days of storage. In particular, cheese appearance, odor, flavor, consistency and overall acceptability were assessed by 5 trained panelists.

The color differences between control and antimicrobial-treated Armola cheese samples are shown in Figure 4.8. The appearance scores given by the panelists for the control and treated Armola cheese samples are presented in Table 4.11. Individual appearance scores showed a similar pattern of decreasing acceptability for the control and treated samples. At the end of storage, appearance scores for GA (treated with Nisaplin®) and GC (treated with Microgard™ 100) samples were 1.7, whereas treatments with Natamax® (GB), Nisaplin® and Natamax® (GD) and Natamax® and Microgard™ 100 (GF) showed major effect on appereance. Some kinds of yeasts were recognized as responsible for the yellow color on the surface of cheeses as a result of associative action. Consequentially, the cheese samples treated with Natamax® exhibited less or no yellow color on the surface of cheeses.

Table 4.11. Appearance scores of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Appearance Scores						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.7±0.14 a,A	4.80±0.00 a,A	4.10±0.14 a,AB	4.10±0.14 a,AB	3.50±0.14 a,AB	3.20±0.00 a,B	2.40±0.00 ab,B
GA	4.7±0.14 a,A	4.50±0.42 a,AB	3.70±0.42 a,ABC	3.70±0.42 a, ABC	3.40±0.28 a,AB	3.00±0.28 a,C	1.70±0.42 a,D
GB	4.7±0.14 a,A	4.50±0.14 a,AB	4.60±0.28 a,AB	4.60±0.28 a,AB	4.10±0.42 a,BCD	3.80±0.28 a,CD	3.30±0.14 b,D
GC	4.7±0.14 a,A	4.30±0.14 a,A	3.50±0.14 a,A	3.50±0.14 a,B	3.40±0.28 a,B	2.80±0.28 a,B	1.70±0.14 a,C
GD	4.7±0.14 a,A	4.40±0.00 a,AB	4.30±0.42 a,AB	4.30±0.42 a,AB	4.20±0.56 a,AB	3.60±0.56 a,AB	3.20±0.28 b,B
GE	4.7±0.14 a,A	4.50±0.4 a,A	3.60±0.28 a,AB	3.60±0.28 a,A	3.70±0.42 a,AB	2.90±0.42 a,B	2.30±0.70 ab,B
GF	4.7±0.14 a,A	4.30±0.42 a,A	4.20±0.84 a,AB	4.20±0.84 a,A	4.20±0.28 a,AB	3.80±0.00 a,AB	3.30±0.14 b,B
GG	4.7±0.14 a,A	3.90±0.14 a,AB	3.70±0.42 a,B	3.70±0.42 a,AB	3.30±0.42 a,B	3.30±0.14 a,B	2.70±0.42 ab,B

a-b: Values within each storage time followed by the same letter are not significantly different ($P > 0.05$).
A-D: Values within each treatment followed by the same letter are not significantly different ($P > 0.05$).
Data are means values ± one standard deviation (n=2).

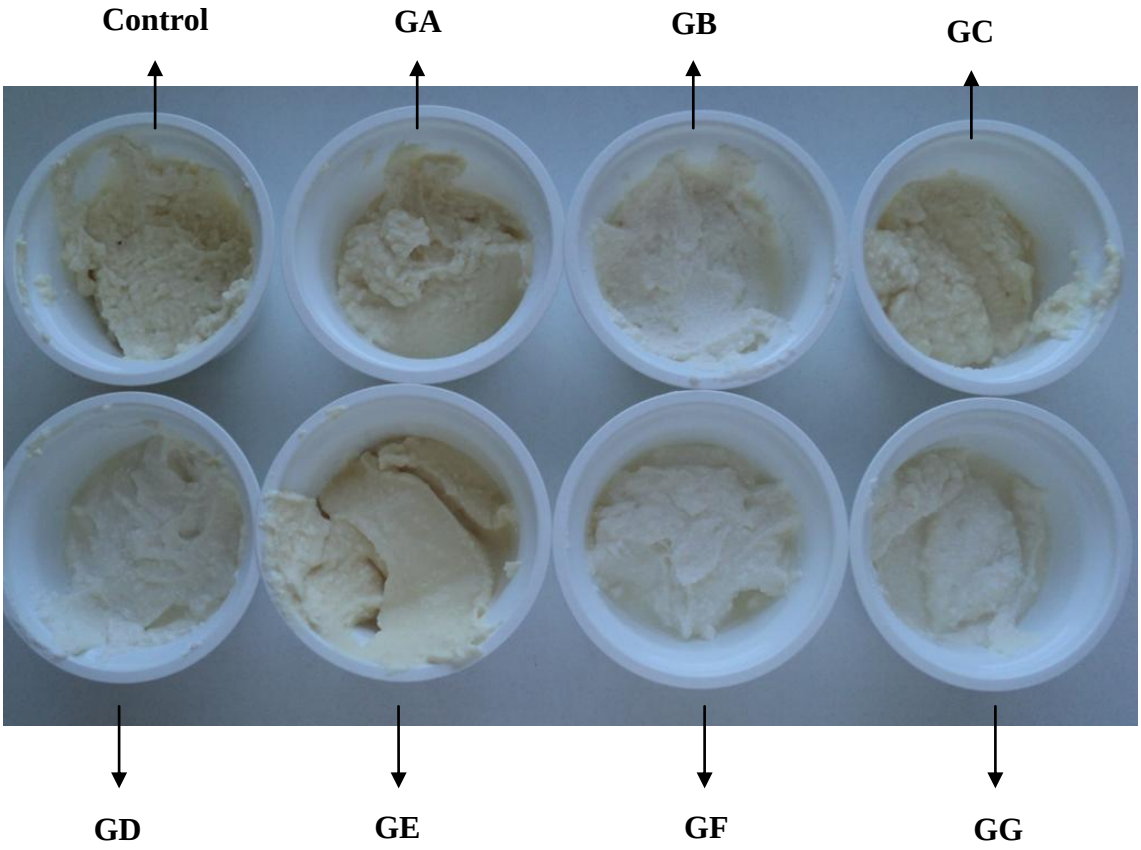

Figure 4.8. Armola cheeses treated with different antimicrobials stored at 4 °C on day 11

The odor scores given by the panelists for the control and treated samples are presented in Table 4.12. The odor scores for each treatment during storage were significantly different ($P < 0.05$). GA (treated with Nisaplin®) and GC (treated with Microgard™ 100) samples had the worst odor acceptabilities. Both of the treatments caused detrimental effect on cheeses. Otherwise, Natamax® and its combination with other antimicrobials (GB (Natamax®), GD (Nisaplin® and Natamax®), GF (Natamax® and Microgard™ 100) and GG (Nisaplin®, Natamax® and Microgard™ 100)) presented better results on odor acceptability of cheeses during storage and there were no significant differences between treatments when compared to control sample.

Table 4.12. Odor scores of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Odor Scores						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.80±0.00 a,A	4.60±0.00 a,AB	3.70±0.42 a,ABC	3.60±0.28 ab,BCD	3.70±0.42 a,ABC	2.50±0.70 a,D	2.60±0.00 bc,CD
GA	4.80±0.00 a,A	4.60±0.28 a,AB	4.30±0.14 bc,ABC	3.90±0.14 ab,ABC	3.30±0.14 a,BC	2.30±1.27 a,C	1.40±0.00 a,D
GB	4.80±0.00 a,A	4.80±0.00 a,AB	4.50±0.14 c,AB	4.40±0.00 b,AB	4.30±0.14 a,BCD	3.90±0.14 a,CD	3.20±0.00 c,D
GC	4.80±0.00 a,A	4.40±0.00 a,A	3.30±0.42 ab,A	3.70±0.42 ab,B	3.80±0.28 a,B	2.70±0.14 a,B	1.50±0.14 a,C
GD	4.80±0.00 a,A	4.60±0.28 a,A	4.30±0.14 bc,A	4.20±0.28 ab,A	4.10±0.14 a,A	4.00±0.56 a,A	3.00±0.56 c,A
GE	4.80±0.00 a,A	4.50±0.14 a,A	3.20±0.00 a,AB	3.30±0.14 a,A	4.10±0.42 a,AB	2.20±0.84 a,B	1.80±0.56 ab,B
GF	4.80±0.00 a,A	4.50±0.14 a,A	4.10±0.42 abc,AB	4.50±0.14 b,A	4.20±0.56 a,AB	4.10±0.14 a,AB	3.30±0.14 c,B
GG	4.80±0.00 a,A	4.30±0.14 a,AB	3.50±0.42 abc,B	4.00±0.56 ab,AB	3.50±0.14 a,B	3.60±0.00 a,B	3.50±0.42 c,B

a-c: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-D: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

The results of the sensory evaluation of the control and treated Armola cheese samples on flavor are presented in Table 4.13. Individual flavor scores showed a similar pattern of decreasing acceptance of flavor for all treatments except for GG (Nisaplin®, Natamax® and Microgard™ 100) treated cheese samples on day 14. The treatment of GG (Nisaplin®, Natamax® and Microgard™ 100) significantly showed the best result. At the end of storage, all of the treatments had undesirable taste and we could say that the cheeses were spoiled. In addition, samples treated with Microgard™ 100 had bitter taste.

