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ABSTRACT

This study explores the presence of *Staphylococcus aureus* (*S.aureus*) in various dairy products produced and marketed by different manufacturing facilities in the Turkish Republic of Northern Cyprus (TRNC). Baird- Parker Agar (BPA) solid medium base has been used for the isolation of *S.aureus* bacteria.

As a result of the analysis of 85 diary product samples produced in different manufacturing facilities in the TRNC and collected from markets in the Nicosia area, it has been determined that; the *S.aureus* amount detected in 1 sample (1%) was within the accepted limits cited in the Turkish Food Codex Regulation on Microbiological Criteria and did not endanger public health; 12 samples (14%) contained *S.aureus* at levels endangering public health; and 72 samples (85%) did not contain *S.aureus.*

It is, therefore, necessary that modern production technologies are used at all manufacturing facilities currently operating in the TRNC; that their personnel are trained on food production safety and personal hygiene; that the Veterinary Department of the Food and Agriculture Ministry takes samples for analysis more frequently from the manufacturing facilities and applies the relevant sanctions in unsuitable cases; and that the Ministry of Health and Local Municipalities carry out regular market inspections in the name of protecting public health, and take the necessary legal actions and apply the relevant sanctions regarding the unsafe food products and their manufacturers.

Keywords: *Staphylococcus aureus,* BPA, milk products, identification, public health

ÖZET

Bu çalışmada, Kuzey Kıbrıs Türk Cumhuriyetinde farklı üretim birimlerinden satışa sunulan çeşitli süt ürünlerinde *Staphylococcus aureus* (*S.aureus*) varlığı araştırılmıştır. *S.aureus* izolasyonu için Braid-Parker (BPA) katı besiyeri kullanılmıştır.

K.K.T.C'de farklı üretim tesislerinde üretilen ve Lefkoşa bölgesi marketlerinde satışa sunulan 85 adet sü türünü örneğinin piyasadan alınan 1 (%1) adetinde saptanan *S.aureus* miktarının Türk Gıda Kodeksi Mikrobiyolojik kriterler tebliğinde belirtilen sınırlar içerisinde olduğu ve halk sağlığını riske atmadığı, 12 (%14) adet örnekte halk sağlığını riske atacak düzeyde *S.aureus* bulunduğu ve 72 (%85) örneğin ise *S.aureus* içermediği saptanmıştır.

K.K.T.C'de mevcut süt işletmelerinde modern üretim teknolojilerinin kullanılması, personelin güvenli gıda üretimi ve personel hijyeni konularında eğitilmesi, Gıda ve Tarım Bakanlığına bağlı Veteriner Dairesinin söz konusu işletmelerden mikrobiyolojik analiz için daha sık numune alıp uygun olmayan durumlarda yasal yaptırımları harekete geçirmesi, Sağlık Bakanlığının ve Yerel Yönetimlerin yani belediyelerin ise halk sağlığı adına düzenli bir şekilde piyasa kontrollerini yapıp güvenli olmayan gıdalar ve üreticileri ile ilgili yasal uygulamalara ve yaptırımlara başvurması gerekmektedir.

Anahtar Kelimeler: *Staphylococcus aureus*, BPA, süt ürünü, tanımlama, halk sağlığı

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LIST OF ABBREVIATIONS

µg: Microgram

µm: Micrometre

nm: Nanometre

Da: Dalton

SE: Staphylococcal Enterotoxin

CFU: Colony-Forming Units

pH: Power of Hydrogen

°C: Degrees Celsius

a_w : Water Activity

NaCl: Sodium chloride

Ng: Nanogram

RIA: Radioimmunoassay

MRSA: Methyllysine Resistant *Staphylococcus aureus*

α: Alfa

β: Beta

δ: Gamma

γ: Delta

ELISA: Enzyme-Linked Immunosorbent Assay

EIA: Enzyme Immunoassay

IDF: International Dairy Federation

TSST: Toxic Shock Syndrome Toxin

ISO : International Organization Standardization

TSE: Turkish Standards Institute

EMS: The most probable number method

BPA: Baird Parker Agar

FDA: Food and Drug Administration

BAM: Bacteriological Analytical Manual

QS: Quorum Sensing Bacteria

L: Litre

TRNC: Turkish Republic of Northern Cyprus

Verda Değirmencioğlu: **DETERMINING THE PRESENCE OF *Staphylococcus aureus* IN VARIOUS DAIRY PRODUCTS PRODUCED AND SOLD IN THE TURKISH REPUBLIC OF NORTHERN CYPRUS**

Approval of Director of Graduate School of Applied Sciences

Prof. Dr. İlkay Salihoğlu

We certify this thesis is satisfactory for the award of the Degree of Masters of Science in Food Engineering

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

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CHAPTER 1

INTRODUCTION

It is still an ongoing debate today whether some of the bacteria which cause intestinal diseases become effective through infection or intoxication. There are two types of bacteria that start to multiply and produce toxins when the suitable conditions become present in foodstuffs. The toxins produced by these bacteria can then penetrate and intoxicate human body through consumption. One of these bacteria is *Clostridium botulinum* and the other one is *Staphylococcus aureus (S.aureus)* (Tunail, 2000).

Staphylococci are the main cause for infections such as folliculitis, endocarditis, furunculosis and staphylococcal scalded skin syndrome (SSS) found in both humans and animals, and the staphylococcal intoxications found in humans. Staphylococcus infections can be caused by both coagulase positive and coagulase negative staphylococci. However, the predominant source of staphylococcal food intoxication is the *S.aureus* (Oliver et al., 2005; Kılıç, 2007).

In terms of invasive infections, *S.aureus* are known to cause wound infections, sinusitis, middle ear infections and mastitis. They share the responsibility with *E.coli* and *Pseudomonas* for causing septicaemia almost in half of the hospitalized patients. The main toxicosis actually caused by *S.aureus* are the intoxications initiated by the consumption of enterotoxin contaminated food. Within a short time, such as couple of hours following consumption, illness starts to progress with initial nausea, followed by vomiting and diarrhoea. The toxic shock syndrome known since 1978 is predominantly caused by strains producing the TSST-1 toxin. The dermatitis exfoliativais caused by staphylococci which generate exfoliatin. The illness outbreaks as wide spread scaling of the skin, often accompanied by itching and hair loss (Tunail, 2009).

According to Wieneke and his colleagues, food poisonings caused by *S.aureus* and other types of staphylococci are among the leading food poisoning cases in terms of importance (Niskanen et al.,1978;Wieneke et al.,1993).

Food poisoning is caused by the intake of toxins and/or intensive amounts of bacteria. Staphylococcal food poisoning is caused by the intake of toxins generated within the foodstuff by these bacteria through the digestive system.

While *S.aureus* was initially kept responsible for all Staphylococcal food poisonings, later research revealed that other staphylococcus types with coagulase positive properties, such as *S.intermedius, S.hycius* and *S.delphini,* also caused food poisoning. *S.aureus* is not only a source of bacterial infection but also causes food poisoning through the enterotoxins it generates. It was also revealed that while some coagulase negative staphylococcus, such as *S.epidermis,* can also generate enterotoxin, not all coagulase positive types produce enterotoxins (Diatek, 2012).

As a result of the foregoing findings, the presumptive identification of staphylococci in foodstuffs cannot be kept limited to *S.aureus.* Accordingly, the new Microbiological Criteria Regulation of the Ministry of Food, Agriculture and Livestock of the Republic of Turkey includes thegeneral citation of coagulase positive staphylococci (Diatek, 2012).

In the Turkish Republic of Northern Cyprus, there are no statistical data on food poisonings caused by *S.aureus* or on food poisoning cases in general. Moreover, no research has ever been carried out in the TRNC on the presence of *S.aureus* in dairy products produced and sold in the country.

Our thesis is the first study carried out on this subject in the Turkish Republic of Northern Cyprus.

1.1. Historical Account

Although Staphylococci were first observed and identified in 1878 by Pasteur and Robert Koch, detailed research on staphylococci was carried out in the years 1880 and 1881 by Ogston, and then in 1884 by Rosenbach. Ogston described micrococci as microorganisms causing suppurative skin inflammation when their activity and spreading area are low, and causing septicaemia and pyemia when they have the capacity to spread, stressing that they have pathogen properties. In 1884 Rosenberg produced pure staphylococci cultures for the

first time and identified them through biochemical analyses. He thought of staphylococci as a family and observed that they formed white and orange coloured colonies. He has identified two different staphylococci sub-types from this family according to their pigmentation properties. Rosenberg named the microorganisms forming orange coloured colonies as *Staphylococcus pyogenes aureus,* and the microorganisms forming white coloured colonies as *Staphylococcus pyogenes albus.*Afterwards, S*taphylococcus pyogenes citreus,* forming yellow coloured colonies was also identified and added. Winslow included the staphylococci to the M*icrococaceae* famil*y* in 1920. In 1957, Evans determined their ability to anaerobically fermentate glucose and classified this family as *Staphylococcus.* A while after, Winslow identified a second type known as *Staphylococcus epidermidis.*Until 1972, *S.aureus* was the only known type. Its main difference from *Staphylococcus epidermidis*is its ability to generatecoagulase. The third type, namely the *S.saprophyticus* was added to the staphylococci in1974. The number of identified types was 13 in 1980 and rose to 20 in 1984. Apart from *S.intermedius* and *S.hyicus,* all of the newly discovered types are coagulase negative (Şardan, 2000; Kılıç, 2007).

1.2. General Information on *Staphylococcus* (Classification and Properties)

The term *Staphylococcus* was first used by Scottish surgeon, Alexander Ogston, because of their characteristic cluster like appearance under microscope, and was derived from the words “staphyle” which means grape bunch and “occus” which means round piece in Ancient Greek.

Staphylococci are listed as members of the Staphyloccaceae family in the last edition of Bergey’s Manual of Systematic Bacteriology (Kılıç, 2007).

However according to older sources, *Staphylococcus* species are members of *Micrococaceae* family which are gram-positive, cocci shaped, still bacteria which do not produce spores and have diameters between 0.5 and 1.5 μm, and also have facultative anaerobic, catalase positive properties (Tükel and Doğan, 2000).

Staphylococcus genus are gram-positive, facultative anaerobic, non-spore producing, still and catalase positive bacteria. This genus includes at least 28 species (strands) and 32 sub-species (sub-strands) (Milci and Yaygın, 2006).

Staphylococci contain guanine and cytosine (G-C) in low percentages (30-39% mol). However, their micrococcus type members contain G-C at levels reaching 68-74% mole. Staphylococcal cell wall is thick (30-60 mm) and has a typical gram-positive microorganism structure. The cell wall of *S.aureus* has a thickness of 120 mm and is composed of peptidoglycan, teichoic acid and proteins. Among these components, proteins contain fibronectin, fibrinogen, laminin and collagen which are very important for binding to eukaryotic cells and adhesion. With the binding of adhesion proteins, bacterial clinging to tissues takes place. The most effective antigenic protein is Protein A which is present in 90-98% of *S.aureus* strains (Aktaş, 2006).

