Manufacture of Selected Cheese Groups from Greece and Presence of Biogenic Amines

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Téma práce:	**Výroba vybraných skupin sýrů z oblasti Řecka a výskyt biogenních aminů**

Zásady pro vypracování:

I. Teoretická část

1. **Vytvořte přehled řeckých sýrů zrajících v solném nálevu.**
2. **Popište technologický proces výroby řeckých sýrů zrajících v solném nálevu.**
3. **Charakterizujte vlastnosti vybraných sýrů zrajících v solném nálevu.**

II. Praktická část

1. **Optimalizujte postup laboratorní výroby sýrů zrajících v solném nálevu.**
2. **Optimalizovaným postupem laboratorně vyrobte sýry zrající v solném nálevu bez a s přídavkem dekarboxyláza-pozitivních laktokoků. S těmito sýry založte zrací pokus.**
3. **Vyrobené vzorky podrobte analýze pH, obsahu sušiny, NaCl, volných aminokyselin, biogenních aminů a dále proveďte texturní profilovou analýzu.**
4. **Výsledky srovnejte s komerčně vyrobenými sýry z oblasti Řecka.**
5. **Výsledky vyhodnoťte a formulujte závěry.**

Rozsah diplomové práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná/elektronická**

Seznam odborné literatury:

[1] Valsamaki, K. ,et al. (2000). Biogenic amine production in Feta cheese. Food Chemistry, 71, 259-266.
[2] Mallatou, H. ,et al. (2003). Changes in free fatty acids during ripening time of Teleme cheese made with ewes, goats, cows or a mixture of ewes and goats milk. International Dairy Journal, 13, 211-219.
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[4] Zerfiridis, G. K. (2001). Technology of dairy products-cheesemaking. 2nd. edition,Thessaloniki.Giahoudis Publisings.

Vedoucí diplomové práce: **Ing. Vendula Pachlová, Ph.D.**
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Ve Zlíně dne 10. února 2012

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ABSTRAKT

Cílem této práce bylo (i) optimalizování technologie výroby bílých sýru zrajících v solném nálevu v podmínkách Ústavu technologie a mikrobiologie potravin, Fakulty technologické, Univerzity Tomáše Bati ve Zlíně, (ii) vyrobit série sýrů s použitím klasické mezofilní smetanové kultury (kontrolní vzorky) a (iii) vyrobit série sýrů, které byly zaočkovány dekarboxylasa-pozitivními laktokoki. Pro srovnání získaných výsledků byl dále založen skladovací pokus s komerčními vzorky sýrů řeckého původu. Experimenty zahrnovaly chemickou analýzu obsahu sušiny, NaCl, volných aminokyselin a biogenních aminů a dále také texturní profilovou analýzu. Doba zrání sýrů vyrobených v laboratorních podmínkách byla 56 dnů při konstantní teplotě (10 ± 2 °C). Bylo prokázáno, že inokulovaný dekarboxylázapozitivní kmen *Lactococcus lactis* subsp. *lactis* je schopen produkovat ve větší míře biogenní aminy i v reálném systému sýra. Celkový obsah volných aminokyselin všech vzorků sýrů značně vzrostl během zrání. Zjištěné celkové obsahy biogenních aminů byly také ve významně vysokých koncentracích v průběhu zrání a to zejména v případě vzorků s přídavkem dekarboxylasa-pozitivní kultury. Na druhou stranu nízké hodnoty pH a vysoký obsah NaCl v bílých sýrech zrajících v solném nálevu nepříznivě ovlivňují dekarboxylaci aminokyselin a následné stanovené obsahy jednotlivých biogenních aminů byly relativně nízké. Na konci zracího procesu tvrdost všech analyzovaných vzorků klesla v důsledku proteolytických dějů.

Klíčová slova: bílý sýr zrající v solném nálevu, Feta sýr, Teleme sýr, volné aminokyseliny, biogenní aminy, texturní profilová analýza.