Table 4.13. Flavor scores of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Flavor Scores						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.80±0.00 a,A	4.60±0.00 a,B	4.10±0.14 bc,AB	3.60±0.56 bc,BC	3.90 ±0.14 a,AB	2,50±0,71 a,C	1,00±0,00 a,D
GA	4.80±0.00 a,A	4.70±0.14 a,A	3.70±0.14 bc,AB	3.70±0.14 bc,AB	3.20 ±0.28 a,BC	2,50±0,71 a,BC	1,50±0,71 a,C
GB	4.80±0.00 a,A	4.70±0.14 a,A	4.40±0.28 c,AB	4.40±0.28 c,AB	4.00 ±0.28 a,AB	3,50±0,71 ab,B	2,00±0,00 a,C
GC	4.80±0.00 a,A	4.40±0.00 a,A	2.50±0.42 a,B	2.50±0.42 a,B	3.30 ±0.42 a,B	3,00±0,00 ab,B	1,00±0,00 a,C
GD	4.80±0.00 a,A	4.50±0.42 a,A	3.60±0.00 bc,AB	3.60±0.00 bc,AB	3.20 ±0.00 a,B	3,50±0,71 ab,B	2,00±0,00 a,C
GE	4.80±0.00 a,A	4.60±0.0 a,AB	2.70±0.14 ab,B	2.70±0.14 ab,B	3.60 ±0.28 a,B	3,50±0,71 ab,B	1,00±0,00 a,C
GF	4.80±0.00 a,A	4.50±0.14 a,AB	3.90±0.14 bc,AB	3.90±0.14 bc,AB	3.80 ±0.28 a,B	4,00±0,00 ab,AB	1,50±0,71 a,C
GG	4.80±0.00 a,A	4.30±0.14 a,AB	3.40±0.56 abc,AB	3.40±0.56 abc,AB	3.50±0.42 a,B	4,50±0,71 b,AB	2,00±0,00 a,C

a-c: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-D: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

The results of the sensory evaluation of the control and treated Armola cheese samples on consistency are presented in Table 4.14. A decrease in consistency was observed for all treatments during storage, but there were no significant differences between treatments on consistency acceptability when compared to control sample. However, the combination of Natamax® and Microgard™ 100 showed the major effect on consistency when compared to other treatments.

Table 4.14. Consistency scores of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Consistency Scores						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.80±0.00 a,A	4.60±0.00 a,A	3.50±0.14 ab,B	3.50±0.14 ab,B	4.10±0.14 ab,AB	3.40±0.28 ab,B	2.30±0.42 ab,C
GA	4.80±0.00 a,A	4.60±0.28 a,A	3.40±0.00 ab,B	3.40±0.00 ab,B	3.50±0.42 ab,B	3.10±0.14 ab,B	2.10±0.14 ab,C
GB	4.80±0.00 a,A	4.70±0.14 a,A	4.40±0.28 a,A	4.40±0.28 a,A	4.30±0.14 ab,A	3.80±0.2 ab,AB	3.10±0.70 ab,B
GC	4.80±0.00 a,A	4.10±0.14 a,AB	3.00±0.28 b,BC	3.00±0.28 b,BC	3.10±0.14 a,BC	2.70±0.70 a,BC	1.90±0.98 a,C
GD	4.80±0.00 a,A	4.60±0.28 a,A	4.10±0.70 ab,AB	4.10±0.70 ab,AB	3.90±0.70 ab,AB	3.40±0.56 ab,AB	2.70±0.14 ab,B
GE	4.80±0.00 a,A	4.70±0.14 a,A	3.10±0.14 b,BC	3.10±0.14 b,BC	3.70±0.14 a,AB	3.00±0.28 ab,BC	2.20±0.28 ab,C
GF	4.80±0.00 a,A	4.40±0.28 a,AB	4.00±0.00 ab,BC	4.00±0.00 ab,BC	4.50±0.42 b,AB	4.10±0.14 b,ABC	3.60±0.00 b,C
GG	4.80±0.00 a,A	4.10±0.14 a,AB	3.30±0.42 ab,BC	3.30±0.42 ab,BC	3.20±0.56 ab,BC	3.10±0.42 ab,BC	2.40±0.56 ab,C

a-b: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-C: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

The results of the sensory evaluation of the control and treated Armola cheese samples on overall acceptability are presented in Table 4.15. The overall acceptability of GA (treated with Nisaplin®), GC (treated with Microgard™ 100), and GE samples indicated that they were statistically similar to each other about reducing liking of cheeses at the end of storage, but surprisingly lower than that of control sample. Otherwise, GB (treated with Natamax®), GD (treated with Nisaplin® and Natamax®), GE (treated with Nisaplin® and Microgard™ 100) and GF (treated with Natamax® and Microgard™ 100) samples presented better results on overall acceptability of cheeses during storage, but there were no significant differences when compared to control sample. As a result, cheeses treated with Natamax® were more effective on overall acceptability of the cheeses by panelists than other treatments without Natamax®.

Table 4.15. Overall acceptability scores of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	Overall Acceptability Scores						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	4.80±0.00 a,A	4.60 ±0.00 a,A	3.80±0.28 abc,AB	3.80±0.28 abc,AB	3.80±0.28 abc,AB	3.10±0.70 a,BC	2.50±0.14 abc,C
GA	4.80±0.00 a,A	4.60±0.28 a,A	3.80±0.00 abc,AB	3.80±0.00 abc,AB	3.00±0.00 a,B	2.90±0.70 a,B	1.70±0.14 a,C
GB	4.80±0.00 a,A	4.70±0.14 a,AB	4.60±0.28 c,D	4.60±0.28 c,D	4.00±0.28 bc,B	3.90±0.14 a,BC	3.40±0.28 c,CD
GC	4.80±0.00 a,A	4.30 ±0.14 a,B	3.10±0.14 a,B	3.10±0.14 ab,B	3.30±0.14 ab,B	2.60±0.28 a,B	1.50±0.42 a,C
GD	4.80±0.00 a,A	4.60±0.00 a,A	3.90±0.42 abc,AB	3.90±0.42 abc,AB	3.80±0.28 abc,AB	3.70±0.70 a,AB	3.10±0.14 abc,B
GE	4.80±0.00 a,A	4.60 ±0.00 a,A	2.90±0.14 a,AB	2.90±0.14 a,AB	3.70±0.14 abc,AB	2.60±0.56 a,CD	2.10±0.70 ab,C
GF	4.80±0.00 a,A	4.40 ±0.28 a,A	4.10±0.42 bc,AB	4.10±0.42 bc,AB	4.20±0.28 c,AB	4.10±0.14 a,AB	3.30±0.14 bc,B
GG	4.80±0.00 a,A	4.20 ±0.00 a,AB	3.40±0.56 ab,AB	3.40±0.56 ab,AB	3.30±0.42 ab,B	3.50±0.14 a,AB	3.00±0.56 bc,B

a-c: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-D: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

Finally, the use of sensory affective tests can help the product developers to understand the behaviour of different consumer groups and, therefore, to understand potential buyers of the product and in which way such product can be inserted into the food market. Data obtained from consumer acceptance tests represent key information in studies of product development, quality control, food product acceptance, and food service evaluation. This type of testing is a very accurate tool in understanding consumer preferences about Armola cheeses with antimicrobials.

4.5.4. Color Analysis

The color of control and treated cheeses was evaluated based on the CIE L^*, a^*, and b^* values using a portable Minolta Colorimeter CR-400 (Tokyo, Japan) during storage. The CIE L^*, a^*, and b^* values correspond to visual lightness (as values increase from 0 to 100), redness to greenness (positive to negative values, respectively) and yellowness to blueness (positive to negative values, respectively) (Rynne et al., 2008).

Color analyses of control and treated cheeses indicated that in general, L^* values remained constant, a^* values and b^* values increased at the end of storage. These trends indicated that the cheeses increased in redness (positive a^* value) and yellowness (positive b^* value) but did not change in whiteness (constant L^* value) during storage. Data for L^*, a^*, and b^* values are shown in Tables 4.16, 4.17 and 4.18, respectively.