Staphylococcal enzymes can be listed as; coagulase, hyaluronidase, Fibrinolysin and Deoxyribonuclease (DNase) produced by *S.aureus;* catalase and penicillinase produced by all staphylococci; and lipase produced by *S.aureus* as well as some coagulase negative staphylococci. Coagulase converts fibrinogen to fibrin and provides for the clustering of bacteria. Hyaluronidase provides for the spreading of bacteria throughout the tissue. Catalase breaks toxic hydrogen peroxide into water and oxygen. Lipase makes it possible for the bacteria to live inside fatty tissues and contributes to the formation of invasive skin infections on subcutaneous tissues. Fibrinolysin breaks fibrin clots. Coagulase forms clots by converting fibrinogen to fibrin and helps the staphylococci to hide from neutrophils. There are two types of coagulase. Bound coagulase, otherwise known as "clumping factor", which can be detected by a slide coagulase test, and free coagulase which can be detected by a tube coagulase test. *S.aureus* possesses both types of coagulase. Hyaluronidase are present in ninety percent of *S.aureus*strands. It helps the spreading of bacteria by breaking hyaluronic acid. Staphylocinase, which is found in all strands of *S.aureus,* is also called Fibrinolysin and it breaks clots. Lipase is produced by all *S.aureus*strands and 30% of coagulase negative staphylococci.

S.aureus is a clumping factor positive and tube coagulase positive microorganism. The clumping factor is a type of coagulase enzyme which exists stuck to the cell wall of staphylococci. Moreover, *S.aureus* produces glycocalyx formations on various surfaces and it is believed that they utilize these formations for spreading and infecting. Glycocalyx is a special material found on the surface of cell membrane and is rich in sugars. It is formed in two ways which is categorized by its relation to the cell membrane; glycocalyx which is attached to the cell membrane and glycocalyx which is not attached to the cell membrane (Tunail, 2009).

Coagulase generation is an important criteria for pathogen *S.aureus* strands but is not an absolute defining factor. Coagulase producing staphylococci are *S.aureus, S.intermedius, S.hycius, S.aureus subsp.anaerobius, S.delphini, and S.schleiferi subsp.coagulans*. Naturally, the important group relevant for food are the staphylococci which are coagulase positive and cause staphylococcal food poisonings (Tunail, 2000).

Staphylococci are mesophyll organisms. They can generate (reproduce) at temperatures between 20-40°C (Tunail, 2009). *S.aureus* is a sphere or oval shaped (0.5-1.5 µm diameter), gram-positive, still, spore-free, usually without capsule, facultative anaerobic, catalase positive, oxidase negative microorganism which optimally generates at 37°C'. The minimum and maximum temperature necessary for them to produce toxins is slightly higher and is between 10 to 48ºC. Cells form individually or in couples or in grape bunch like clusters (Carey et al., 2004; Madigan et al., 2009).

Staphylococcus aureus, which belongs to the family, is highly sensitive to all applications used for the reduction of microorganisms, including and especially, to heat applications (Milci and Yaygın, 2006).

Staphylococci are one of the most resistant microorganisms to surrounding conditions and disinfectants among the spore-free bacteria. They can be preserved for 2 to 3 months at a temperature of 4°C, and for 3 to 6 months at a temperature of 20°C in culture form. At a temperature of 60°C they can last for 30 minutes of processing (Yüce, 1992; Kloos and Bannerman, 1995; Ekici et al., 2008).

The heat resistance of *S.aureus* varies according to the texture and characteristics of the food product it habitats. For example, its heat resistance in milk is determined to be 3,1 to 3,4 minutes at 60 ℃ (Tunail,2009). They create resistance to antibiotics very rapidly. They eliminate the effect of penicillin as a result of their penicillinase property (Yüce, 1992; Kloos and Bannerman, 1995; Ekici et al., 2008).

Staphylococci generate heat resistant enterotoxins which cause food poisoning in humans. Staphylococcal enterotoxins (SE) are single-strand proteins which contain large amounts of lysine, tyrosine, aspartic and glutamic acid and which have a molecular weight varying between 26900 and 29600 Da. (Milci and Yaygın, 2006).

Enterotoxins are a kind of exotoxin which are synthesized when the staphylococci count reaches 10^6 CFU/grand above (Cowell et al., 2002; Sağun and Alişarlı,2003).

Staphylococcal enterotoxins are single-strand proteins with low molecular weight (26-34 kDa) which can be produced during all phases of proliferation but are mainly produced in the middle or at the end of the exponential phase. They are resistant to proteolytic enzymes such as pepsin, trypsin, chymotrypsin, rennin and papain, and are relatively resistant to heat (Balaban and Rasooly, 2000).

They require temperatures between 10°C to 48℃ to produce toxins. In order to produce toxins in food, their minimum pH requirement is slightly above their vegetative reproduction requirements (9-5.1 pH). A similar situation is present for water activity value as well. The minimum water activity value for aerobic reproduction is 0,83-0,86; and the minimum water activity value for anaerobic reproduction is 0,90. However, they require higher water activity values to generate toxins (Tunail, 2009).

While the optimum water activity value for *S.aureus*growth is below 0,99 it is known to have a considerably wide a_w range for growth compared to other food based pathogens. While its minimum a_w value is generally 0,86 it is known to drop as low as 0,83 for some strands. In anaerobic conditions, minimum a_w value is slightly higher at around 0,90 (Atıcı,1999; Reginald et al., 2001).

Heat resistance is the most important property of staphylococcal enterotoxins (SE) and it is reported that food enterotoxins cannot be completely inactivated through pasteurization or other heat applications. It is reported, however, that SEA and SEB are completely inactivated at an application of 100℃ for 90 minutes, or at an application of 120℃for 30 minutes. SEC is reported to be completely inactivated at an application of 100℃ for 180 minutes, or at an application of 120℃' for 60 minutes (Erol and İşeri, 2004).

The enterotoxin known to cause most of food poisonings is the SEA (Tsai and Li, 2008). Staphylococcal enterotoxins have an extreme resistance to gamma radiation. Staphylococcal enterotoxin based food poisonings become symptomatic within 30 minutes to 8 hours following intoxication. The symptoms are nausea, diarrhoea, vomiting, stomach cramps and fatigue (Gomes et al., 2007).

SE are emetic toxins which cause the symptoms of staphylococcal food poisonings in humans. SE are classified as members of the pyrogenic toxin super antigen family in respect of their biologic activities and structural forms. SE are divided into five stereotypes (SEA,SEB,SEC,SED, and SEE) on the basis of antigenicity. As a result of research carried out in the recent years some new types have been reported. (SEG, SHE, SEI, SEJ, SEK, SEL, SEM, SEN, SEO) (Omoe et al., 2002).

Moreover, the detailed structure of sek, sel, sem, sen, seo, sep, seq, ser, seu genes have been determined with the introduction of new sequence analysis methods (Leterle et al., 2003; Omoeet al.,2003; Orwin et al., 2003). It is stated that some of these genes do not have emetic activity (Omoe et al., 2003).

There are other strands apart from *S.aureus* that generate enterotoxin. These are *S.haemo*lyticus, *S.xlosus, S.equorium, S.lentus, S.capitis and S.intermedius* (Tunail, 2000).

Enterotoxin A is the most toxic one among the *S.aureus* enterotoxins which cause intoxication based food poisonings. The one most resistant to heat is enterotoxin B. The intake of 20-25 µg enterotoxin B, and the intake of 1µg or even 0,1-0,2 µg enterotoxin A is enough to cause intoxication. On the other hand, it is necessary that bacteria multiply and

increase their cell number to 6 log CFU/g or above within the food product for *S.aureus* intoxications to set off (Tunail, 2000).

Staphylococci reproduction on liquid media form a residue and blurriness. They can easily reproduce in aerobic and anaerobic conditions on solid media. Gram positive staphylococci can show gram negative properties in mature cultures (Yüce, 1992).

S.aureus form a golden colony on blood agar medium and for that reason the name of its strand type was derived from the Latin word aureus which means gold.

S.aureus form flat, shiny, circular convex shaped colonies on nonselective medium. While they grow well in NaCl concentrations up to 10%, their growth is very weak in15% NaCl concentrations. Their optimum reproduction take place at 30-40°C and 7-7,5 pH value (Demiret and Karapınar, 2000).

S.aureus show resistance to some chemicals such astelluride, mercury, chloride, sodium azide and some antibiotics such as neomycin and polymyxin (Tunail, 2000).

While they can metabolize many carbohydrates and produce acid in anaerobe conditions, they do not produce acid from arabinose, cellobiose, dextrin, inositol, raffinose and xylose. The property separating *S.aureus* from other types is their ability to fermentate glucose and produce α-toxin in anaerobic conditions (Atıcı, 1999).

While there are staphylococci that are human pathogens, the most typical example for those which are used as starters in manufacturing different food products, is *S.cornosus* which is used in the production of sausages (Öztan, 2003).

The human body is actually the natural source for *S.aureus* strands, which cause many infections (such as skin and tissue infections, bacteraemia, toxic shock syndrome, and endocarditis) and food poisoning (Demir et al., 2003).

S.aureus is a saprophytic bacteria which can be detected in general at a rate of 50-30% and at a rate of 20% in nostrils. (Tomi et al., 2005).While it is predominantly a nosocomialbacteria, *S.aureus* can alsoexist commensally in human skin flora (Tunail, 2009).

In terms of general public health, enterotoxin generating staphylococci are the most important subject of study. The reason for this is the fact that entero-toxigenic staphylococci cause toxin based food poisoning. Food poisoning is caused by toxins produced by bacteria and, as this chemical activity does not cause any detectible changes in foodstuffs, consumers do not realize that they are eating contaminated food. *S.aureus* and coagulase positive S.*hyicus* and *S.intermedius* are predominantly responsible for most staphylococcal food poisonings. However, the fact that the enterotoxin synthesizing ability of the other two types are much lower than *S.aureus*, this microorganism stands out among other staphylococci in terms of their role in food poisonings (Yüce, 1992).

In addition to food poisoning, staphylococci cause other illnesses in both humans and animals. They can be the cause of illnesses such as infected wounds on skin, maternity fever, meningitis and septicaemia. Moreover, it is estimated that 50% of the mastitis cases detected in animals are currently caused by staphylococci. This bacteria can penetrate into milk and subsequently to milk products if the necessary hygiene measures are not taken at the milking areas. They can cause food poisoning, nausea, vomiting and diarrhoeaas a result of the large amount of enterotoxin they produce. Animal carers and milking personnel can also directly transfer these bacteria to other humans. *S.aureus* is a particularly important microorganism in dairy sector (Tunail and Köşker, 1989).

S.aureus can also be transferred to food by cross contamination and can stay alive even after heat treatment. If enterotoxins have already been generated in food, inactivation of these toxins might not be possible through applications such as heat treatment. While it is initially possible to eliminate bacteria in dairy products by pasteurizing, it is also possible that microorganisms penetrate the product during the other phases of production through cross contamination. Cross contamination can occur through human hands, tools and machinery, air, additives, water, etc. Afterwards, if the suitable conditions become present,

the microorganisms start generating enterotoxins. In particular, *S.aureus* strands which penetrate dairy products after pasteurization are known to reproduce and start generating toxins much faster (Selçuk, 1991).

S.aureus has a high virulence factor and is very frequently the source of human infection. After the discovery of penicillin in 1929 by Alexander Fleming and the commencement of its utilization in 1945, important success has been achievedin the treatment of staphylococcal infections. But, as a result of the widespread use of penicillin, staphylococci strands with the ability to break penicillin emerged. Penicillin resistance in staphylococci started increasing rapidly from mid-1940, and in 1950's resistance to other antibiotics such as tetracycline, erythromycin and streptomycin was also detected. In 1960, a semi synthetic enzyme called methicillin was developed. It was resistant to penicillinase which was the enzyme generated by staphylococci to break penicillin. As a result, a second important victory was achieved in the fight against staphylococci based infections. However in 1961, methicillin resistance was detected in staphylococci. From the end of 1970's and start of 1980's, it has been detected that multiple antibiotic resistance started in MRSA (Methicillin Resistant *Staphylococcus aureus*) strands (Gülay, 2002).