ABSTRACT

The objectives of this work were (i) the optimization of cheese making technology of white brined cheeses under conditions provided by the Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata Univesity in Zlin, (ii) the manufacture of cheese series using classic mesophilic culture (control samples) and (iii) the manufacture of cheese series which were vaccinated with decarboxylase positive lactococci. For the comparison of the results was performed storage experiment of commercial Greek cheese samples. The experiments included chemical analysis of dry matter, NaCl, free amino acid, biogenic amine contents and texture profile analysis. The ripening period of the cheeses made in the laboratory was 56 days at constant temperature (10 ± 2 ° C). It has been shown that inoculated decarboxylase positive lactococci of *Lactococcus lactis* subsp. *lactis* strain, is capable of producing biogenic amines at higher level in a real system-cheese. The total content of free amino acids of all samples showed a significant increase during ripening, especially in the case of cheese samples with the addition of decarboxylase positive lactococci culture. The determined contents biogenic amines also increased significantly during ripening. On the other hand the low pH and the high NaCl content of white brined cheeses adversely affect the decarboxylation of amino acids and the determined content of biogenic amines was relatively low. At the end of the ripening process the hardness of all the analyzed cheese samples decreased due to proteolysis.

Keywords: white brined cheeses, Feta cheese, Teleme cheese, free amino acids, biogenic amines, texture profile analysis

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I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

White brined cheeses are the group of cheese varieties ripened and preserved in brine for a considerable amount of time, i.e. until consumption (Alichanidis and Polychroniadou, 2008, Moatsou and Govaris, 2011). From technological point of view ripening is a very important process; during which cheese submit specific microbiological and biochemical changes. Changes which affect organoleptic and texture properties of the cheese (Pachlová et al., 2012). The biochemistry of cheese ripening involves three primary processes; glycolysis, lipolysis and proteolysis. The length of the ripening depends on the cheese variety. White brined cheeses usually ripen at least 15 days (Greek Codex Alimentarious, 2011). The principal role of proteolysis in the production of flavor compounds is the liberation of amino acids as precursors for a complex series of catabolic reactions (Katsiari et al., 2000a, 2000b, Hayaloglu et al., 2003, Fox et al., 2004, Mallatou et al., 2004, 2005, Bontinis et al., 2011). Amino acids are considered to be precursors for biogenic amine production. Biogenic amines are basic nitrogenous compounds with low molecular weight formed by microbial decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Silla-Santos, 1996, Valsamaki et al., 2000, Innocente et al., 2007, Buňková et al., 2009, 2010, Pachlová et al., 2011, Buňka et al., 2012). Biogenic amines can be expected in all foods containing proteins or free amino acids and are subject to conditions enabling microbial and biochemical activity (Silla-Santos, 1996). Consumption of food containing high concentrations of biogenic amines (>100 mg/kg) may cause toxic or some deleterious effects. The presence of biogenic amines is highly related with food spoilage and also influences the food safety and quality (Silla Santos, 1996, Valsamaki et al., 2000, Buňková et al., 2009, 2010, Tao Tang et al., 2011). The aim of this work was the presentation of the optimization process of cheese making technology of white brined cheeses similar to Greek. The experiment included chemical analyses of dry matter, NaCl, free amino acid and biogenic amine contents of commercial Greek cheeses and cheeses made under laboratory conditions during a ripening period of 56 days under constant temperature and moisture conditions. Rheological properties of commercial Greek cheeses and of cheeses made under laboratory conditions during ripening of 56 days under constant temperature and moisture conditions were examined.

I. THEORY

1 INTRODUCTION TO CHEESEMAKING

For the reader of this master thesis it is useful having a general overview about Greek cheese, its composition and position in human nutrition. Therefore, basic information data will be mentioned about Greek cheeses, cheese making history, cheese production and consumption in Greece, their nutritional value and their classification.Subheading