Table 4.16. L* (lightness) values of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	L* (lightness) Values						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	82.52±0.54 a,A	82.34±0.00 abc,A	82.81±0.23 a,A	82.96±0.16 a,A	82.97±0.10 a,A	82.59±0.21 abc,A	83.12±0.42 a,A
GA	82.52±0.54 a,A	81.77±0.40 ab,A	82.70±0.16 a,A	82.91±0.07 a,A	82.61±0.15 a,A	82.30±0.06 a,A	82.48±0.06 ab,A
GB	82.52±0.54 a,A	82.22±0.08 c,A	82.84±0.03 a,A	82.97±0.09 a,A	82.78±0.53 a,A	82.96±0.01 cd,A	83.03±0.08 ab,A
GC	82.52±0.54 a,A	82.92±0.01 cd,A	82.61±0.08 a,A	82.12±0.04 a,A	82.71±0.16 a,A	82.53±0.06 ab,A	82.51±0.13 ab,A
GD	82.52±0.54 a,A	83.18±0.00 d,A	82.96±0.07 a,A	82.74±0.39 a,A	83.25±0.05 a,A	83.02±0.16 d,A	83.02±0.18 ab,A
GE	82.52±0.54 a,A	82.85±0.04 bcd,A	82.27±0.88 a,A	82.64±0.12 a,A	82.83±0.20 a,A	82.24±0.17 a,A	82.34±0.28 b,A
GF	82.52±0.54 a,A	82.69±0.36 bcd,A	83.04±0.40 a,A	83.05±0.03 a,A	83.19±0.06 a,A	82.93±0.06 bcd,A	82.98±0.10 ab,A
GG	82.52±0.54 a,A	82.72±0.04 bcd,A	82.88±0.20 a,A	82.76±0.01 a,A	82.64±0.21 a,A	82.64±0.03 abcd,A	82.54±0.03 ab,A

a-d Values within each storage time followed by the same letter are not significantly different ($P > 0.05$). A Values within each treatment followed by the same letter are not significantly different ($P > 0.05$). Data are means values ± one standard deviation (n=2).

Table 4.17. a* (redness-greenness) values of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	a* (redness-greenness) Values						
	Day 0	**Day 1**	**Day 5**	**Day 8**	**Day 11**	**Day 14**	**Day 20**
Control	-1.46±0.02 a,AB	-1.53±0.00 ab,A	-1.41±0.00 a,ABC	-1.38±0.01 abc,ABC	-1.30±0.07 ab,BCD	-1.22±0.09 bcd,D	-1.20±0.06 b,D
GA	-1.46±0.02 a,A	-1.50±0.01 ab,A	-1.44±0.00 a,AB	-1.42±0.00 a,AB	-1.32±0.00 ab,B	-1.14±0.04 cd,C	-1.20±0.08 b,C
GB	-1.46±0.02 a,AB	-1.56±0.00 a,A	-1.44±0.08 a,AB	-1.48±0.00 a,AB	-1.42±0.01 a,B	-1.43±0.00 a,B	-1.41±0.00 a,B
GC	-1.46±0.02 a,A	-1.44±0.03 b,A	-1.44±0.05 a,A	-1.20±0.01 d,B	-1.19±0.03 b,B	-1.06±0.00 d,C	-1.19±0.02 b,B
GD	-1.46±0.02 a,AB	-1.49±0.06 ab,A	-1.45±0.03 a,AB	-1.37±0.00 bc,ABC	-1.38±0.05 a,ABC	-1.36±0.00 ab,BC	-1.31±0.02 ab,C
GE	-1.46±0.02 a,A	-1.48±0.00 ab,A	-1.32±0.01 a,AB	-1.30±0.00 c,AB	-1.20±0.07 b,BC	-1.07±0.10 d,C	-1.00±0.09 c,C
GF	-1.46±0.02 a,A	-1.46±0.01 b,A	-1.40±0.00 a,C	-1.44±0.00 ab,AB	-1.40±0.00 a,BC	-1.27±0.00 abc,E	-1.33±0.00 ab,B
GG	-1.46±0.2 a,AB	-1.53±0.01 ab,A	-1.44±0.05 a,AB	-1.41±0.07 abc,AB	-1.37±0.03 a,B	-1.37±0.01 ab,B	-1.36±0.04 ab,B

a-d: Values within each storage time followed by the same letter are not significantly different (P > 0.05).
A-D: Values within each treatment followed by the same letter are not significantly different (P > 0.05).
Data are means values ± one standard deviation (n=2).

Table 4.18. b* (yellowness-blueness) values of Armola cheeses treated with different antimicrobials during storage of 20 days at 4 °C

Treatments	b* (yellowness-blueness) Values						
	Day 0	Day 1	Day 5	Day 8	Day 11	Day 14	Day 20
Control	7.86±0.04 a,AB	7.80±0.01 a,A	8.23±0.17 a,AB	8.20±0.06 a,AB	8.45±0.23 a,B	8.41±0.28 ab,AB	8.28±0.24 ab,AB
GA	7.86±0.04 a,A	7.90±0.23 a,A	8.17±0.00 a,AB	8.29±0.00 a,ABC	8.22±0.02 ab,AB	8.62±0.10 a,BC	8.79±0.31 c,C
GB	7.86±0.04 a,A	7.83±0.00 a,A	8.17±0.10 a,C	8.12±0.03 a,BC	7.98±0.00 bc,AB	7.88±0.04 b,A	8.23±0.06 ab,C
GC	7.86±0.04 a,A	8.06±0.04 a,AB	8.39±0.07 a,BC	8.31±0.09 a,BC	8.23±0.15 ab,AB	8.27±0.05 ab,BC	8.60±0.11 bc,C
GD	7.86±0.04 a,A	7.87±0.09 a,A	7.89±0.04 a,A	8.08±0.05 a,B	7.86±0.04 bc,A	8.05±0.01 b,A	8.09±0.00 ab,B
GE	7.86±0.04 a,A	8.07±0.02 a,A	8.09±0.21 a,A	8.31±0.18 a,AB	8.15±0.11 ab,AB	8.64±0.17 a,B	8.59±0.06 bc,B
GF	7.86±0.04 a,A	8.03±0.03 a,A	8.05±0.10 a,A	8.11±0.02 a,A	8.03±0.00 c,A	8.10±0.27 ab,A	7.92±0.16 a,A
GG	7.86±0.04 a,A	8.13±0.09 a,B	8.21±0.04 a,BC	8.30±0.05 a,BC	8.28±0.00 abc,BC	8.35±0.11 ab,BC	8.40±0.01 abc,C

a-c: Values within each storage time followed by the same letter are not significantly different ($P > 0.05$). A-C: Values within each treatment followed by the same letter are not significantly different ($P > 0.05$). Data are means values ± one standard deviation (n=2).

Yeasts contribute to the color change of cheeses. The color of smear cheeses is thought to be mainly due to bacterial microbiota, such as *Brevibacterium* or *Arthrobacter* spp. However, a recent study on the indirect effects of yeasts on the color of *B. linens* showed that the color development depended on the yeast used for cheese deacidification (Leclercq-Perlat et al., 2004).

The a^* values of GB (treated with Natamax®) samples did not significantly change, but the a^* values of the other treated cheese samples significantly ($P<0.05$) increased when compared to control sample. In addition to this, GD (treated with Nisaplin® and Natamax®), GF (treated with Natamax® and Microgard™ 100), and GG (treated with Nisaplin®, Natamax® and Microgard™ 100) samples had little increasing effect on the a^* values. Moreover, GB (treated with Natamax®), GD (treated with Nisaplin® and Natamax®), and GF (treated with Natamax® and Microgard™ 100) samples remain unchanced ($P>0.05$) on day 11 when compared to control sample, then the b* values of these cheeses increased for other days. These changes are indicative of

a more redness and yellowness of cheese as a result of deacidification effect of yeast. The cheeses treated with Natamax® and its combination with other antimicrobials tended to be less yellow (lower b* values) than control and cheeses without Natamax® because of antimycotic effect. The combination of Natamax® and Microgard™ 100 (GF) was more effective on reducing yellowness than Natamax® alone. The Natamax® treatment remained constant on b* value on the 14th day, thereafter significantly increased on the 20th day. Changes in a* and b* values of cheese samples during storage are given in Figure 4.9. and Figure 4.10.