1.2.1. Staphylococcal enterotoxins

Enterotoxins were first detected in 1914 when Barber drunk milk obtained from a cow infected with mastitis caused by staphylococcus. While acute gastrointestinal infection symptomatic with nausea was observed, the cause and progress of infection could not exactly be identified until Duck verified the existence of enterotoxins in 1930's. Enterotoxins are in fact exotoxins (Marth and Halpindohnalek, 1989).

The toxins generated by staphylococci are divided into two groups as exotoxins and endotoxins. Exotoxins consist of haemolysins, leucocidins, leucocidin cytotoxins, exfoliative toxin, pyrogenic toxin and toxic shock syndrome toxin 1. Haemolysins have cytotoxic effects on erythrocytes, leukocytes, hepatocytes and human diploid fibroblasts and are grouped as α, β,Б,γ hemolysins. Although staphylococcus can be phagocyted through leucocidins, they continue to live within the phagocyting cells. Another exotoxin is the exfoliative toxin which causes staphylococcal scalded skin syndrome (SSS), also

known as the Ritter Disease, in new born and breast-feeding children. This condition presents itself as the crusting of the intradermal tissue and the fissuring of skin. Pyrogenic toxin and toxic shock syndrome toxin 1 are very dangerous exotoxins, which together cause the toxic shock syndrome, giving rise to a very serious pathologic condition (Anderson and Stone, 1955).

SE are single-chain proteins which contain large amounts of lysine, tyrosine, aspartic acid and glutamic acid and with molecular masses varying from 26900-29600. They have seven widely seen different types. These are named as; A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED) and E (SEE). The most important property of these enterotoxins is their high resistance to heat treatment. Research showed that they remain 50% active after a ten minute heat treatment at 100℃. It has also been determined that they only became inactive after a heat treatment of 1-2 minutes at 121℃.They are not only resistant to heat but also to proteolytic enzymes such as pepsin and trypsin. These properties provide for their passing through the digestive tissues without losing their active effects (Yaygın and Milci, 2006).

The heat treatment application necessary for the inactivation of *S.aureus* enterotoxins is reported asa temperature of 100℃for 1-3 hours, or a temperature of 120℃ for 10-40 minutes (Tunail, 2000).

While enterotoxins are strain particular, it is known that one strain has the capability to produce more than one type of toxin. While 7 toxins (A, B, C, C2, C3, D, and E) arelisted serologically, TST-1 is the toxin which causes toxic shock syndrome. Together with the recently identified toxins G and H, it is reported that ten different types of enterotoxins exist. In *S.aureus* strains isolated from humans A, B, C type enterotoxins are most commonly seen.In strains isolated from cattle, C type enterotoxins and in strains isolated from chicken,D type enterotoxins are seen. While Enterotoxin A synthesis occurs during the logarithmic and stationary phases of bacterial growth; Enterotoxin B synthesis occurs only during the period when the stationary phase of bacterial growth starts. Although strains generating staphylococcal enterotoxin C grow well in their media, the internal

factors within food and certain organisms present in natural flora are known to delay the enterotoxin C generation (Yüce, 1992).

The intensity of intoxication depends on the amount of toxin intake. According to Evenson and colleagues, the intake of 100-200 ng enterotoxin A results in food intoxication. Raj and Bergdoll, cites the amount necessary for intoxication as 20-25 μg for enterotoxin B. Many researchers have reported that food intoxications are mostly caused by A type toxin, followed by B and D toxins. The two leading clinical symptoms of staphylococci based food poisonings are vomiting and diarrhoea. Poisoning presents itself within 1-6 hours following the intake of food contaminated with enterotoxin. The duration of symptoms varies according to the amount of the enterotoxin and the age and weight of patient. The condition offsets with symptoms such as vomiting, abdominal pain and salvation which usually last for 24-48 hours. Blood in the stool and vomitare occasionally observed and death very rarely occur (Yüce, 1992).

Acute gastroenteritis caused by food poisoning gives rise tomucosal hyperaemia and mucosal erosion; irrigational muscle spasms; local edema; and mitochondrial damage toepithelium cells of the jejunum mucosa. Enterotoxins cause diarrhoea as a result of the transfer of factors which inhibit the absorption of water through the lumen of the intestine. Cases of intestinal necrosis and pseudo membranous enterocolitis caused by enterotoxin synthesis has also been reported in patients using wide spectrum antibiotics for *S.aureus* bacteria intoxication (Selçuk, 1991).

The factors affecting toxin production of *S.aureus* are the pH, a_w and atmosphere conditions as well as the presence of other organisms. In recent years, it has been determined that the toxin generation mechanism of *S.aureus* was interrelated to other bacteria reaching sufficient cell density. It has also been determined that this interrelation occurs through a system of stimulus and response correlated to population density. This system is called “Quorum Sensing” (QS) and is cited as one of the means of communication between bacteria as well as coordinating social behaviour amongst bacteria (Bilge and Karaboz, 2005).

1.2.1.1. Factors affecting staphylococcal enterotoxin generation

1.2.1.1.1. Contamination level

In contaminated food**,** *S.aureus* generated toxins at an amount less than 1.0 μg is sufficient to set off the symptoms of staphylococcal intoxication. The sufficient amount of toxin for causing food poisoning is a subject of discussion and the minimum amount varies according to toxin type. In the case of *S.aureus,* it is reported thatthe generation of sufficient toxin amount for intoxication is reached when the *S.aureus*count is more than100.000 CFU/g-mL. In other words, if the *S.aureus* count is 5x105 CFU/g-mL within a food product, it is definitely risky. However, a low *S.aureus* count in a food product does not necessarily mean that it is safe (Tükel and Doğan, 2000).

1.2.1.1.2. pH and NaCl

The optimum pH value for the generation of enterotoxins is between pH 6.0-7.0. In comparison to SEB generation, the generation of SEA is more tolerant to pH variations. In comparison to salt free conditions, 5% NaCl concentrations help increase *S.aureus* proliferationrate. On the other hand, NaCL concentrations at 7.5% and10%levels are known to decrease growth rate to a certain extend (Erol and İşeri, 2004).

1.2.1.1.3. Temperature

The optimum temperature for *S.aureus* growth is 37ºC, whereas the optimum temperature for enterotoxin generation varies between 40-45ºC (Erol and İşeri, 2004).

1.2.1.1.4. Competitive property

Staphylococci are easily inhibited by other microorganisms in mixed cultures. *Enterococcus*, *Lactococcus* and *Leuconostoc are* known to be important inhibiting bacteria. In addition, *E. coli*, *Pseudomonas*, *Serratia* and *Aerobacter* also have an inhibiting effect on *S.aureus* growth (Ekici et al., 2008).

1.2.1.2. Methods of identification of staphylococcal enterotoxins

The identification of enterotoxins in foodstuffs is proportional to the amount of enterotoxin necessary for causing illness in humans. This dose is 100-200 ng (FDA, 2001). Various

methods of immunologic and serologic analysis have been developed for the identification of staphylococcal enterotoxins in foods. The immunologic analysis methods are sensitive and are based on the specific identification of enterotoxins. However, the identification of certain uncharacterized staphylococcal enterotoxins is carried out on the basis of generating the special emetic activities found only in monkeys. It has been reported that in 50% of young Rhesus monkeys, 5-20 μg toxin amount generated emetic reaction (List Biological, 1999).

1.2.1.2.1. Immunologic methods

Radioimmunoassay (RIA), is a widely used method for enterotoxin identification in culture filtrates and food extracts.This method is based on the competing of an unlabelled toxin in the sample with a standard radioactive labelled toxin to the adhesion parts of antibody molecules. This method can generally identify rapid (3-4 hours) toxins under 1-10 ng/g level. Its disadvantages can be listed as non-specific reactions, the need for highly purified enterotoxins, adverse effects that may arise during the labelling of antibody epitopes, the short half-life period of radioisotopes, the harming effects of radionuclides to human health and the need for expensive identification equipment (Brett, 2006).

Enzyme-Linked Immunosorbent Assay (ELISA) or Enzyme Immuno Assay (EIA) has been used for a long time for the identification of antigens and antibodies. ELISA is rapid and sensitive just like the RIA. It is based on catalysing chromogenic substrates through enzymes and their visual observance and evaluation. The equipment necessary for ELISA test can easily be found in many laboratories and enzyme antibody conjugates can be preserved at –20º C for a long period (Çırak, 1999; Brett, 2006). In recent years, commercial test kids such as RIDASCREEN, TEVRA, TRANSIA, VIDAS, SET-EIA and RPLA are being used for the analysis of various foods. (Brett, 2006).In their study carried out for the comparison of VIDAS SET, VIDAS SET2 and TRANSIA kids, Vernozy-Rozand and colleagues (2004) analysed food which they contaminated with SEA, SEB, SEC2, SED and SEE . The study determined that VIDAS SET2 produced more specific and more sensitive results in comparison to VIDAS and TRANSIA.

1.3. *Staphylococcus aureus* and Food Poisoning

Food Poisoning has been described by the World Health Organisation as a disease which occur as a result of consuming contaminated food or water. 250 different types of poisoning have been reported for food poisoning which is listed among the most important illnesses affecting the world in general. It has also been reported that 2/3 of these different types of poisoning have been caused by bacteria. Staphylococcal food poisoning is brought about by the presence of staphylococcal enterotoxins in food (Loir et al., 2003; Kumar et al., 2009). More than 60-90% of food poisonings are caused by bacteria present in nature. Bacterial food poisonings are divided into two groups on the basis of how they affect human organism. The two groups are called toxi-infection type food poisonings and toxic type food poisonings.

The bacteria which provoke food poisoning through intoxication, in other words those which cause food poisoning through proliferating and secreting exotoxin within food are *C. Botulinum* and *S.aureus*. In addition, the bacteria whose own mass density, in other words, whose own existence and their endotoxins, cause food poisonings progressing with symptoms of gastroenteritis are *C. perfringens*, *B. cereus*. The bacteria which cause food poisoning through infection are *Salmonella* spp., *Shigella* spp., and *E. coli.* And there is *Proteus* spp. and *Pseudomonas* spp. which are known to cause food poisoning but their etiology has not yet been explained (Belitz et al, 2009).

1.4. Symptoms Caused by Staphylococcal Intoxications in Humans

In Staphylococcal food poisoning cases symptoms usually progress quite rapidly. The symptoms set off within 1-6 hours following intoxication. On average symptoms progress within three hours. The predominant and serious symptom is vomiting and it follows severe nausea. Other symptoms are stomach pain, fever, dizziness, shivering, headache, abdominal cramps and diarrhoea. Symptoms usually disappear within 1-2 days of diagnosis and although death rate is extremely low, cases resulting with death have been reported (İşeri and Erol, 2009)

It has been reported that, in general, the presence of at least 1μg toxin in 100g food is necessary for food poisoning to occur as a result of consuming contaminated food.

However, it has also been reported in a food poisoning case caused by chocolate milk amongst school children that only 0.2 μg SEA set off the poisoning (Loir et al., 2003).

1.5. The Significance of *Staphylococcus aureus* in Cheese

The contamination of foodstuffs can frequently happen for different reasons and during any stage starting from preparation, manufacturing, packaging, transportation to stocking.As a result, not only the quality of the food product is undermined causing economic loss, but various clinical symptoms as well as food poisoning can be seen in people consuming the product. Milk and milk products constitute a very suitable growth medium for microorganisms. Microorganisms transferred to milk from different sources can multiply quite rapidly (Demiret and Karapınar, 2000).