1.1 History of cheese making

Milk belongs among the best and most valuable food for the human. But it has a big disadvantage; fresh milk does not have very long shelf-life. This is the main reason for milk transformation into another form, which will have longer shelf-life and the same high biological value as has milk. Cheese is this form, whose discovery was certainly accidental. Possible discovery area of cheese might be around the Mediterranean Sea. According to a very old myth, cheese was made when an Arab merchandiser put milk into a bag, made of sheep's stomach, while he was traveling through a desert. Natural rennet contented in fresh milk and combined with high temperatures caused coagulation of milk and its separation in curd and whey. According to this myth, one of the highest quality food started being produced (Zerfiridis, 2001). Diodorus, the Greek historian from Sicily refers that due to Greek mythology, Aristeus son of Apollo and grandson of Zeus, learned the art of cheese making from his nannies known as nymphs and was sent by the gods of mountain Olympus to pass the knowledge of cheese making to the ancient Greeks. Given the value of cheese as food, it is not surprising that the ancient Greeks considered cheese as a divine invention and special gift (Litopoulou-Tzanetaki and Tzanetakis, 2011). In Homer's epic poem Odyssey is referred that Ulysses and his faithful companions during their return from the Trojan war, entered into the cave of Cyclops Polyphymus and discovered containers full of milk and whey and shelves full of cheese (Zerfiridis, 2001). Homer describes a technique of cheese making which has many similarities to those which are used nowadays. The main type of cheese which was produced in ancient Greece was white cheese (Zerfiridis, 2001). The cheese whose production was described by Homer is probably an ancestor of a well-known Greek cheese named Feta and it is the main cheese manufactured in Greece from the ancient times until today (Litopoulou-Tzanetaki and Tzanetakis, 2011). Production technology of this cheese, expanded throughout Europe during the Roman Empire. In the Middle

Ages cheese production was principally made by monks in European monasteries. At this period Italy was the major center of cheese production in Europe (Zerfiridis, 2001).

1.2 Cheese production and consumption in Greece

Cheese making in Greece has a tradition of centuries, which is related to specific geographical areas (Anifantakis, 1998). Meanwhile, various cheese types evolved through the centuries, so that nowadays each area in Greece, almost every island has its individual unique tradition in cheese manufacture (Litopoulou-Tzanetaki and Tzanetakis, 2011). It is known that ewes'milk is the most adequate for cheese manufacture, because of its high content in fat, proteins, particularly in caseins (Moatsou and Govaris, 2011). According to Pappa et al., 2006 the composition of raw ewes' milk before standardization is fat 8.1 %, lactose 4.9 %, proteins 5.9 %, pH value 6.6 and titrable acidity 0.265 % in lactic acid (11.8 °SH). Composition of raw goats milk is fat 4.8 %, lactose 5.0 %, proteins 3.6 %, pH value 6.5 and titrable acidity 0.185 % in lactic acid (8.2 °SH). Composition of raw cows' milk is fat 3.6 %, lactose 4.9 %, proteins 3.4 %, pH value 6.6 and titrable acidity 0,180 % in lactic acid (8.0 °SH). The casein content is in ewes', goats', cows'milk 4.6 %, 2.8 % and 2.5 % respectively (Pappa, et al., 2006). Those values are better shown below in Table 1.

Tab. 1: Typical composition of raw ewes', goats'and cows'milk (Pappa, et al., 2005).

	Milk Type		
Component (w/w %)	*Ewes'*	*Goats'*	*Cows'*
Fat	8.1	4.8	3.6
Lactose	4.9	5.0	4.9
Proteins	5.9	3.6	3.4
Caseins	4.6	2.8	2.5

As we can see from the table above ewes' raw milk has the highest content in fat, proteins and caseins, those values are almost double in contrast to goats' and cows' raw milk. Likewise the value of lactose does not varied significantly between those three types of raw milk. The composition of goat's milk from indigenous Greek breeds has a remarkable high fat and casein content, resulting from the abundance of "strong" α_{s1}-casein variants (Moatsou et al., 2002, 2004, 2006, 2007, Moatsou and Govaris, 2011). Greek cheeses are mostly produced from sheep's milk or a mixture of sheep's and goat's milk, the latter being about 20–30 % of the cheese milk mixture (Moatsou and Govaris, 2011). An indicative example