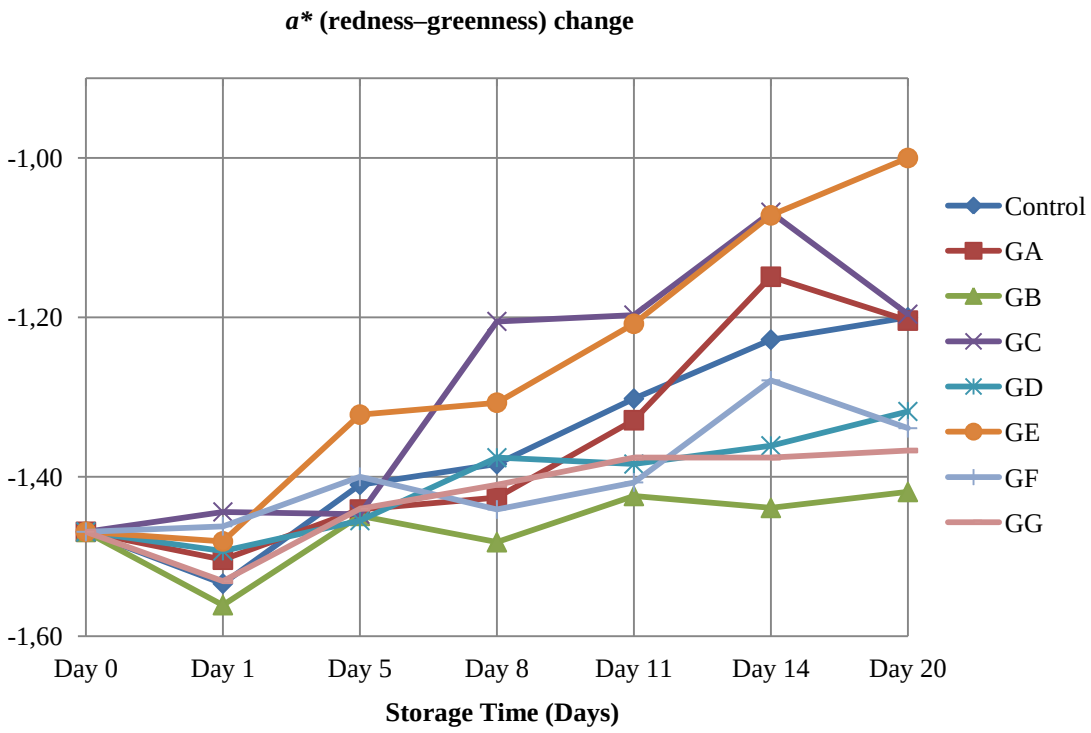

Figure 4.9. Change in a*(redness-greenness) values for control and treated cheese samples during storage at 4 °C

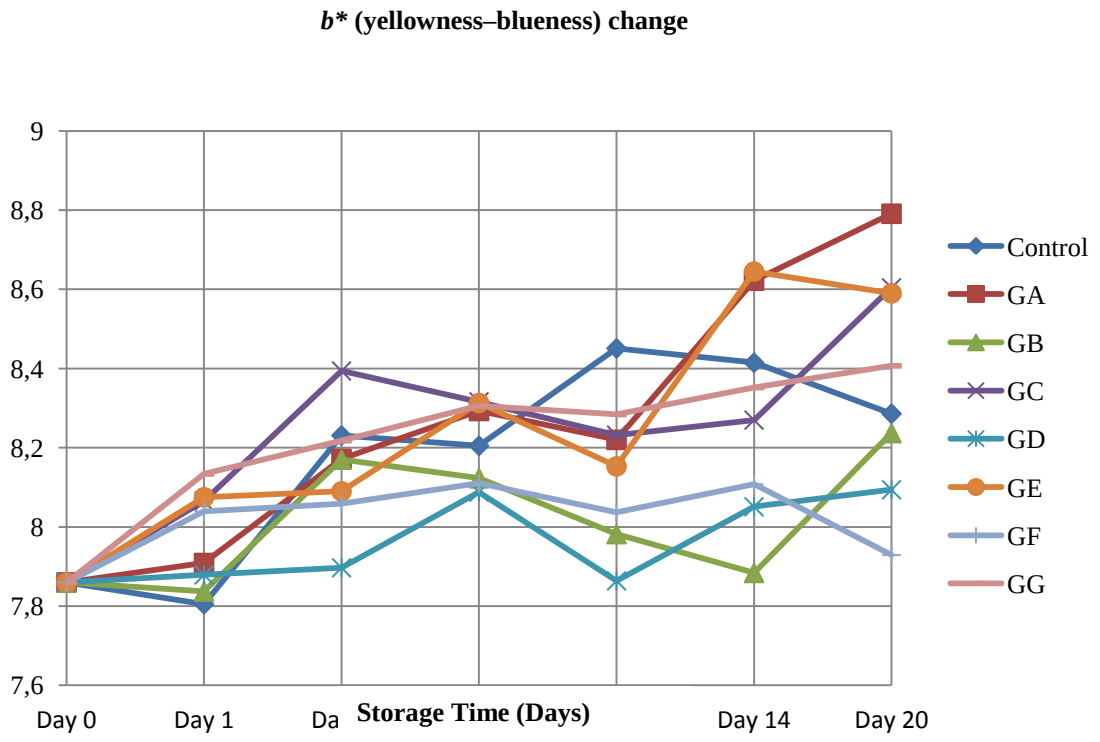

Figure 4.10. Change in b*(yellowness-blueness) values for control and treated cheese samples during storage at 4 °C

CHAPTER 5

CONCLUSION

In conclusion, in this study, the physicochemical, microbiological, and sensory quality of forty Armola cheese samples collected from Seferihisar, İzmir were investigated. In addition, the lactic acid bacteria flora of cheese samples was identified using genotyping method (16S-rRNA gene sequencing).

In these samples the average total solid, fat, and protein contents (%), pH value, titratable acidity (%), water activity and salt content (%) of these samples were 37.26, 19.52, 10.87, 4.70, 0.95, 0.91, and 2.51, respectively. The differences of physicochemical composition between samples were mostly due to the fact that there was no standard manufacturing method applied by dairies. As a result, production of Armola cheese must be standardized. The average microbial counts were found as follows: total aerobic mesophilic bacteria, 7.82; psychrotrophic bacteria, 6.98; coliform bacteria, 4.56; lactococci, 7.55; lactobacilli, 7.87; enterococci, 6.17; yeast 7.33; mold <1.00; *Staphylococcus* spp., 5.94; and *Listeria* spp., 2.94 cfu/g. The microbial results showed the presence of high counts of microorganisms and the poor hygienic quality of Armola cheese. In order to improve the microbial quality of Armola cheese, it should be produced under good hygienic conditions and stored at refrigeration temperatures until consumption.

As a result of the descriptive sensory analysis, dominant flavors were salty and sour as basic tastes; however, creamy, cooked and whey tastes were as aromatics. Sensory evaluation is key to the successful introduction of new food products and the reliable monitoring of existing food products in the marketplace.

As a result of sequencing analyses of 87 selected bacteria, *Enterococcus ratti, Enterococcus durans, Enterococcus hirae, Streptococcus lutetiensis, Streptococcus equines, Streptococcus luteciae, Lactobacillus paracasei subsp. tolerans, Lactobacillus casei subsp. casei, Lactobacillus zeae, Lactobacillus paracasei subsp. paracasei* were found as dominant bacteria.

Due to the short shelf-life of Armola cheese, it was needed to extent the shelf-life of cheese. Therefore, Nisaplin®, Natamax®, and Microgard™ 100 and their

combinations were added to Armola cheese samples. Based primarily on flavor and appereance scores and yeast counts, all treatments, especially including Natamax®, resulted in an extension of shelf-life of Armola cheese. Natamax®, used as an antimicrobial, was able to prevent yeast growth in cheese during storage. On the other hand, the usage of antimicrobials in combination gave more effective results than the usage of antimicrobials alone. As a result, by adding the antimicrobials, the shelf-life of Armola cheese was approximately extended to 20 days.

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APPENDIX A

CHEMICAL USED

Table A.1. Chemical Used

No	Chemical	Code
1	AgNO3	Merck 1.0512.0025
2	Potassium chromate	Merck 1.04951.1000
3	Phenol ftalein	Merck 1.07233.0100
4	Sulfuric acid	Merck 1.00729.2500
5	n-Amyl Alcohol	Merck 8.07500.1000
6	Kjeltabs-catalysts	Delta
7	Silicon antifoaming agent	Merck 1.07743.0100
8	Boric acid	Sigma B6768
9	HCl	Reidel-de Haen 07102
10	M17 agar	Merck 1.15108.0500
11	MRS agar	Fluka 69964
12	Violet Red Bile agar	Difco 211695
13	Plate Count agar	Merck 1.05463.0500
14	Skim milk	Difco 232100
15	Baird Parker agar base	Difco 276840
16	Kanamycin esculin azide agar	Merck 1.05222.0500
17	Yeast extract glucose chloromphenical agar	Difco 219001
18	Palcam Listeria selective agar	Merck 1.11755
19	MRS broth	Fluka 69966
20	M17 broth	Biolab M11320500
21	Tyrptic soy broth	Merck 1.05459.0500
22	Peptone water	Merck 1.07228.0500
23	Nisaplin®	Danisco, Denmark
24	Natamax®	Danisco, Denmark
25	Microgard™	Danisco, Denmark

APPENDIX B

SENSORY EVALUATION SHEETS

Name;
Date; / / 2011
Age;

Give score to Armola cheese samples according to personal liking
(1= worst ☹ ……5= is best ☺)