The proliferation and toxin generation of *S.aureus* strains are particularly rapid especially if they are transferred to pasteurized milk and milk products after pasteurization. The reason for this is that the microorganisms which compete with *S.aureus* bacteria are already destroyed during pasteurization, completely leaving the medium to *S.aureus* strains which have been transferred after pasteurization (Selçuk, 1991).

Various staphylococcus types and many biotype *S.aureus* strains are transferred to milk from the animal during milking. In particular, milk acquired from animals with mastitis constitute an important source for enteropathogenic *S.aureus* strains. While the main source of transfer are humans and animals, the microorganisms can also be transferred from soil, water sources, septic water, vegetable surface, dust and air, where these microorganisms exist. In addition, the transfer from contaminated animals to healthy ones through the milking equipment is inevitable (Demiret and Karapınar 2000).

In general, raw milk already contains toxigenic staphylococcus resistant to penicillin and if cheese is produced under any favourable conditions to their proliferation, toxin secretion starts. When milk is heated to 25-30 °C during cheese production, an ideal medium is prepared for staphylococcal toxic effect if the bacteria in the starter culture do not become active on time and start producing acids. The toxic effect becomes even faster with low rate of humidity loss (Ünlütürk et al., 1991).

The low pH value of coagulating milk also provides for the staphylococcus to proliferate within a short period. In this respect, inadequately pasteurized milk is dangerous. Owing to their diverse properties different cheese types have differing tendencies in terms of staphylococcal growth (Metin, 1977).

The issue of production safety becomes even more important as the proliferation of toxigenic staphylococcus in the milk used for cheese production can easily cause food poisoning. All studies aimed at enhancing production safety for cheese are based on the principle of adequate pasteurization of milk. While this procedure leads to slow maturation of cheese, it prevents bacterial contamination (Metin, 1977).

Initially pasteurization values were kept low to help protect the taste of milk. However, it has been determined that such pasteurization was not enough for killing all pathogens. In effect, fifteen seconds of pasteurization at 66 ℃ is sufficient for staphylococcus to die. However, for a guaranteed result it is usually advised that pasteurization is applied for 15 seconds at 68 ℃. Moreover, in some countries 15 seconds at 71 °C is adapted as usual practice of pasteurization. This pasteurization norm is, in fact, sufficient to kill *Streptococcus pyogenes, Salmonella typhimurium, Brucella abortus* and *S.aureus*. However, *Streptococcus faecalis* may stay alive at these heat treatment values. Apart from *Streptococcus pyogenes,* all the above cited bacteria can continue their activity for different periods in various cheese types (Metin, 1977). As the pasteurization of milk at low temperature and for short periods enhance productivity, these norms are preferred in most dairy farms.

Staphylococcal poisonings emanating from cheese consumption are seen from time to time and the investigation of these cases show that *S.aureus* proliferation and generation of toxin takes place during the manufacturing stages of cheese. The slow activity rate of the starter culture added to milk during cheese production and the resulting low acidity (0,4% or lower) prepares a suitable medium for S.aureusgrowth.The proliferation of the microorganism continues during the filtering of curd (4-5 hours or more) and its count can reach amounts sufficient for toxin generation(such as 107 CFU/g). However, the presence of oxygen within the medium inhibits toxin generation. In any case, the fact that low

S.aureus amount is detected in a cheese does not necessarily mean the absence of toxin. *S.aureus* which proliferates to numbers sufficient for toxin generation during manufacturing, can in time decrease in number within the host cheese. In order to keep staphylococcal poisoning under control, sufficient heat treatment, ensuring normal starter activity and the implementation of a good sanitation program is necessary (Ünlütürk et al., 1991).

In a study where brined white cheese was produced from raw milk, pasteurized milk and pasteurized milk with starter culture, significant amounts of microorganism in terms of food safety have been detected in the brined white cheese during maturation. As a result of the analysis of 15 day old brined white cheese, the *Staphylococcusaureus* count in cheese made from pasteurized milk was determined to be 5,39 log CFU/g, in cheese made from pasteurized milk with starter culture was 4,54 log CFU/g and in cheese made from raw milk was determined to be 5,90 log CFU/g (Selçuk, 1991).

Another important factor in the proliferation of *S.aureus* and the generation of enterotoxin in cheese is the activity rate and amount of the starter culture added to the milk used for production. An inhibited starter activity increases *S.aureus* proliferation rate by 5-10 times in comparison to normal conditions. The possibility of *S.aureus* proliferation and generation of toxin is much higher in cheese produced from raw milk. As a result of the studies carried out on this subject it has been reported that enterotoxigenic *S.aureus* strains were isolated from samples of Cheddar, Gouda, Ras, Camambert, Brick, Colby, Swedish type, Mozzarella and goat cheese. It has also been reported that *S.aureus* livelihood continued for 98-154 days in Cheddar cheese produced from milk contaminated with mastitis, and that the livelihood period prolonged up to 210 days in Swedish type cheese produced from milk contaminated with mastitis.In another study, it has been reported that *S.aureus* generated toxin in correlation to starter culture activity in Swedish type and Brickcheese, while it did not produce toxin in Mozzarella and in Blue cheese types. It has also been stated that in Spain, the toxin generation differed in Manchego type cheese according to the activity and amount of starter culture. Moreover, it has been determined that *S.aureus* continued to be an important pathogen in cheese produced in different parts of the world such as Iraq, Canada and Portugal (Demiret and Karapınar, 2000).

In Turkey, milk and milk products are usually produced in small establishments and dairy farms in the absence of any surveillance or control mechanisms. This situation inevitably increases the risk of infection and food poisoning emanating from consuming milk and milk products. In the TRNC the same increased risk is present for exactly the same reasons. It has been determined by many studies that *S.aureus* is very often present in milk and milk products produced in Turkey.

In a study carried out on pasteurized milk sold in Ankara, coagulase positive staphylococcus presence at counts above 1,30 log CFU/ml level were identified in 44% of the samples. It should be noted, in this context, that unsuitable keeping conditions carry serious risks in terms of public health as they may cause the multiplication of microorganisms (Demiret and Karapınar, 2000).

S.aureus should preferably be not present or be present in very small amounts in cheese and other milk products. In Turkey, all stages of production, from the animal providing milk to the distribution and preservation of the product should be kept under control with the necessary measures intact, in order to avoid the economic damage, including the loss of manpower, suffered from *S.aureus*poisonings caused by milk and milk products.(Demiret and Karapınar, 2000). The same situation and the same needs apply to the TRNC.

At a time when Turkey is being closely monitored as a candidate for European Union membership, it is very important that food products produced in the country are safe in terms of human health and are on par with international standards in terms of quality. In order to achievethese goals it is necessary that; quality raw produce is manufactured; starter cultures are utilized in dairy production; milk is transferred via cold chain; food engineers and other qualified workers are employed; sanitation and hygiene protocols are strictly applied; HACCP system is designed for each product and monitored closely; consumers are educated; and supervising authorities become more efficient (Coşkun and Öztürk, 2000).

In a study carried out on identifying and eliminating sources of contamination during white cheese production, it has been determined that the critical control stages were the

pasteurization treatment, materials used during production such as presses and bale clothes, and air and personnel hygiene (Kasımoğlu, 1999).

S.aureus,is a bacteria not welcome in food. Its presence in directly consumed food products such as cheese should not actually be accepted or tolerated. However, its presence in low amounts in cheese is allowed as a general international standard in line with manufacturing technology protocols. Accordingly, both TS 591 (Anonymous, 2006a) and Turkish Food Codex (Anonymous, 2011), cite n c m M values as 5;*2;*1,0*x*10^2CFU/g; 1,0x10^3CFU/g respectively.

1.6. Analysis of *Staphylococcus aureus*

Research to develop faster and more sensitive methods of microbiological analysis of foodstuffs is a continuous effort. While the inhibition of competitive flora at the highest level is targeted for increasing sensitivity, it is a requirement to a certain extent that the bacteria is not harmed through this process. Accordingly, the identification of the microorganism through the use of various chromogenic or floragenic substrate is also applied in addition to inhibiting competitive flora (Baron et al., 1995; Anonymous, 2005).

1.6.1. Enumeration

The standard method used for the enumeration of*S.aureus*is the Baird-Parker Agar medium. (Anonymous, 2001a).Spreading culture method is used for cultivation. It is usually advised, especially for analysing solid food, that 1 mL solution derived from 10–1 diluted sample is spread equally to 3 standard size petri-dish boxes. Or it is advised that the 1 mL solution is directly spread over large size petri-dish box with 14 cm diameter. With this procedure, even a low bacteria presence at a count level of 10 CFU/g can be detected (Duncan et al., 2004; Laird et al., 2004; Anonymous, 2006b).

If the *S.aureus* presence in the food to be analysed is lower than this level, it is advised that the MPN technique is utilized. In other words, MPN technique is a more sensitive method of selective isolation of *S.aureus*. In this method, the sample is firstly cultivated in Giolitti-Cantoni Broth medium, and following incubation, test results are confirmed through spreading cultures from positive tubes to Baird-Parker Agar medium (Anonymous, 2006b).

For suitable foods, membrane filtration method can also be used for *S.aureus* analysis (Tükel and Dogan, 2000).

However, International Dairy Federation (IDF) and International Organization for Standardization (ISO), and accordingly, the Türk Standartları Enstitüsü (TSE), all advise that the Giolitti-Cantoni Broth and Baird-Parker Agar combination method is used for the analysis of foodstuffs (Anonymous, 2004; Anonymous, 2006b).

1.6.2. Utilized media

A great number of selective media have been developed for the analysis of *S.aureus.* While the tolerance of this bacteria to high salt concentration as well as its resistance to lithium chloride and tellurite is used to inhibit competitive flora, its ability to reduce tellurite to tellurium and form blackening colonies, present itself as an important selective property in both solid and liquid media (Atlas, 1996; Anonymous, 2005).

The most widely used liquid medium is Giolitti-Cantoni Broth. It is used in Baird Broth (staphylococcus) enrichment or MPN technique. Baird-Parker Agar is listed as a leading solid medium in most international standards. In addition, Chapman Agar (Staphylococcus Medium 110), Mannitol Salt Agar (Mannitol Salt Phenol Red Agar) and Vogel-Johnson Agar are also used (Anonymous, 2005). In recent times, direct identification of coagulase is preferred whereby rabbit plasma fibrinogen is utilized instead of egg yolk emulsion in Baird-Parker Agar solid medium (Anonymous, 2001b). A study carried out on the enumeration of *S.aureus* in cheese, 12 different selective media have been used and Staphylococcus Medium 110 and Mannitol Salt Agar Medium have been identified as superior to Baird-Parker Agar in general terms. However, it was also an interesting findingthat the superiority of different mediawere observed during different stages of maturation. It was also proven that strain difference can also play an important role (Stiles, 1977).

In another study, 80 samples of coagulase positive enterotoxigenic *S.aureus* were utilized and it has been established that Baird-Parker Agar medium had the highest return of isolates amongst the tested media which were Baird-Parker Agar, Calf-blood Agar, Baird-

Parker Agar, Carter Agar, Vogel-Johnson Agar, Mannitol-salt Agar and Staphylococcus Medium 110 (Niskanen and Aalto, 1978). Short information is provided below on only Baird-Parker Agar, Mannitol Salt Agar and Giolitti-Cantoni Broth media.