is that in the year 2003 production of all type cheeses in Greece reached 240 550 tons. From this amount 125 000 tons were from sheep's milk, 48 000 from goat's milk and 35 000 tons from cow's milk (Greece Dairy Annual, 2011). Milk production and manufacture of dairy products have major economic importance for Greece. In the year 2008 nearly 189 000 tons of milk were produced, 41 % of this was cow's, 37 % was sheep's and 22 % goat's. Except milk which is intended for direct human consumption, among mainly dairy products belong various cheeses and traditional yogurts. More than 80 % of produced milk is used in cheese making (Moatsou and Govaris, 2011). The total production of cheese in Greece for year 2008 was approximately 185 000 tons and from this amount 2/3 were from sheep's or goat's milk. Cheese named Feta represents 50 % of this amount. It is interesting to mention that were produced 10 500 tons of yogurt made from sheep's milk and 310 tons of yogurt made from goat's milk (Moatsou and Govaris, 2011). In the year 2010 cheese production reached 221 000 tons and consumption reached 276 500 tons. In the year production reached 210 000 tons and consumption reached 265 390 tons. Production of soft brined cheeses reached 150 000 tons (EL.STAT., 2012). Main cheese representatives in Greece are Feta cheese and Teleme cheese. It is very important to refer that Greek consumer by the term cheese has in mind Feta cheese. This cheese belongs among 20 Greek cheeses designated as Protected Designation of Origin (PDO), which can be manufactured only in Greece (EU, 1996, 2002, Moatsou and Govaris, 2011). Cheese consumption in Greece per person per year is approximately 25 kg, which is the highest in te world. In contrast to this comes consumption of milk in Greece, which is nearly 69 l per person per year, one of the lowest in the world. High cheese consumption eliminates the low milk consumption (Zerfiridis, 2001). In second place at cheese consumption is France, where cosnumption per person per year reaches 23 kg; third place takes Germany with consumption 20 kg per person per year. In Czech Republic cheese consumption per person per year is 14 kg (Zerfiridis, 2001, Fox et al., 2004).

In Czech Republic typical Greek cheeses (Feta, Telemes, Batzos, Kefalograviera, Graviera, Kasseri, Manouri, etc.) are not produced. Those cheeses are mainly imported from neighboring countries, like Germany. Greek cheese under the name Feta is quite known among consumers in Czech Republic, but unfortunately its consumption is very low. This happens because Czech consumers have different nutritional preferences and habits. Another possible reason might be the high price of Feta cheese. Import of Feta cheese to

Czech Republic is primarily from Germany (50 % of the total volume), follows Denmark, Italy, while Greece exports only 3.5 % of the total volume (Ziogas, 2008).

1.3 Cheese production and consumption in Greece

Consumption of cheese is undoubtedly an essential part of daily human diet and due to this it is important to understand its nutritional value. Zerfiridis, 2001 mentions that consumption 100 g of white brined cheese (pickled) like Feta or Teleme cheese can satisfy the daily needs of an adult in proteins, vitamin A, riboflavin (B_2) and calcium to a ratio 1/3. Cheese protein content varies from 3 % to 40 % and depends on the type of cheese (e.g. cream cheese-3.1 % and Parmesan-39.4 %) (Fox et al., 2000, 2004). Proteins of cheese are almost 100% digestible. They are also good suppliers of energy and that relates with their fat content. Fat-soluble vitamin concentration is influenced by the same factors that affect its fat content. Concentration of water-soluble vitamins in cheese is characteristically lower than in milk, because of losses in the whey. Most cheeses are satisfying sources of vitamin A, riboflavin (B_2) and cyanocovalamin (B_{12}) (Fox et al., 2004). On the other hand cheeses are poor in iron (Fe) and vitamin D (kalsciferol) (Zerfiridis, 2001). Also they are an excellent source of calcium, phosphorus and magnesium. The sodium content is variable and depends on the added NaCl during cheese making (Fox, et al., 2004). Cheeses are manufactured in various forms and can satisfy the needs in flavor, protein, calories, etc. They can be also eaten unchanged and well combined with more kinds of food. Different types of cheese that have high biological value due to their protein digestibility can be part of children's diet. Eating cheese is important and suitable for children because it helps body and skeleton construction. Cheese consumption is suitable for the elderly people because it helps the replenishment of damaged body tissues (Zerfiridis, 2001). In the following table are presented the values of nutritional components contained in 100 g of Feta cheese.

Tab. 2: Values of nutritional components contained in 100g of Feta cheese (Anifantakis, 1991, Tamine, 1993, Fox et al., 2000).