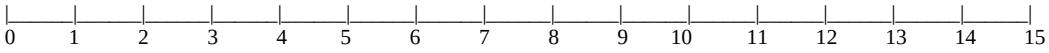
Armola sample	Appearance	Odor	Flavor	Consistency	Overall acceptability
320					
274					
986					
671					
576					
735					
127					
404					
813					
689					
311					
515					
472					
765					
348					
691					

Figure B.1. Sensory evaluation sheet of consumer acceptance test for Armola cheese

Aromatics

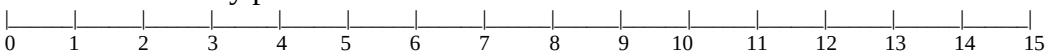
1. Cooked

Referance = sterilized milk



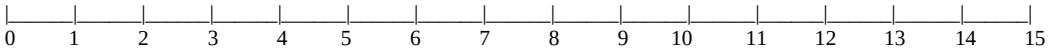
2. Whey

Referance = Whey powder



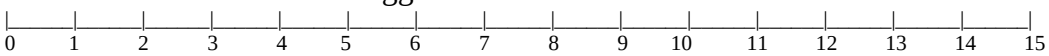
3. Creamy

Referance = Butter



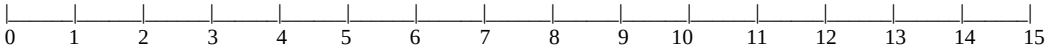
4. Sulfur

Referance = Boiled mashed egg



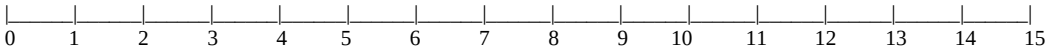
5. Free-fatty acid

Referance = Butyric acid



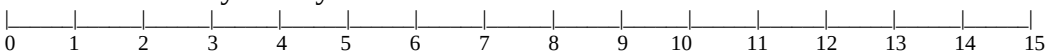
6. Animalic

Referance = Na-Caseinate



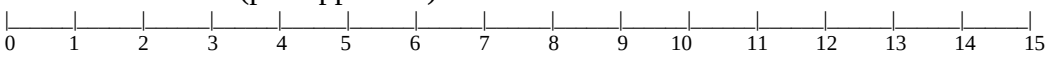
7. Moisty Cloth

Referance = Dirty moisty cloth



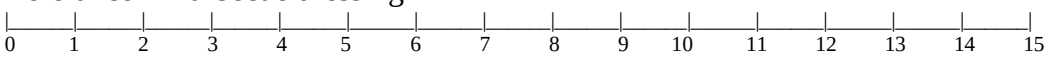
8. Yeasty

Referance = Fruit (pineapple etc.)



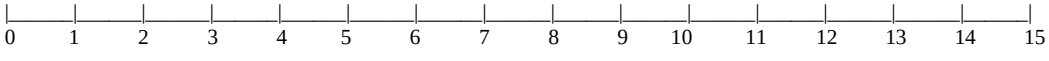
9. Smokey

Referance = Barbecue dressing



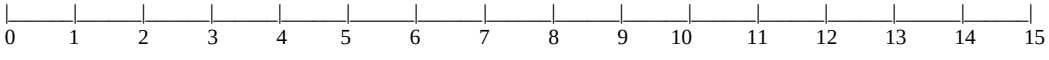
10. Brothy

Referance =Agar



11. Fermented

Referance = Yoghurt



Basic Tastes

1.Sweet

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=%2 sugar=2, %5 sugar=5, %10 sugar=10

2.Salty

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=%0.2 NaCl=2.5, %0.35 NaCl=5, %0.5 NaCl=8.5

3.Sour

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=%0.05 citrik acid=2, %0.08 citrik acid=5, %0.15 citrik acid=10

4.Bitter

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=%0.05 cafein=2, %0.08 cafein=5, %0.15 cafein=10

5.Umami

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=%0.5 Monosodium glutamate=3, %1 Monosodium glutamate=6

6.Bite

|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|_____|

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Referance=Mineral water=8-10

Figure B.2. Sensory evaluation sheet of flovar profile analysis for Armola cheese

APPENDIX C

CONTENTS OF ANTIMICROBIALS

Nisaplin® is composed of ;	
Nisin (E234) / Nisin preparation	min. 1000 IU/ mg
+	
Sodium chloride	min. 50 %
Natamax® is composed of ;	
Natamycin (E235)	min. 50 %
+	
Lactose	max. 50 %
All percentages are by weight.	

Figure C.1. The content of Nisaplin® and Natamax®

APPENDIX D

16S rRNA SEQUENCES OF ISOLATES

ref|NR_041933.1| *Enterococcus ratti* strain ATCC 700914 16S ribosomal RNA, partial sequence
Length=1503

Score = 974 bits (527), Expect = 0.0
Identities = 542/548 (99%), Gaps = 5/548 (1%)
Strand=Plus/Plus

```
Query  35   ACGCCCGCGATGTAGTG-AGAAGGCTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC  93
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Sbjct  388  ACG-CCGCG-TG-AGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC  444

Query  94   AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTA  153
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTA  504

Query  154  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  213
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  505  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  564

Query  214  AGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT  273
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  565  AGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT  624

Query  274  CATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  333
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  625  CATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  684

Query  334  AAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGA  393
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  685  AAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGA  744

Query  394  CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  453
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  745  CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  804

Query  454  AAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA  513
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  805  AAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA  864

Query  514  AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  573
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  865  AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  924

Query  574  CGCACAAG  581
             |||||||
Sbjct  925  -GCACAAG  931
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☑ ref|NR_036922.1| *Enterococcus durans* strain 98D 16S ribosomal RNA, partial sequence
Length=1534

Score = 974 bits (527), Expect = 0.0
Identities = 542/548 (99%), Gaps = 5/548 (1%)
Strand=Plus/Plus

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Sbjct  399  ACG-CCGCG-TG-AGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC  455

Query  94   AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTA  153
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Query  154  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  213
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Sbjct  516  CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA  575

Query  214  AGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT  273
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Sbjct  576  AGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT  635

Query  274  CATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  333
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Sbjct  636  CATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTG  695

Query  334  AAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGA  393
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  696  AAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGA  755

Query  394  CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  453
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  756  CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  815

Query  454  AAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA  513
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Sbjct  816  AAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA  875

Query  514  AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  573
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  876  AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC  935

Query  574  CGCACAAG  581
             |||||||
Sbjct  936  -GCACAAG  942
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☑ ref|NR_037082.1| *Enterococcus hirae* strain R 16S ribosomal RNA, partial sequence
Length=1535

Score = 974 bits (527), Expect = 0.0
Identities = 542/548 (99%), Gaps = 5/548 (1%)
Strand=Plus/Plus

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Query  35   ACGCCCGCGATGTAGTG-AGAAGGCTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC  93
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Query  94   AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTA  153
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Query  574  CGCACAAG  581
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Sbjct  935  -GCACAAG  941
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ref|NR_040789.1| *Enterococcus faecalis* strain JCM 5803 16S ribosomal RNA, partial sequence
Length=1517

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Identities = 550/554 (99%), Gaps = 3/554 (1%)
Strand=Plus/Plus

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Sbjct  389  CGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTG  448

Query  73   TTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAA  132
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Query  133  AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG  192
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Query  193  ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGC  252
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Sbjct  569  ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGC  628

Query  253  TCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATT  312
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Sbjct  629  TCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATT  688

Query  313  CCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCT  372
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  689  CCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCT  748

Query  373  CTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT  432
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  749  CTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT  808

Query  433  GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCT  492
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  809  GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCT  868

Query  493  GCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG  552
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  869  GCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG  928

Query  553  AATTGACGGGGGCC  566
            ||||||||||||||
Sbjct  929  AATTGACGGGGGCC  942
```

☑ ref|NR_037096.1| *Streptococcus lutetiensis* strain HDP90246 16S ribosomal RNA, partial sequence
Length=1473

Score = 1029 bits (557), Expect = 0.0
Identities = 565/568 (99%), Gaps = 3/568 (1%)
Strand=Plus/Plus

```
Query  7    TCGGC-ATGGGGGC-ACCCTGACCGAGC-ACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  63
            ||||| |||||||| |||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  345  TCGGCAATGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  404

Query  64   GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  123
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  405  GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  464

Query  124  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  465  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  524

Query  184  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  525  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  584

Query  244  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  585  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  644

Query  304  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  363
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  645  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  704

Query  364  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  705  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  764

Query  424  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  483
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  765  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  824

Query  484  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  825  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  884

Query  544  AAACTCAAAGGAATTGACGGGGGCCCGC  571
            ||||||||||||||||||||||||||||
Sbjct  885  AAACTCAAAGGAATTGACGGGGGCCCGC  912
```

ref|NR_042052.1| *Streptococcus equinus* ATCC 9812 strain ATCC 9812 16S ribosomal RNA, complete sequence
Length=1433