Baird-Parker Agar Medium: While this medium contains lithium chloride and tellurite for the inhibition of competitive flora, the pyruvate and glycine present in its composition selectively stimulate staphylococcal growth. If high level of Proteus contamination is suspected in the sample to be analysed, it is advised that filter sterilized sulphamethacin is added in amounts ensuring a concentration level of 50 mg/L after autoclaving.The differentiation of *S.aureus* colonies is based on their two characteristic properties.One is their property to form typical zones and circles around the colonies as a result of lipolysis and proteolysis, and the other is their property to form blackening colonies as a result of the reduction of tellurite to tellurium.At the end of a 24 hour long incubation at 37 °C, *S.aureus* forms shiny convex colonies with a diameter of 1-1,5 mm. The colony diameter becomes 1,5-2,5 mm at the end of a 24 hours incubation period. Egg Yolk reaction and tellurite reduction usually take place with positive coagulase reaction. In Baird-Parker Agar medium, haemolysis test can also be carried out by adding blood plasma instead of egg yolk. Human sourced *S.aureus* produces α-haemolysis whereas cattle sourced ones produce ß-haemolysis. Haemolyse reaction can be more definitely identified after leaving the medium at room temperature or preferably in fridge for one night following incubation at 37 °Cfor a period of 24 or 48 hours (Anonymous, 2006b).

Giolitti-Cantoni Broth Medium: While staphylococcal growth is stimulated by the pyruvate, glycine and high doses of mannitol present in the medium composition, gram negatives and gram positives present in the competitive flora are inhibited by lithium chloride and tellurite respectively. The inhibition of micrococcus, on the other hand, is partly achieved by anaerobic incubation. The proliferation of Staphylococcus is determined by the black colour which appears as a result of the reduction of tellurite to metallic tellurium. This medium is used for the enumeration of *S.aureus* in foodstuffs by using the MPN technique or for the presence/absence test of *S.aureus* in a given amount (volume or weight) of the analysed food. The results are confirmed by spreading the samples from

blackening tubes to Baird-Parker Agar base plates. This is the medium advised by ISO for the identification of low-count staphylococcus by MPN technique (Anonymous, 2006b).
Mannitol Salt Agar: This medium is a modification of Chapman Agar (Staphylococcus Medium 110) medium. Its high salt concentration inhibits competitive flora growth. Mannitol stimulates *S.aureus* growth and provides for the formation of a yellow zone around the colony marked by fenol red. Mannitol positive *S.aureus* forms abright yellow colony, in mannitol negative *S. epidermidis* and others no colour changes occur and very weak growth is observed (Anonymous, 2006b).

1.6.3. Confirmation

S.aureus strains form typical colonies in their selective media. Confirmation of colonies is not usually necessary in everyday use. While it is necessary to confirm in questionable cases, it is also advised that confirmation is frequently carried out in laboratories performing routine microbiological analysis. The most widely utilized method for confirmation is the coagulase test. Coagulase is an enzyme which coagulate blood plasma. There is close correlation between the coagulase and enteric toxin produced by *S.aureus.* Accordingly, it is accepted that coagulase positive *S.aureus* has the ability to produce toxins. Isolates are tested with Brain-Heart Broth culture, whereby 0,1mL culture is added to a tube containing 0,3 mL of rabbit plasma and is left to incubate at 37 °C. Every hour the presence and/or state of clotting is controlled by slowly tilting the tube. However, special care should be taken to ensure that the tube is not excessively tilted or shaken during these controls. The formation of distinctclotting (75% clot) is considered as a positive result. Positive result is usually observed within 4-6 hours. In case of negative result, the incubation should be continued for 24 hours (Reginald et al., 2001; Anonymous, 2006b).

Coagulase test can also be applied as Latex Test. Moreover, coagulase test can also be carried out by directly adding rabbit plasma fibrinogen to Baird-Parker Agar medium instead of egg yolk. However it is known that some strains of *S.aureus* produce weak coagulase and it is possible that some isolates give negative results despite the fact that they are typical. It is therefore advised that other tests such as lysostafin sensitivity test,

haemolysin generation test, thermostabil nuclease test and mannitol test, are also applied in suspicious cases (Reginald et al., 2001; Anonymous, 2005).

1.6.4. Identification of *S.aureus*

Identification of S.*aureus* isolated from a sample is possible by coagulase test. However if the isolated microorganism is not *S.aureus,* coagulase test cannot help determine its real identity. Moreover, it has already been mentioned above that coagulase negative *S.aureus* strains exist. In addition, there is always the possibility of a typical colony isolated form its selective medium not be *S.aureus* or, it is also possible that an atypical colony is in fact *S.aureus.* Genetics and immunology based methods are increasingly being used for determining the true identity of typical and/or atypical colonies. Nevertheless, it is obvious that classic culture methods are going to be utilized for a while longer.

CHAPTER 2

MATERIAL AND METHOD

2.1. Material

In this study, 85 samples of various dairy products collected from markets in the Nicosia area in the TRNC have been used as material. The dairy products have been collected by random sampling directly from the market stands, transported to laboratory environment via unbroken cold chain and have been analysed within a short time for *S.aureus* presence.

2.1.1. Media and test kids utilized for the isolation and identification of bacteria.

Peptone Water (Oxoid CM0009) has been used for dilution during the isolation phase. 15 g of ready combination from the medium has been diluted with 1000 mL distilled water and set at pH 7.2±0.2. The prepared medium base has been distributed to 9‘ a mL tubes (second dilution) and 90’ar mL bottles (first dilution) and has been left for cooling after auotoclave sterilisation at 121ºC for 15 minutes.

Baird- Parker (BPA) Agar Base (Oxoid CM0275) was used as the selective medium for the identification of *Staphylococcus aureus.* (FDA/BAM, 2001). Following isolation, Staphytect Plus (OXOID DR 0850) Latex Agglutination Test Kid, composed of DR851M Staphytect Plus Test Reageny (5,6 ml), DR852M Staphytect plus Control Reagent (5,6 ml) and 35 DR500G Reaction Cards, was utilized for the confirmation of probable *Staphylococcus* aureus colonies.

2.2. Method

2.2.1. Cultivation in solid medium base and evaluation of colonies (classic technique)

The identification of coagulase positive staphylococci by classic technique has been carried out in accordance with FDA/BAM (2001). 10 g test sample has been taken in a manner to represent the whole of the sample product and placed in stomacher bag. Then, 90 mL peptone water has been added and homogenised in stomacher for 60 seconds. 1 mL solution was taken by pipette from the homogenate (10^{-1}) and equally distributed and cultivated on pre-prepared BPA medium plates by spread plate technique. The petri dishes

were, then, left for incubation in anaerobe environment at 35 ±1ºC'd for 24-48 hours. The same method and procedure has been repeated for each of the 85 samples.

At the end of the incubation period, the shiny black-grey colonies with a diameter of 2-3 mm and circled by narrow, convex shaped, smooth, shiny zones were considered as probable *S.aureus* colonies and counted.

2.2.2. Confirmation

Latex confirmation test was applied to probable *S.aureus* colonies which proliferated as a result of BPA medium cultivation.Latex reagent has been brought to room temperature and homogenised by shaking. Afterwards, 1 drop of test reagent has been poured on one of the test coils on the reaction card; five suspicious colonies were taken from the petri dish with sterile loop; and were smeared over the test coil whereby they mixed with the test reagent. The reaction card has been shaken in circular movements for 20 seconds and observed for agglutination. As agglutination was observed by the testing of all colonies, the result was considered positive. We proceeded to the second phase of confirmation and repeated all the above mentioned stages with a control reagent. The testing of the analysed colonies resulted in agglutination with latex test reagent, but no agglutination was observed with the control reagent. It was, thus, confirmed that the isolated *S.aureus* colonies were, in fact, coagulase positive strains.

CHAPTER 3

RESULTS

As a result of the analyses of 85 diary product samples (34 Hellim Cheese, 10 Talar Cheese, 20 Young Cheddar Cheese, 5 Fresh Lor Cheese, 5 Cokelek Cheese, 5 Kaymak Cream, 6 Cheddar Cheese) produced in different manufacturing facilities in the TRNC and collected from markets in the Nicosia area, it has been determined that; the *S.aureus* amount detected in one sample (1%) was within the accepted limits cited in the Turkish Food Codex Regulation on Microbiological Criteria and did not endanger public health; 12 samples (14%) contained *S.aureus* at levels endangering public health; and 72 (85%) samples did not contain *S aureus. S aureus* colonies were isolated from 2(6%) of the 34 Hellim Cheese samples; from 2(10%) of the 20 Young Cheddar Cheese samples; from 2(40%) of the 5 Cokelek Cheese samples; from 4(80%) of the 5 Fresh Lor Cheese; from 2(20%) of the 10 Talar Cheese samples and from 1(20%) of the 5 Kaymak Cream samples. No proliferation was detected in the 6 Cheddar Cheese samples (Table 3.1.).

The samples have been collected from the market by random sampling. The shiny black-grey colonies, with a diameter of 2-3 mm and circled by narrow, convex shaped, smooth, shiny zones, which formed in 13 of the BPA petri dishes after the incubation period were considered to be *S.aureus* colonies.

The *S.aureus* colonies could be counted in one dish, but enumeration was not possible in the remaining 12 petri dishes because of high bacteria growth intensity (Figures 3.1, 3.2, 3.3).

No proliferation was observed in the remaining 72 petri dishes.

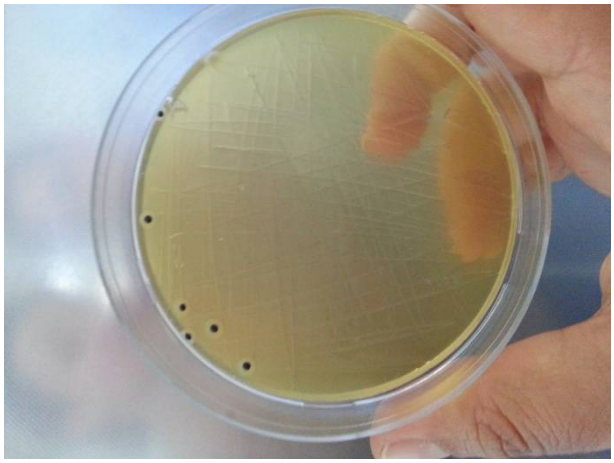

Figure 3.1 : Below limit *S.aureus*colonies isolated from Hellim Cheese sample and counted in BPA medium base