Component		**Component**	
Water (g)	58	**Minerals (mg)**	
Proteins (g)	20	*Sodium*	1,440
Fat (g)	21	*Potassium*	95
Cholesterol (mg)	70	*Calcium*	360
Energie (kJ)	1,048	*Magnesium*	20
Vitamins (µg)		*Phosphorus*	280
A		*Iron*	0.2
D	0.5	*Copper*	0.07
E	370	*Zinc*	0.9
Thiamin	40	*Sulfur*	
Riboflavin	210	*Chlorine ions*	2,350
Niacin	200		
Pyridoxine	70		
Cobalamin	1.1		
Folate	23		
Pantothenic acid	360		
Biotin	2.8		

As we can see from the table above Feta cheese has a high content in fat and proteins, approximately 20%. From the vitamins we observe a domination of vitamin E, pantothenic acid, riboflavin and niacin. On the other hand we can clearly notice the absence of vitamn A, biotin and cobalamin are in very low levels. From the minerals dominate are chlorine anions and sodium; also noteworthy is the content of calcium and phosphorus.

1.4 Main groups of Greek cheeses

The following table (Table 3) is showing the types of well-known Greek cheeses labeled as PDO (Protected Designation of Origin). According to the Official Journal of the European Union, PDO is a quality sign. The PDO sign covers agricultural products and foodstuff which are produced, processed and prepared in a given geographical area using recognized

know-how. Also this quality sign is aiming at pointing at the link between the quality characteristics of agricultural products, foodstuffs and their territorial origin.

PDO is considered to be an important protection and promotion tool for producers in order to increase their product value and market power. (EU, 1996, 2002, Thenvsen, et al., 2007).

Tab. 3: Main groups of Greek cheeses (Moatsou and Govaris, 2011).

Groups of cheese		Name
Brined cheeses		
white other		Feta , Telemes , Kalathaki Limnou, Sfela , Batzos
Hard type		Kefalotyri , Kefalograviera , Ladotyri Mytilinis , Foramella Parnasou
Gruýere type		Graviera Kritis , Graviera Agrafon
Pasta Filata type		Kasseri
Soft type (fresh or matured)		Anevato , Galotyri , Katiki Domokou, Pichtogalo Chanion
Various		Xinotyri, Touloumotyri
Whey cheeses		Myzithra , Manouri , Xinomyzithra Kritis

1.4.1 White brined cheeses

Cheeses ripened (matured) in brine are one of the oldest known group of cheeses. By the term “Brined cheeses” it is described the group of cheese variety ripened and preserved in brine, for a notable amount of time, until consumption (Alichanidis and Polychroniadou, 2008). White brined cheeses is a sub-category of Brined cheeses (presented in Table 3), made from curd-whey mixture that is not subjected to any heat treatment (cooking), like Feta cheese, Teleme cheese or other similar varieties produced in Greece. They have particular sensory and textural characteristics (Fox et al., 2000, Moatsou and Govaris, 2011). Their texture is smooth, soft, compact and easily can be cut into slices. Their color may be whitish to yellow. The color is pure white, when they are made from ewe’s, goat’s or buffalo’s milk. When cow’s milk is used only, some milk discoloration techniques have to be to achieve the desirable white color (Zerfiridis, 2001, Fox, et al., 2004). Their flavor is slightly acid, salty and sometimes turns to be rancid piquant. Cheese mass has no rind, no