Score = 1029 bits (557), Expect = 0.0
Identities = 565/568 (99%), Gaps = 3/568 (1%)
Strand=Plus/Plus

```
Query  7    TCGGC-ATGGGGGC-ACCCTGACCGAGC-ACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  63
            ||||| |||||||| |||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  270  TCGGCAATGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  329

Query  64   GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  123
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  330  GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  389

Query  124  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  449

Query  184  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  450  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  509

Query  244  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  510  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  569

Query  304  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  363
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  570  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  629

Query  364  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  630  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  689

Query  424  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  483
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  690  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  749

Query  484  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  809

Query  544  AAACTCAAAGGAATTGACGGGGGCCCGC  571
            ||||||||||||||||||||||||||||
Sbjct  810  AAACTCAAAGGAATTGACGGGGGCCCGC  837
```

ref|NR_042051.1| *Streptococcus luteciae* strain NEM 782 16S ribosomal RNA, partial sequence
Length=1461

Score = 1029 bits (557), Expect = 0.0
Identities = 565/568 (99%), Gaps = 3/568 (1%)
Strand=Plus/Plus

```
Query  7    TCGGC-ATGGGGGC-ACCCTGACCGAGC-ACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  63
            ||||| |||||||| |||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  299  TCGGCAATGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC  358

Query  64   GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  123
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  359  GTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACACAGTGACGGT  418

Query  124  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  419  AACTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCG  478
```

```
Query  184  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  479  AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTT  538

Query  244  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539  AAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACTGTTAGACTTGAGTGCAGAAGGGG  598

Query  304  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  363
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  599  AGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCG  658

Query  364  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  659  AAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA  718

Query  424  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  483
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  719  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGG  778

Query  484  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  779  GGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTG  838

Query  544  AAACTCAAAGGAATTGACGGGGGCCCGC  571
            ||||||||||||||||||||||||||||
Sbjct  839  AAACTCAAAGGAATTGACGGGGGCCCGC  866
```

ref|NR_043660.1| *Streptococcus pasteurianus* strain CIP 107122 16S ribosomal RNA, partial sequence
Length=1470

Score = 758 bits (410), Expect = 0.0
Identities = 495/535 (93%), Gaps = 10/535 (2%)
Strand=Plus/Plus

```
Query  47   GTG-AGAAGGCCTTCGGATCGT-AAGCTCTGTTGTCAGAGAAGAACGTGTGTGAGATTGG  104
            ||| ||||||  |||||||||| |||||||||||| ||||||||||||||||||| |||
Sbjct  379  GTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGG  438

Query  105  AAAGTTCACACAGTGACGGTCACTTACCAGAAAGGGCCGGTTAA-TTCCTGGCCACCACC  163
            |||||||||||||||||| |||||||||||||| ||| ||| | | | |||| || |
Sbjct  439  AAAGTTCACACAGTGACGGTAACTTACCAGAAAGGGACGGCTAACTACGT-GCCAGCAGC  497

Query  164  CG-GGGAATTCCGAAGGTGCCGAGGGTTGTCCGAATTTCTGGGGCGTAAAGCGAGCCCAG  222
            || || || | || |||| ||||| |||||||| |||| | ||||||||||||||| |||
Sbjct  498  CGCGGTAA-TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAG  556

Query  223  GGGGTTTATTAATTCTG-AGTGTAAAGGCCGGGGCTTACCCGTTGTTCGCTTTGGAAACT  281
            | ||||||| ||| |||| ||| ||||||| | |||||| || ||||||||||||||||||
Sbjct  557  GCGGTTTAATAAGTCTGAAGT-TAAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACT  615

Query  282  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  341
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  616  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  675

Query  342  TATCTGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  401
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  676  TATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  735

Query  402  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  461
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  736  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  795

Query  462  GCTAGGTGTTAGGCCCTTTCCGGGGCTTATTGCCGCAGCTAACGCATTAAGCACTCCGCC  521
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  796  GCTAGGTGTTAGGCCCTTTCCGGGGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCC  855

Query  522  TGGGAGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCC-CACA  575
```

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            |||| ||||||||||||||||||||||||||||||||||||||||||| ||| ||||
Sbjct  856  TGGG-GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  909
```

☑ ref|NR_041781.1| *Streptococcus alactolyticus* strain ATCC 43077; DSM 20728 16S ribosomal RNA, partial sequence
Length=1437

Score = 758 bits (410), Expect = 0.0
Identities = 495/535 (93%), Gaps = 10/535 (2%)
Strand=Plus/Plus

```
Query  47   GTG-AGAAGGCCTTCGGATCGT-AAGCTCTGTTGTCAGAGAAGAACGTGTGTGAGATTGG  104
            ||| ||||||  |||||||||| |||||||||| |||||||||||||||||||| |||
Sbjct  396  GTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGG  455

Query  105  AAAGTTCACACAGTGACGGTCACTTACCAGAAAGGGCCGGTTAA-TTCCTGGCCACCACC  163
            |||||||||||||||||||| |||||||||||||| ||| ||| | | | |||| || |
Sbjct  456  AAAGTTCACACAGTGACGGTAACTTACCAGAAAGGGACGGCTAACTACGT-GCCAGCAGC  514

Query  164  CG-GGGAATTCCGAAGGTGCCGAGGGTTGTCCGAATTTCTGGGGCGTAAAGCGAGCCCAG  222
            || || || | || |||| ||||| |||||||| |||| | ||||||||||||||| |||
Sbjct  515  CGCGGTAA-TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAG  573

Query  223  GGGGTTTATTAATTCTG-AGTGTAAAGGCCGGGGCTTACCCGTTGTTCGCTTTGGAAACT  281
            | |||||| ||| |||| ||| ||||||| | |||||| || ||||||||||||||||||
Sbjct  574  GCGGTTTAATAAGTCTGAAGT-TAAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACT  632

Query  282  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  341
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  633  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  692

Query  342  TATCTGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  401
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  693  TATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  752

Query  402  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  461
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  753  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  812

Query  462  GCTAGGTGTTAGGCCCTTTCCGGGGCTTATTGCCGCAGCTAACGCATTAAGCACTCCGCC  521
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  813  GCTAGGTGTTAGGCCCTTTCCGGGGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCC  872

Query  522  TGGGAGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCC-CACA  575
            |||| ||||||||||||||||||||||||||||||||||||||||| ||| ||||
Sbjct  873  TGGG-GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  926
```

☑ ref|NR_037002.1| *Streptococcus macedonicus* strain LAB617 16S ribosomal RNA, partial
sequence
Length=1542

Score = 758 bits (410), Expect = 0.0
Identities = 495/535 (93%), Gaps = 10/535 (2%)
Strand=Plus/Plus

```
Query  47   GTG-AGAAGGCCTTCGGATCGT-AAGCTCTGTTGTCAGAGAAGAACGTGTGTGAGATTGG  104
            ||| ||||||  |||||||||| |||||||||| |||||||||||||||||||| |||
Sbjct  407  GTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGG  466

Query  105  AAAGTTCACACAGTGACGGTCACTTACCAGAAAGGGCCGGTTAA-TTCCTGGCCACCACC  163
            |||||||||||||||||||| |||||||||||||| ||| ||| | | | |||| || |
Sbjct  467  AAAGTTCACACAGTGACGGTAACTTACCAGAAAGGGACGGCTAACTACGT-GCCAGCAGC  525

Query  164  CG-GGGAATTCCGAAGGTGCCGAGGGTTGTCCGAATTTCTGGGGCGTAAAGCGAGCCCAG  222
```

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            || || || | || |||| ||||| |||||||| |||| | ||||||||||||||| |||
Sbjct  526  CGCGGTAA-TACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAG  584

Query  223  GGGGTTTATTAATTCTG-AGTGTAAAGGCCGGGGCTTACCCGTTGTTCGCTTTGGAAACT  281
            | |||||| ||| |||| ||| ||||||| | |||||| || ||||||||||||||||||
Sbjct  585  GCGGTTTAATAAGTCTGAAGT-TAAAGGCAGTGGCTTAACCATTGTTCGCTTTGGAAACT  643

Query  282  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  341
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  644  GTTAAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA  703

Query  342  TATCTGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  401
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  704  TATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC  763

Query  402  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  461
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  764  GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT  823

Query  462  GCTAGGTGTTAGGCCCTTTCCGGGGCTTATTGCCGCAGCTAACGCATTAAGCACTCCGCC  521
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  824  GCTAGGTGTTAGGCCCTTTCCGGGGCTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCC  883

Query  522  TGGGAGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCC-CACA  575
            |||| |||||||||||||||||||||||||||||||||||||||||| ||| ||||
Sbjct  884  TGGG-GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  937
```

ref|NR_042778.1| *Streptococcus thermophilus* strain ATCC 19258 16S ribosomal RNA, complete sequence
Length=1539