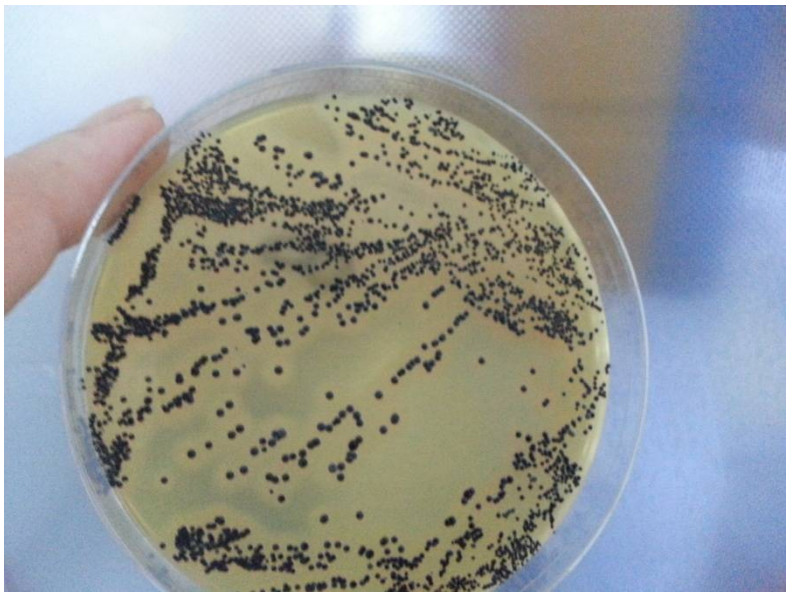

Figure 3.2: Above limit *S.aureus* colonies isolated from Hellim Cheese sample

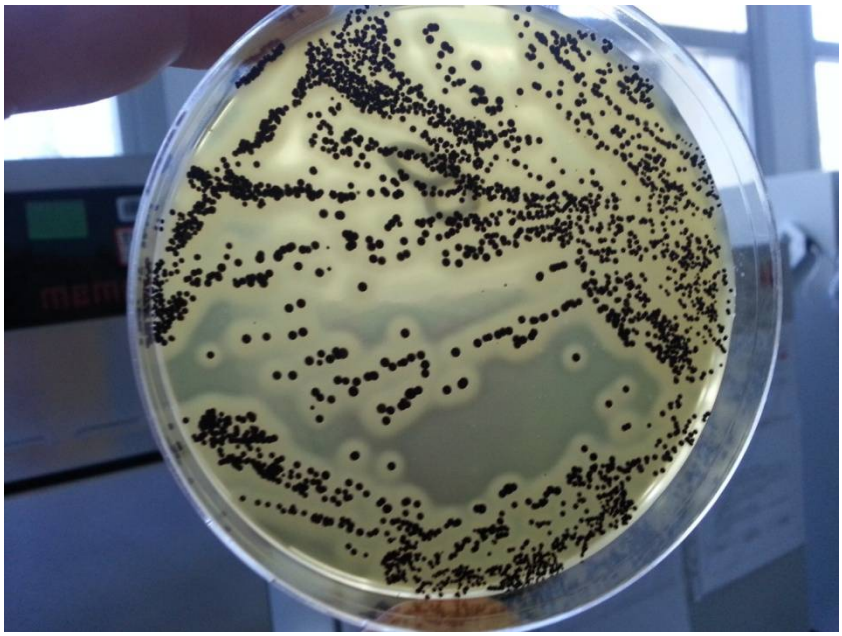

Figure 3.3: Above limit *S.aureus* colonies isolated from Young Cheddar Cheese sample

3.1. Confirmation

It was confirmed by Oxoid Staphytect Plus type latex test that all the suspected colonies from the BPA medium were in fact *S.aureus* colonies. The results of the latex agglutination test are summarised in Table 3.1 according to sample distributions.

Table 3.1: Latex agglutination test and culture results of the dairy products produced in TRNC and sold in Nicosia area

Product Name	**Local**	**Import**	**Area**	***S.aureus* Latex Test Result**	***S.aureus*Colony Count (CFU/ml)**
Hellim Cheese	+		Nicosia	Positive	$>1.10^3$
Hellim Cheese	+		Nicosia	Positive	$\leq 1.10^3$
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative

Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Hellim Cheese	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Positive	$>1.10^3$
Young Cheddar	+		Nicosia	Positive	$>1.10^3$
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative

Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Young Cheddar	+		Nicosia	Negative	Negative
Cokelek Cheese	+		Nicosia	Positive	$>1.10^3$
Cokelek Cheese	+		Nicosia	Positive	$>1.10^3$
Cokelek Cheese	+		Nicosia	Negative	Negative
Cokelek Cheese	+		Nicosia	Negative	Negative
Cokelek Cheese	+		Nicosia	Negative	Negative
Fresh Lor Cheese	+		Nicosia	Positive	$>1.10^3$
Fresh Lor Cheese	+		Nicosia	Positive	$>1.10^3$
Fresh Lor Cheese	+		Nicosia	Positive	$>1.10^3$
Fresh Lor Cheese	+		Nicosia	Positive	$>1.10^3$
Fresh Lor Cheese	+		Nicosia	Negative	Negative
Kaymak Cream	+		Nicosia	Positive	$>1.10^3$
KaymakCream	+		Nicosia	Negative	Negative
Kaymak Cream	+		Nicosia	Negative	Negative
Kaymak Cream	+		Nicosia	Negative	Negative
Kaymak Cream	+		Nicosia	Negative	Negative
Cheddar Cheese	+		Nicosia	Negative	Negative
Cheddar Cheese	+		Nicosia	Negative	Negative
Cheddar Cheese	+		Nicosia	Negative	Negative
Cheddar Cheese	+		Nicosia	Negative	Negative
Cheddar Cheese	+		Nicosia	Negative	Negative

Cheddar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Positive	$>1.10^3$
Talar Cheese	+		Nicosia	Positive	$>1.10^3$
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative
Talar Cheese	+		Nicosia	Negative	Negative

CHAPTER 4

DISCUSSION

The microorganism contamination of foodstuffs can frequently happen for different reasons during the preparation, manufacturing, packaging, transportation and stocking stages. As a result, not only the quality of the food product is undermined causing economic loss, but various clinical symptoms as well as food poisoning can be seen in people consuming the product. Milk and milk products constitute a very suitable growth medium for microorganisms. Microorganisms transferred to milk from different sources can multiply quite rapidly (Demiret and Karapınar, 2000).

The proliferation and toxin generation of *S.aureus* strains are particularly rapid especially if they are transferred to pasteurized milk and milk products after pasteurization. The reason for this is that the microorganisms which compete with *S.aureus* bacteriaare already destroyed during pasteurization, completely leaving the medium to *S.aureus* strainswhich have been transferred after pasteurization (Selçuk, 1991).

Microorganism penetration as a result of cross contamination during the preparation, production, packaging, transportation and stocking of foods is a widely seen occurrence.

S.aureus, is one of the leading causes of mastitis in milk producing animals. Accordingly, *S.aureus* is frequently present in the milk of animals with mastitis. In addition, *S.aureus* can be transferred to milk from the milking personnel.

It has been determined that humans are the main source of contamination transferring *S.aureus* to food in food based intoxications. Humans act as a carrier and transfer this bacteria to other humans and foods. However, the fact that this bacteria can easily be isolated from air, dust, sewage and water, shows there are many different sources of contamination threatening foodstuffs.

In their study Donelly andcolleagues (1968), determined that *S.aureus* proliferation and toxin generation was better in pasteurized milk with light microorganism presence compared to raw milk with heavy microorganism presence. They also observed that in raw milk *S.aureus* toxingeneration started after its count reached 106 CFU/mL, whereas in pasteurized milk toxin generation started 12 hours after its count reached 104 CFU/mL.

In their study carried out with 26 samples of Erzincan Tulum Cheese, Özalp and colleagues (1978), detected presence of staphylococcal enterotoxin, although they did not identify presence of staphylococci in any of the samples.

Özalp andcolleagues (1978) also analysed 70 milk powder samples, 36 of which were of local and 34 were of foreign origin. They detected *S.aureus* presence in one of the local samples and presence of enterotoxin in two local samples. They reported that the presence of any microorganism was not observed in any of the foreign samples.

In their study carried out with homemade cheese in Chile, Castro and colleagues (1986), isolated 103 *S.aureus* from their samples. They reported that the coagulase and thermonuclease activity of all samples were positive, but no enterotoxin presence was detected in any of the samples.

As a result of his study on *S.aureus* contamination in various foodstuffs, Ewald (1987) reported that 20 of the isolates were coagulase negative, and that one of these isolates which was identified as*S. Haemolyticus* could produce C and D type enterotoxins at the same time. He also reported that 38 of the 150 isolates identified as *S.aureus* were enterotoxic and could produce either one and/or a couple of A, B, C, D, E type enterotoxins, and that C type enterotoxin was the mostly produced toxin.

In their study carried out with goat cheese produced in England, Bone and colleagues (1989) determined SEA presence, although they did not find any live pathogen bacteria in any of the samples.

Wieneke and colleagues (1993), determined 359 food poisoning cases caused by *S.aureus* enterotoxins between the years 1969 and 1990 in the city London. The analysis they carried out on the food products consumed by the said patients determined the average *S.aureus* count in the analysed food samplesas 3.0x107 CFU/g. Moreover, they isolated staphylococcal enterotoxin from the cheese samples consumed by two patients who were hospitalised for food poisoning, although no presence of live *S.aureus* bacteria were identified in any of the cheese samples.

Mutluer and colleagues (1993) investigated the ability of enterotoxigenic *S.aureus* strains to proliferate and produce toxins in White Turkish Cheese. They analysed white cheese produced from pasteurised milk and white cheese produced from raw milk contaminated with *S.aureus* strains (at 105 CFU/mL contamination level) producingA, B, C, D type toxins. As a result, they determined that the *S.aureus* count in cheese produced from raw milk were higher than the count in cheese made from pasteurised milk during all phases of production and maturing. Enterotoxin A stimulation was observed at the very first day of maturation in cheese produced from raw milk.

In their study carried out in Ankara, Kısa and colleagues (1996) identified coagulase positive staphylococci presence; in 73.3% of the plain cream cake samples at an average level of 6.3x102 CFU/g, and in all of the cacaoand fruit flavoured cream cake samples at an average level of 1.7x103 CFU/g. Moreover they detected coagulase (+) staphylococci count as 105 CFU/g which is at risk level for enterotoxin generation. They also determined that the coagulase (+) staphylococci isolated from a total of 25 (26 %) cream cake samples were able to produce enterotoxin. Four (36.4%) of the said samples were plain cream cake samples; 12 (22.6%) were cacao flavoured cream cake samples; and 9 (28.1%) were fruit flavoured cream cake samples.

Rosec and colleagues (1997), isolated 213 *S.aureus* strains from 121 food samples they analysed in France. They reported that 15.9% of the *S.aureus* strains isolated form cheese samples produced from raw milk stimulated enterotoxin, and that 43% of the *S.aureus* strains isolated from other foods had enterotoxigenic property.

In their study carried out with ice cream marketed at Bursa provincial centre, Özcan and Kurdal (1997) determined *Staphylococcus aureus* presence at an average level of 10,11x103 CFU/g in lemon flavoured ice cream; at an average level of 8,01x103 CFU/g in sour cherry flavoured ice cream; and at an average level of 11,46x103 CFU/g in strawberry flavoured ice cream.

Erol and colleagues (1998), carried out the microbiological analysis of 100 ice cream samples marketed in Ankara. As a result, they determined coagulase positive staphylococci presence in 20-30, 8% of the samples at 102-104 CFU/g level, and isolated E. *coli* and *Salmonella* bacteria from 2% of the samples.

Evrensel and Güneş (1998), carried out a microbiological study on plain ice cream marketed in Bursa. As a result, they detected coagulase positive *S.aureus* presence at 1.0x102-6.3x105 CFU/g level.

Rasooly and Rasooly (1998), carried out a microbiological analysis study on various food groups (mushroom, milk, potato salad, ice cream and meat products) sold in the United States of America. They reported that they detected SEA presence in the samples at a minimum level of 100 pg/mL.

Toklu and Yaygın (2000) carried out a study with 69 ice cream samples marketed in the Antalya province and determined that *coliform* was present in 88.40%; *E. coli* was present in 69.5%, and *S.aureus* was present in 49.27% of the samples. Yücel and Çıtak (2000) carried out a study with 30 ice cream samples marketed in Ankara and determined the lowest and highest level of *S.aureus* presence in the samples as$1.0x10^2$ and $3.0x10^3$ CFU/mL respectively.

Akineden and colleagues (2001) carried out a study with 103 milk samples marketed in Germany and isolated *S.aureus* colonies from all of the samples. They reported that 17 samples contained SEI; 21 samples contained SEG and SI; 21 samples contained SED and SEJ; 15 samples contained SEC+SEG+SEI and TSST-1; and 1 sample contained SEA+SEC and TSST-1.

Bostan and Akın (2002), reported that as a result of the study they carried out in İstanbul, they did not detect *Salmonella* spp., *S.aureus* and *E.coli* colonies in any of the 300 packaged ice cream samples they analysed.

Cowel and colleagues (2002), carried out a microbiological analysis of the foods consumed by 42 people who were hospitalised in Queensland, Australia, for diarrhea and vomiting. The initial analysis revealed SE presence. The further analyses of the chicken, cakes and salads consumed by the patients determined that the *S.aureus* level was >2.5x106 CFU/g.

Mukan and Evliya (2002) did not isolate any *S.aureus* colonies from any of the ice cream samples they analysed in Adana. However, they isolated coagulase negative staphylococci from all of the samples. They identified the isolates as *S.epidermidis* colonies and reported the average bacteria count as 2.4x105 CFU/g.

Alişarlı and colleagues (2002), carried out a study on the ability of enterotoxigenic *S.aureus* strains to proliferate and generate toxin in cream cakes. As a result, they determined that Enterotoxin-A generating *S.aureus* strains were able to stimulate toxin even at low temperatures.

Küplülü and colleagues (2002), carried out a study on SE presence in pasteurised milk marketed in Ankara and detected SEA presence in two of the 250 tested samples at a level >0.1 ng/mL.

Alişarlı and colleagues (2003), carried out a microbiological study with pudding and creamcakes and isolated *S.aureus* in 10% of 100 pudding types and 27% of the cream cakes. A type enterotoxin was detected in 7 samples, C type enterotoxin was detected in 2 samples, and A/B mixed enterotoxin was detected in 2 samples. It has also been reported that all of the enterotoxin producing *S.aureus* also had thermonuclease activity.

Becker and colleagues (2003), carried out a study with patients carrying *S.aureus* bacteria at the Munster University Hospital in Germany. They determined that 50.8% of the

S.aureus colonies isolated from 429 patients contained exfoliative toxin, whereas73% contained staphylococcal enterotoxin.

Günşen and Büyükyörük (2003), analysed 125 samples of vacuum packaged young cheddar cheese marketed in Bursa. As a result, they detected *S.aureus* presence in four samples and reported highest *S.aureus* count as 1.8x103 CFU/g and lowest *S.aureus* count as 1.0x102CFU/g.

In their study carried out in Bursa, Evrensel and colleagues (2003), detected coagulase(+) staphylococcus in raw milk samples and determined the bacteria count as 1.9x104 CFU/mL, detected staphylococcus/micrococcus count in pasteurised milk samples and determined the bacteria count as 103-104 CFU/mL, and determined coagulase(+) staphylococcus count in brine as 5.0x104 CFU/g.

As a result of the microbiological analysis of 75 ice cream samples marketed in Van, Ağaoğlu and Alemdar (2004), isolated coagulase (+) *S.aureus* in 13.3% of the samples.

Normanno and colleagues (2005), carried out a study on various food groups (meat, UHT milk, cheese, ice cream, cake, egg, fish) sold in a market in Italy.As a result, they detected 541 coagulase positive staphylococcus colonies, 537 of which was identified as *S.aureus.* They determined that 298 of the *S.aureus* colonies (%55.5 of the total) contained one or more enterotoxin, and reported the enterotoxin type distribution as 33.9% SEC, 26.5% SEA, 20.5% SEA+SED, 13.4% SED, 2.7% SEB, 1.7% SEA+SEB, 7% SEC+SED, 0.3% SEA+SEC and SEB+SEC.

Fueyo and colleagues (2005), isolated 269 *S.aureus* colonies from handmade foodstuffs (cheese, cream, ice-cream) marketed in Spain, and reported that 57 isolates contained at least one of the four enterotoxins, listed as SEA, SEB, SEC and SED.

Korel and colleagues (2005), carried out a microbiological study with 85 ice cream samples marketed in Manisa.They did not detect *S.aureus* in any of the 15 packaged and 70 unpackaged ice cream samples they tested.

In their study on identifying *S.aureus* on the skin and in the nostrils of patients with Atopic Dermatitis (AD), Psoriasis (PS), erythroderma, skin infections and septitis, where a healthy control group was also included, Tomi and colleagues (2005), isolated *S.aureus* from the lesion areas of the skin of 22 patients from a total of 25 patients with AD, and from 15 of 25 patients with PS.

Fujikawa and Morozumi (2006), showed that the amount of toxin present in milk increased proportionately with increasing *S.aureus* count, after its level reached 106.5 CFU/mL, and also determined that toxin generation increased between 14and 32ºC.

In their study carried out with the milk of large and small dairy animals in Italy, Cremonesi and colleagues (2006), determined coagulase positive *S.aureus* in all of the 111 tested samples. 95 (%86) of the samples contained at least one type of enterotoxin. 58 (%79) of the 73 large dairy animal samples and 37 (%97) of the 38 small diary animal samples contained enterotoxin.

In their study carried out in Kahramanmaraş, Erdoğrul and colleagues (2006), did not determine *S.aureus* presence in any of the mayonnaise, colza, skimmed yoghurt and bulgur samples they analysed.

In their study carried out in the Isparta area with whey cheese (tort cheese) made from cow, sheep and goat white cheese whey, Şimşek and Sağdıç (2006), did not determine *S.aureus* presence in any of the samples they analysed.

Soejima and colleagues (2007), intentionally contaminated skimmed milk with *S.aureus* and following churning left the milk to incubate at 35 ºC. As a result, they reported that these conditions increased the rate of *S.aureus* proliferation and SEA generation.

In their study carried out with plain ice cream samples taken from 55 different selling points in İstanbul, Keskin and colleagues (2007), determined that 12.7% of the samples were not suitable in terms of *S.aureus* presence according to the regulation on

microbiological criteria for foodstuffs. They reported that none of the samples contained toxin.

In a study carried out by Gülbandılar (2009), at Kütahya Public Health Laboratory, nose cultures were taken over a period of almost one year (May 2006-June 2007) from a total of 3048 people who came for other analyses but whose job either involved food handling (cook, baker, etc.) or necessitated direct contact with general public (barber, coiffeur, etc.). *S.aureus* colonies were isolated from 217 samples. 37 (17.05 %) of these isolates came from samples acquired from female donors and 180 (82.9%) came from samples acquired from male donors.

Kumar and colleagues (2009), determined *S.aureus* presence in one sample out of 10 milk samples they analysed in India and reported the *S.aureus* count as 4.5x101 CFU/g.

In a study carried out at Soxony Hospital in Germany, Monecke and colleagues (2009), determined the presence of enterotoxin in 45.8% of the 155 *S.aureus* contaminated samples they analysed, and identified 17.42% of these enterotoxins as SEA.

In a study carried out with 207 samples at 3 different hospitals in Bronx, United States, Varshney and colleagues (2009), identified 19 different types of staphylococcal enterotoxins, namely SEA, SEB, SEC, SED, SEE, SEG, SEH, SEİ, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SEV, and TSST.

Garcia and colleagues (2009), reported that they determined presence of *S.aureus* in all of the 75 milk samples collected from various dairy farms in Spain.

Önganer and Kırbağ (2009), carried out a microbiological study in Diyarbakırwith 30 Cokelek Cheese samples. They detected *S.aureus* in all of the samples and reported the lowest *S.aureus* count as 6x106 CFU/g and the highest count as 10.28x106 CFU/g.

In their study carried out in Kayseri province with 60 raw milk samples, Yılmaz and Gönülalan (2010), determined *S.aureus* presence in 30 samples and SE presence on 37 samples.

The acceptable limit for the presence of coagulase positive staphylococcus in foodstuffs ready for consumption is given as $\leq 1.10^3$ in Annex 3-Pathogen Microorganism Limits of the Turkish Food Codex Regulation on Microbiological Criteria (Anonymous, 2011).

In the TRNC, *S.aureus* is accepted as one of the most important pathogens that are transferred to humans through the consumption of contaminated foodstuffs and seriously threaten human health. It poses an important risk particularly for dairy products industry as a considerable amount of milk is transported in large copper jugs in the absence of cold chain conditions and cattle are widely milked in regular farm surroundings with traditional methods. Although its resistance to heat treatment is quite low, bad sanitation conditions, inadequate pasteurization, and the risk of being re-transferred to foods through cross-contamination after pasteurization and/or production, constitute extrarisk factors in the TRNC which make *S.aureus* a significant pathogen threatening public health. The results of many microbiological studies on milk and milk products support the considerable risk of *S.aureus* contamination in diary industries and the possible risks it poses to human health.

In a study they carried out in Ankara, Küplülü and colleagues (2002), detected staphylococcal enterotoxins, although they did not determine presence of *S.aureus* colonies in the pasteurized milk samples they analysed. Similarly, Wieneke and colleagues (1993), reported that in their analysis of the cheese samples consumed by two patients hospitalised for food poisoning, they did not detect live *S.aureus* bacteria but isolated staphylococcal enterotoxin in the samples.

Staphylococci are the microorganisms which form the natural fora present in the upper respiratory system and skin of human beings. It is for this reason that the food industry personnel are specified as the most important risk factor for staphylococcal contamination of foodstuffs. Inadequate pasteurizing conditions and contamination after pasteurisation are also cited as important factors in the staphylococcal contamination of pasteurized milk.

Staphylococci are extremely sensitive to heat treatment and are completely eliminated within 30 minutes of heat treatment at 60ºC. In contrast, staphylococcal enterotoxins are resistant to heat treatment (Erol andİşeri, 2004). As a result, if *S.aureus* colonization occurred and stimulated enterotoxin generation in foodstuffs prior to heat treatment, staphylococcal enterotoxin can be detected after UHT and pasteurisation although no live *S.aureus* are present. Unfortunately, the heat treatment methods utilized in the food industry fall short of inactivating staphylococcal enterotoxins at an adequate level.

In their microbiological studies, Özalp and colleagues (1978), reported that they detected staphylococcal enterotoxin in Tulum Cheese samples although no staphylococci presence was determined in any of the 26 samples; Bone and colleagues (1989), reported that although no live pathogen was detected, staphylococcal enterotoxin was determined in the sheep milk cheese samples they analysed. On the other hand, Cremonesi and colleagues (2006), determined *S.aureus* presence in all of the 111 milk samples they analysed and detected enterotoxins in 95 samples.

S.aureus is a leading cause of clinical or sub-clinical mastitis.It is therefore frequently present in and can be isolated from raw milk. In the light of the fact that staphylococcal enterotoxins are thermostable and not destroyed at pasteurisation temperatures, it is undeniable that *S.aureus* contaminated milk carry potential health risks even after pasteurisation (Küplülü et al., 2002).

In a study aimed at determining the possible presence of coagulase positive staphylococcus in 140 samples of various dairy products (23 of which were Turkish White Cheese), the presence of this bacteria was identified in 18.6% of the samples as a result of the analysis carried out with Baird-Parker Agar medium (Başтepe, 1977). In another study also carried out on Turkish White Cheese, coagulase positive staphylococcus was identified in exactly 1/3 of the 60 samples. Baird-Parker Agar medium was utilized as the main selective medium in this study as well (Aşkın, 1983). In another study, BPA results gave the *S.aureus* count as 7 log CFU/g or above for 3 (7.5%) of the 40 analysed cheese samples in total. This result is very significant in terms of food safety as toxin generation can be expected at this level of contamination (Ünlütürk et al., 1991).