Score = 992 bits (537), Expect = 0.0
Identities = 552/558 (99%), Gaps = 5/558 (1%)
Strand=Plus/Plus

```
Query  15   GGGGGC-A-CCTGA-CGAGC-ACGCCGCGTGAGTG-AGAAGGTTTTCGGATCGTAAAGCT  69
            |||||| | ||||| ||||| ||||||||||||||| |||||||||||||||||||||||
Sbjct  380  GGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCT  439

Query  70   CTGTTGTAAGTCAAGAACGGGTGTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACC  129
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  CTGTTGTAAGTCAAGAACGGGTGTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACC  499

Query  130  AGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGT  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  AGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGT  559

Query  190  CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGATAAGTCTGAAGTTAAAGGCTG  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  560  CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGATAAGTCTGAAGTTAAAGGCTG  619

Query  250  TGGCTCAACCATAGTTCGCTTTGGAAACTGTCAAACTTGAGTGCAGAAGGGGAGAGTGGA  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  620  TGGCTCAACCATAGTTCGCTTTGGAAACTGTCAAACTTGAGTGCAGAAGGGGAGAGTGGA  679

Query  310  ATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGC  369
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  680  ATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGC  739

Query  370  TCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATAC  429
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  740  TCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATAC  799

Query  430  CCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGATCCTTTCCGGGATTCAGT  489
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  800  CCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGATCCTTTCCGGGATTCAGT  859

Query  490  GCCGAAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAA  549
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  860  GCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAA  919
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```
Query  550  AGGAATTGACGGGGGccc  567
            ||||||||||||||||||
Sbjct  920  AGGAATTGACGGGGGCCC  937
```

☑ ref|NR_041054.1| *Lactobacillus paracasei subsp. tolerans* strain NBRC 15906 16S ribosomal RNA, partial sequence
Length=1497

Score = 979 bits (530), Expect = 0.0
Identities = 545/552 (99%), Gaps = 2/552 (0%)
Strand=Plus/Plus

```
Query  19   AGTCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGCTTTCGGATCGTAAAACTCTGTTGT  77
            ||||||||||||| |||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  381  AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGT  440

Query  78   TGGAGAAGAATGGTCGTGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  137
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  TGGAGAAGAATGGTCG-GCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  499

Query  138  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  197
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  559

Query  198  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTCA  257
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  560  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTA  619

Query  258  ACCGGGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  317
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  620  ACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  679

Query  318  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTG  377
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  680  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTG  739

Query  378  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  437
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  740  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  799

Query  438  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  497
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  800  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  859

Query  498  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  557
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  860  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  919

Query  558  TGACGGGGcccc  569
            |||||||| |||
Sbjct  920  TGACGGGGGCCC  931
```

☑ ref|NR_041893.1| *Lactobacillus casei subsp. casei* ATCC 393 strain ATCC 393 16S ribosomal RNA, partial sequence
Length=1517

Score = 979 bits (530), Expect = 0.0
Identities = 545/552 (99%), Gaps = 2/552 (0%)
Strand=Plus/Plus

```
Query  19   AGTCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGCTTTCGGATCGTAAAACTCTGTTGT  77
            ||||||||||||| |||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  393  AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGT  452

Query  78   TGGAGAAGAATGGTCGTGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  137
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  453  TGGAGAAGAATGGTCG-GCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  511

Query  138  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  197
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  512  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  571

Query  198  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTCA  257
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  572  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTA  631

Query  258  ACCGGGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  317
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  632  ACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  691

Query  318  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTG  377
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  692  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTG  751

Query  378  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  437
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  752  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  811

Query  438  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  497
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  812  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  871

Query  498  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  557
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  872  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  931

Query  558  TGACGGGGcccc  569
            |||||||| |||
Sbjct  932  TGACGGGGGCCC  943
```

ref|NR_037122.1| *Lactobacillus zeae* strain RIA 482 16S ribosomal RNA, partial sequence
Length=1522

Score = 979 bits (530), Expect = 0.0
Identities = 545/552 (99%), Gaps = 2/552 (0%)
Strand=Plus/Plus

```
Query  19   AGTCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGCTTTCGGATCGTAAAACTCTGTTGT  77
            ||||||||||||| |||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  381  AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGT  440

Query  78   TGGAGAAGAATGGTCGTGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  137
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  TGGAGAAGAATGGTCG-GCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  499

Query  138  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  197
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  559

Query  198  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTCA  257
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  560  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTA  619

Query  258  ACCGGGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  317
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  620  ACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  679

Query  318  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTG  377
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  680  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTG  739

Query  378  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  437
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  740  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  799

Query  438  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  497
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```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  800  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  859

Query  498  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  557
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  860  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  919

Query  558  TGACGGGGcccc  569
            |||||||| |||
Sbjct  920  TGACGGGGGCCC  931
```

ref|NR_025880.1| *Lactobacillus paracasei subsp. paracasei* strain R094 16S ribosomal RNA, partial sequence
Length=1522

Score = 979 bits (530), Expect = 0.0
Identities = 545/552 (99%), Gaps = 2/552 (0%)
Strand=Plus/Plus

```
Query  19   AGTCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGCTTTCGGATCGTAAAACTCTGTTGT  77
            ||||||||||||| |||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  381  AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGT  440

Query  78   TGGAGAAGAATGGTCGTGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  137
            ||||||||||||||| |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  TGGAGAAGAATGGTCG-GCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCAGAAAGC  499

Query  138  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  197
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT  559

Query  198  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTCA  257
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  560  TATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTA  619

Query  258  ACCGGGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  317
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  620  ACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA  679

Query  318  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTG  377
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  680  TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTG  739

Query  378  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  437
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  740  GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT  799

Query  438  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  497
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  800  AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCA  859

Query  498  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  557
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  860  GCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAAT  919

Query  558  TGACGGGGcccc  569
            |||||||| |||
Sbjct  920  TGACGGGGGCCC  931
```

ref|NR_029018.1| *Lactobacillus coryniformis subsp. torquens* strain 30 16S ribosomal RNA, complete sequence
Length=1521

Score = 992 bits (537), Expect = 0.0
Identities = 555/563 (99%), Gaps = 3/563 (1%)
Strand=Plus/Plus

```
Query  8    CAATGGA-GCAAGTCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGTTTTAGGATCGTAA  65
            ||||||| | |||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  392  CAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTAGGATCGTAA  451

Query  66   AACTCTGTTGTTGGAGAAGAACAGGGACTAGAGTAACTGTTAGTCCTTTGACGGTATCCA  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  452  AACTCTGTTGTTGGAGAAGAACAGGGACTAGAGTAACTGTTAGTCCTTTGACGGTATCCA  511

Query  126  ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGC-AAGCG  184
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  512  ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAAGCG  571

Query  185  TTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAG  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  572  TTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAG  631

Query  245  CCTTCGGCTTAACCGAGGAACTGCATTAGAAACTGGCAAACTTGAGTGCAGAAGAGGACA  304
            ||||||||||||||| ||| ||||||||||||||| ||||||||||||||||||||||||
Sbjct  632  CCTTCGGCTTAACCGAAGAAGTGCATTAGAAACTGGGAAACTTGAGTGCAGAAGAGGACA  691

Query  305  GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAG  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  692  GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAG  751

Query  365  GCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGTATGGGGAGCGAACAGGATTA  424
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  752  GCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGGAGCGAACAGGATTA  811

Query  425  GATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCT  484
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  812  GATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCT  871

Query  485  TCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAA  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  872  TCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAA  931

Query  545  CTCAAAGGAATTGACGGGGGcccc  567
            |||||||||||||||||||||||||
Sbjct  932  CTCAAAGGAATTGACGGGGGCCC  954
```

ref|NR_042728.1| *Lactobacillus delbrueckii subsp. lactis* DSM 20072 strain ATCC 12315 16S ribosomal RNA, partial sequence
Length=1505

Score = 1026 bits (555), Expect = 0.0
Identities = 560/562 (99%), Gaps = 2/562 (0%)
Strand=Plus/Plus

```
Query  8    CAATGGA-GC-AGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTCTTCGGATCGTAA  65
            ||||||| || ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  CAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTCTTCGGATCGTAA  440

Query  66   AGCTCTGTTGTTGGTGAAGAAGGATAGAGGCAGTAACTGGTCTTTATTTGACGGTAATCA  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  AGCTCTGTTGTTGGTGAAGAAGGATAGAGGCAGTAACTGGTCTTTATTTGACGGTAATCA  500

Query  126  ACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  501  ACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT  560

Query  186  TGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAATGATAAGTCTGATGTGAAAGC  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  561  TGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAATGATAAGTCTGATGTGAAAGC  620

Query  246  CCACGGCTCAACCGTGGAACTGCATCGGAAACTGTCATTCTTGAGTGCAGAAGAGGAGAG  305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  621  CCACGGCTCAACCGTGGAACTGCATCGGAAACTGTCATTCTTGAGTGCAGAAGAGGAGAG  680

Query  306  TGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGG  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  681  TGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGG  740

Query  366  CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAG  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  741  CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAG  800

Query  426  ATACCCTGGTAGTCCATGCCGTAAACGATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCT  485
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  801  ATACCCTGGTAGTCCATGCCGTAAACGATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCT  860

Query  486  CAGTGCCGCAGCAAACGCATTAAGCGCTCCGCCTGGGGAGTACGACCGCAAGGTTGAAAC  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  861  CAGTGCCGCAGCAAACGCATTAAGCGCTCCGCCTGGGGAGTACGACCGCAAGGTTGAAAC  920

Query  546  TCAAAGGAATTGACGGGGGCCC  567
            |||||||||||||||||||||||
Sbjct  921  TCAAAGGAATTGACGGGGGCCC  942
```

ref|NR_029106.1| *Lactobacillus delbrueckii subsp. indicus* strain NCC725 16S ribosomal RNA, partial sequence
Length=1515