In a study carried out with Civil cheese samples collected from Ankara market, staphylococcal presence was found to be under identifiable level (<100 CFU/g), although the microbiological quality of the analysed samples was evaluated to be low,(Polat, 2000).

Altın and Tekinşen (2002), carried out a microbiological study on the quality of white pickled cheeses sold in Konya area and its surrounding settlements. As a result, aerobic *mesophyll microorganism*, *coliform bacteria, Staphylococcus spp*., *Lactobacillus spp*., *yeast* and *mould* counts were respectively determined as 1,60x108 CFU/g, 1,75x105 CFU/ g, 1,69x103 CFU/g, 2,68x107 CFU/ g and 2,46x105 CFU/g. It was also reported that 60% of the cheese samples were not on par with white cheese standards in terms of *coliform bacteria* presence, whereas 66 % of the samples were not on par with white cheese standards in terms of yeast and mould presence. It was, thus, concluded that a standard method was not used for production of white pickled cheese in Konya and its surrounding settlements, and/or effective quality control was not applied during their production and marketing phases.

Usca and Erol (1998), carried out a microbiological study on 50 Hellim cheese samples produced and marketed in the TRNC. They determined presence of live *aerobe mesophyll* in 96% (104 CFU/g) of the samples; presence of *enterobacter* in 64% (104 CFU/g) of the samples; presence of *coliform* in 26% (103CFU/g) of the samples; presence of *enterococcus* in 52% (103 CFU/g) of the samples; and presence of *yeast/mould* in 66 % (103 CFU/g) of the samples. They also detected 103CFU/g coagulase (+) staphylococcus presence in 26% and 103 CFU/g *E.coli* presence in 12% of the samples.

In the TRNC the authority to collect raw milk produced throughout the country and its distribution to local manufacturers at a set price is vested solely in the TRNC Milk Industry Organization. In this respect, the following data acquired from the Milk Industry Organization is helpful to a certain extent for evaluating the changes in the microbiological analysis results of our present study in comparison to the results of the study carried out by Usca and Erol (1998) in terms of *staphylococcus aureus* presence.

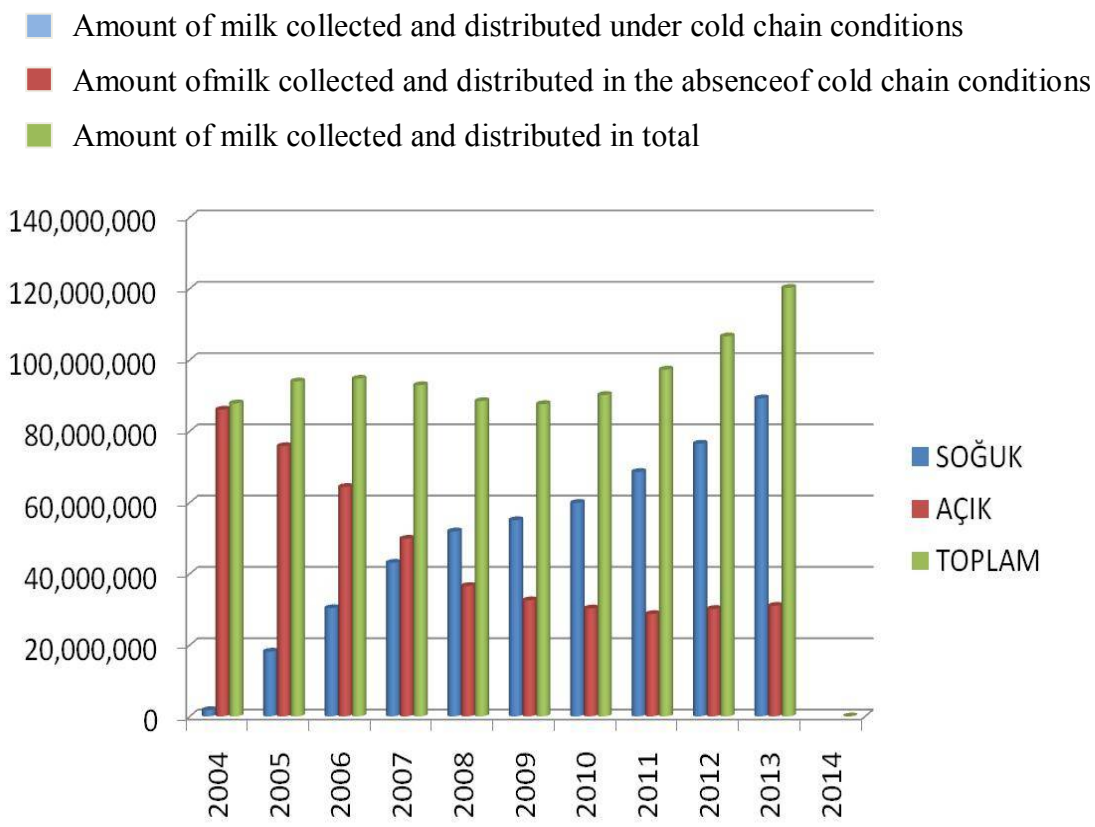

Figure 4.1: Milk Industry Organization 2004 - 2013 Milk Amount Distribution (L/year)

Table 4.1: TRNC Milk Industry Organization's Data on the number of milk producers and The amount of Milk collected in October 2004 and October 2014

	Cold Chain Collection and Distribution		**Absence of Cold Chain Collection and Distribution**	
	Number of Manufacturers	**Milk Amount(L)**	**Number of Manufacturers**	**Milk Amount(L)**
October 2004	16	337.459	2007	6.737.816
October 2014	181	8.267.227	569	2.455.729

As a result of the analysis of 34 Hellim Cheese samples produced in the TRNC in 2014 and collected from the markets in the Nicosia area, we did not detect any *S.aureus* presence in 32(94%) samples but detected *S.aureus* presence in 2 samples (6%), the count of which was at a level posing risk to public health in 1 (3%) sample and was within

acceptable limits in the other 1 sample (3%) according to the Turkish Food Codex Microbiological Criteria.

Contrary to our present study, Usca and Erol (1998), did not report *S.aureus* presence at levels posing risk to public health. However, the comparison of the results of the two studies can also be interpreted as a decrease from 26% to 6% in the general *S.aureus* presence in Hellim Cheese in 16 years. In any case, it is not really possible or reliable to directly correlate the results of the two independent studies, and our following evaluation and comments were not based on such a comparison.

The general evaluationof the foregoing facts, the documented improvement in the collection and distribution conditions of milk, and the reported modernization of the manufacturing methods and facilities in the TRNC, lead as to the probable conclusion that there is, in fact, a general positive development in terms of *S.aureus* contamination of Hellim Cheese produced in the country. In this context, it is assumed that in the case of the sample we found to be containing risky amount of *S.aureus*, cross-contamination was most probably involved during the manufacturing phases following heat treatment.The reasons in favour of this interpretationcan be summarized as follows:

1. The Milk Industry Organization introduced cold chain application to its operations on 16.10.2004. Before that date all the milk produced in the country was collected and distributed with old methods. As can be seen from Table 4.1; while only 5% of the total amount of 705275 L milk acquired from 2023 producers were collected and distributed under cold chain conditions in October 2004; 77% of the total amount of 10722956 L milk acquired from 750 producers were collected and distributed under cold chain conditions in October 2014. In other words, over the 16 year period that elapsed between 1998 and 2014, majority of the raw material, namely milk, for all dairy products started to be collected and distributed under appropriate conditions.
2. It is also a statistically documented fact that over the years, the amount of milk with mastitis considerably decreased in the TRNC.
3. The export of dairy products, in general, and Hellim Cheese in particular, overwhelmingly increased over the last ten years. Accordingly, the manufacturers started

to develop and modernize their production facilities (with European Union aid) in order to meet the microbiological analysis criteria demanded by the importing countries.

4. The EU supported education programs provided to the concerned groups on food production safety, made the manufacturers more conscious and sensitive about this issue.

5. Similarly, the competent authorities increased the supervisions and sanctions aimed at increasing food safety in general.

CHAPTER 5
CONCLUSİON AND RECOMMENDATİONS

The utilization of high quality raw material and the application of the necessary production process are not sufficient for ensuring microbiological safety of dairy products. Microbiological contamination from air, tools and machinery, personnel and other sources is a serious possibility. Contamination after pasteurisation is of particular importance in terms of product quality and consumer health.

Although the *staphylococcus aureus* presence detected in13 samples (15%) as a result of our present study can be presumed to be proportionally low, in essence it still carries a potential risk for public health which can theoretically result in simple food poisoning to occasional death. In this respect, the results of the present study once again shows that the sanitation and hygiene requirements are not sufficiently met.

In addition to utilization of quality raw material and adequate production techniques, it is important that the following criteria are also met in order to ensure that the bacterial quality of the dairyproducts in the TRNC do not threaten public health.

- Production should be carried out at modern facilities,
- Personnel should undergo regular health checks,
- Personnel should adequately be trained on microbiological contamination risks,
- Personnel should be trained on personal hygiene,
- Heat conditions, which are of particular importance for microbial growth at production facilities, should be strictly controlled by relevant authorities, especially during the very hot summer months in the TRNC,
- Necessary legal sanctions should strictly be applied for all punishable conditions.

While the proper application and control of the foregoing criteria diminish the risks threatening food safety and public health, aiming to reach 100% microbiological food safety and evaluating the possibility of achieving this aim,requires a wide range of additional measures, particularly those related to the determination and elimination of the source of food poisonings.

In the TRNC, statistical data on food poisonings and the type of microorganism causing the poisoning should be collected by both public and private laboratories. The private laboratories should work in close coordination and cooperation with the TRNC Ministry of Health. Accordingly, the data collecting should be taken a step further, so that the Ministry can get into direct contact with the patients in order to carry out the necessary investigation to determine the source product causing the food poisoning. Once the source of poisoning is determined, it is of uttermost importance that the Ministry of Health takes all the measures necessary to ensure food safety.

In every country, the national health statistics provide the primary source of data for determining the epidemiology of infectious diseases. Reliable health statistics are being kept for many years in developed countries and are available in detail for public research. In undeveloped countries and, unfortunately owing to negligence and other shortcomings, in our country as well, no health statistics on infectious diseases exist.

The TRNC Ministry of Health has made the notification of certain infectious diseases obligatory by law in order to be able to keep track of and to take the necessary measures for the prevention of these diseases. In accordance with the 3. Sub-clause of the 2. Clause of 1932 Law no 17, food poisonings (Salmonella, Staphylococcus and other microorganisms) are listed among the diseases which are obligatory to be notified. However, this law is not being applied in practice, although it is currently in force in the TRNC. The said Law also requires detailed information on the patient and possible causes/sources of infection to be supplied upon the notification of the listed diseases. Hence, if it is practically and strictly applied, it will be possible to collect statistical data identifying both the type and manufacturer of products in food poisoning cases.

Moreover, the TRNC Parliament has recently adopted a new Food Law on par with relevant EU legislation which covers all aspects of food safety. At this juncture in time,we believethat the proper application of the relevant legislation and existing legal sanctionswill provide the sufficient ground for the TRNC to aim and to achieve 100% microbiological food safety of dairy products within a short period of time.

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