Score = 992 bits (537), Expect = 0.0
Identities = 551/557 (99%), Gaps = 4/557 (1%)
Strand=Plus/Plus

```
Query  26   TCTGATGGAGC-ACGCCGCGTGAGTG-AGAAGGTTTTCGGATCGT-AAGCTCTGTTGTTG  82
            |||||||||||| ||||||||||||||| |||||||||||||||| |||||||||||||||
Sbjct  375  TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTG  434

Query  83   GAGAAGAAGGATAGAGGCAGTAACTGGTCTTTATTTGACGGTAATCAACCAGAAAGTCAC  142
            | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435  GTGAAGAAGGATAGAGGCAGTAACTGGTCTTTATTTGACGGTAATCAACCAGAAAGTCAC  494

Query  143  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  202
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  554

Query  203  TGGGCGTAAAGCGAGCGCAGGCGGAATGATAAGTCTGATGTGAAAGCCCACGGCTCAACC  262
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555  TGGGCGTAAAGCGAGCGCAGGCGGAATGATAAGTCTGATGTGAAAGCCCACGGCTCAACC  614

Query  263  GTGGAACTGCATCGGAAACTGTCATTCTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGT  322
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  615  GTGGAACTGCATCGGAAACTGTCATTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGT  674

Query  323  GTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTC  382
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  675  GTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTC  734

Query  383  TGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT  442
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  735  TGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT  794

Query  443  CCATGCCGTAAACGATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCTCAGTGCCGCAGCA  502
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  795  CCATGCCGTAAACGATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCTCAGTGCCGCAGCA  854

Query  503  AACGCATTAAGCGCTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGA  562
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  855  AACGCATTAAGCGCTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGA  914

Query  563  CGGGGGCCCCGCACAAG  579
            |||||||||  |||||||
Sbjct  915  CGGGGGCCC-GCACAAG  930
```

☑ ref|NR_042394.1| *Lactobacillus plantarum* strain NRRL B-14768 16S ribosomal RNA, partial sequence
Length=1474

Score = 1007 bits (545), Expect = 0.0
Identities = 548/549 (99%), Gaps = 1/549 (0%)
Strand=Plus/Plus

```
Query  25   TCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  83
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375  TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  434

Query  84   AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  143
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435  AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  494

Query  144  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  203
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  554

Query  204  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  263
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  614

Query  264  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  323
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  615  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  674

Query  324  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  383
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  675  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  734

Query  384  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  443
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  735  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  794

Query  444  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  503
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  795  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  854

Query  504  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  563
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  855  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  914

Query  564  CGGGGGCCC  572
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Sbjct  915  CGGGGGCCC  923
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☑ ref|NR_025447.1| *Lactobacillus paraplantarum* strain DSM 10667 16S ribosomal RNA, partial sequence
Length=1502

Score = 1007 bits (545), Expect = 0.0
Identities = 548/549 (99%), Gaps = 1/549 (0%)
Strand=Plus/Plus

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Query  25   TCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  83
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Sbjct  384  TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  443
```

```
Query  84   AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  143
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Sbjct  444  AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  503

Query  144  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  203
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Sbjct  504  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  563

Query  204  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  263
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  564  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  623

Query  264  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  323
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Sbjct  624  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  683

Query  324  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  383
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Sbjct  684  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  743

Query  384  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  443
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Sbjct  744  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  803

Query  444  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  503
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  804  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  863

Query  504  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  563
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  864  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  923

Query  564  CGGGGGCCC  572
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Sbjct  924  CGGGGGCCC  932
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ref|NR_029133.1| *Lactobacillus pentosus* strain 124-2 16S ribosomal RNA, partial sequence
Length=1519

Score = 1007 bits (545), Expect = 0.0
Identities = 548/549 (99%), Gaps = 1/549 (0%)
Strand=Plus/Plus

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Query  25   TCTGATGGAGC-ACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  83
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  382  TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA  441

Query  84   AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  143
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  442  AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCAC  501

Query  144  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  203
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  502  GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT  561

Query  204  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  263
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  562  TGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACC  621

Query  264  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  323
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  622  GAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGT  681

Query  324  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  383
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  682  GTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC  741

Query  384  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  443
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  742  TGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGT  801

Query  444  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  503
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Sbjct  802  CCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCT  861

Query  504  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  563
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  862  AACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  921

Query  564  CGGGGGCCC  572
            |||||||||
Sbjct  922  CGGGGGCCC  930
```

APPENDIX E

RESULTS OF SENSORY PROFILE ANALYSIS

Table E.1. Results of Sensory Profile Analyses

			AROMATICS						
Yeasty	**Fermented**	**Brothy**	**Moisty cloth**	**Animalic**	**Free fatty acids**	**Sulfur**	**Creamy**	**Whey**	**Cooked**
0.00	2.40	1.55	0.20	0.93	2.30	0.05	3.90	2.65	3.25
0.00	2.70	1.15	0.20	0.85	2.05	0.00	3.05	2.50	2.85
0.00	2.80	0.80	0.45	0.80	2.08	0.00	2.45	2.90	3.20
0.00	2.30	1.10	0.35	0.96	2.00	0.00	2.88	2.45	2.48
0.00	2.65	1.20	0.40	1.30	3.60	0.00	2.60	2.35	2.70
0.40	1.78	1.26	0.00	0.93	0.48	0.45	3.25	2.25	3.40
0.30	2.05	0.80	0.00	0.70	0.56	0.68	3.40	1.70	3.00
0.35	2.35	1.15	0.05	1.03	1.10	0.60	3.20	2.18	3.65
0.73	2.20	0.65	0.10	1.18	2.45	0.65	3.05	2.10	3.35
1.98	1.75	0.85	0.05	1.30	2.68	0.65	3.40	2.43	3.25
1.40	2.60	0.75	0.35	1.80	1.85	0.95	4.20	2.85	3.40
0.30	2.55	0.65	0.30	1.65	3.05	1.25	3.90	2.55	3.20
0.35	2.40	0.45	0.35	1.90	1.85	1.35	2.60	2.25	2.65
0.35	2.65	1.30	0.50	2.55	1.25	0.65	2.65	2.85	3.55
0.55	1.80	0.50	0.30	2.20	1.50	1.45	3.25	5.05	3.25
0.48	2.20	0.41	0.13	0.73	3.88	1.44	3.35	2.44	2.04
0.60	1.89	0.25	0.13	0.63	3.06	1.38	3.06	2.60	2.16
0.56	1.98	0.29	0.13	0.66	3.23	1.44	3.33	2.48	2.25

BASIC TASTES							
Samples	**Bite**	**Umami**	**Bitter**	**Sour**	**Salty**	**Sweet**	**Smokey**
1	0.30	0.56	0.18	2.41	2.98	1.60	0.00
2	1.16	0.30	0.30	2.85	3.10	1.35	0.00
3	0.50	0.30	0.05	3.00	3.55	1.10	0.00
4	0.60	0.30	0.28	2.65	2.90	1.50	0.00
5	1.50	0.33	0.53	3.35	5.20	1.15	0.00
6	0.05	0.05	0.05	1.48	3.55	2.08	3.40
7	0.20	0.00	0.10	1.50	3.00	1.86	0.25
8	0.15	0.15	0.05	1.50	3.18	1.68	0.10
9	0.40	0.10	0.20	2.35	4.85	1.43	0.10
10	0.35	0.00	0.38	2.76	5.25	1.15	0.15
11	1.20	0.85	0.25	1.50	5.20	1.25	0.05
12	0.75	0.80	1.25	0.95	4.05	1.85	0.30
13	1.30	0.60	0.15	2.60	4.45	0.75	0.65
14	1.10	0.65	0.10	1.45	4.30	0.70	0.25
15	1.30	0.70	0.15	1.50	8.90	0.85	1.05
16	0.44	0.56	0.38	1.30	3.31	2.38	0.38
17	0.35	0.31	0.38	1.39	3.76	2.41	0.19
18	0.31	0.56	0.31	1.31	3.51	2.35	0.19