gas holes or other openings, except for some small mechanical openings (Zerfiridis, 2001, Alichanidis and Polychroniadou, 2008, Moatsou and Govaris, 2011). According to Greek Codex Alimentarius, 2011, the maximum moisture content of these cheeses is 56 % and fat in dry matter 43 %. The development of cheese curd is made by the use of rennin (chymosin) or other milk clotting enzymes with similar function. Milk clotting enzymes are originally obtained by extraction from the stomachs of ruminants and namely calf rennets. Enzymes with similar function are proteases, which are capable of initiating the proteolysis of κ-casein, known as aspartic proteinases. Sources of these proteinases can be from plant origin, from microbiological origin or from other ruminants (outside calfs) (Kehagias, 2005). Fermentation of *Rhizomucor miehei*, *Rhizomucor pusillus* and *Cryphonectria parasitica* was used for the production of aspartic proteinases. Also recombinant DNA technology was used to clone the gene of chymosin, hosting organisms were mainly *Escherichia coli, Aspergilus niger Kluveromyces lactis*. Interesting are coagulants extracted from the flowers of thistle *Cynara carduncelus*, used in the production of artisanal cheeses in the Iberian Peninsula (Fox, et al., 2004). According to Greek Codex Alimentarius, 2011, white brined cheeses made from not heat-treated milk (not pasteurized), must ripen at least two months before consumption. According to Greek Codex Alimentarius, 2011, white brined cheeses made from pasteurized must ripen at least 15 days before consumption. The high amount of salt that contain these cheeses delivers better organoleptic characteristics and fulfills the salt losses during human body sweating. Therefore white brined cheeses are mainly consumed in countries where the climate conditions are hot (Zerfiridis, 2001). On the other hand high consumption of salt can cause negative long term outcomes, like strokes, cardiovascular disease, high blood pressure (hypertension), stomach cancer, cardiac enlargement, hypernatremia, osteoporosis, etc (Aly, 1995, Katsiari et al., 2000a, 2000b, Dumler, 2009, Taylor, 2011). Generally it can be said that salt acts like a natural preservative. Salt has the ability to inhibit the development of unwanted microorganisms and retard the development of wanted (starter and non-starter) microorganisms. Salt can create intense osmotic pressure or in other words said has the ability to reduce water activity $A_w=1-0{,}033\times m$, where *m=molarity of NaCl* (moles of NaCl per liter of H_2O). Cheese microflora is developing in the cheese's water phase where is also salt. If osmotic pressure is high, the bacterial cell cannot receive or exclude substances. Cell dehydration or even cell destruction are happened (Zerfiridis, 2001). White brined cheeses have many names in different countries. In Table 4 are presented some of these cheese names.

Tab. 4: White brined cheese names in different countries (Zerfiridis, 2001).

Country	White brined cheese name
Egypt	Beda, Kariesch
Bulgary	Bjalo salamureno sirene, Telemea bulgarsca
Greece	Feta, Telemes
Izrael	Djibne
Romania	Telemea sarata
Turkey	Teleme, Beyaz peynir
Syria	Sirena
Czech Republic	Akawi, Jadel , Balkan cheese

Traditionally, for centuries white brined cheeses were manufactured in small scale. However in the recent decades, production increased to a higher scale, standardized and mechanized. It is estimated that ultrafiltered Feta cheese represents 56 % of the total ultrafiltered cheeses. Ultrafiltration is a method used for milk condensation. In cheese making this technique is applied to reduce the amount of cheese-making milk and increase the yield of cheese (Zerfiridis, 2001). Due to high concentration of whey proteins in ultrafiltered cheeses they ripen more slowly than the traditional ones (un-ultrafiltered) (Mistry and Maubios, 1993). Most of these cheeses are stored in well-sealed containers or in containers that do not allow access to gases (Fox et al., 2004).

1.4.2 Classification of white brined cheeses

Most of the cheeses ripened in brine are not well described so this causes serious problems with their classification. In many cases cheeses are described as white pickled cheeses, this is a common name which can be easily applied to all cheeses matured in brine. For this reason the classification is necessary. A general classification system of cheeses ripened in brine is shown in Table 5 (Fox et al., 2004).

Tab. 5: General classification system of cheeses matured in brine (Fox, et al., 2004).

Soft cheeses (moisture 55-65 %)	**Name**	**Country**
Acid coagulation	Mish	Egypt
Rennet coagulation		
curd salting	Feta	Greece
	Teleme	Greece, Romania
	Brinza	Russia, Izrael
	Bli-sir-U-kriskama	Serbia
	Bjalo nebo Belo Saramunero sirene	Bulgary
	Chanakh	Russia, Izrael
	Beyaz peynir	Turkey
	Akawi	Syria
	Baida	Lebanon
milk salting	Domiati	Egypt
	Dani	Egypt
	Gibna bayda	Sudean
Semihard cheeses (moisture 45-55 %)	Halloumi	Cyprus
	Braided Meddafara	Syria, Sudan
	Magdula	Syria, Sudan
	Nabulsi	Jordan

2 MANUFACTURE TECHNOLOGY OF FETA AND TELEME CHEESE

2.1 Feta cheese

2.1.1 Milk

First of all milk must become from healthy animals, be microbiologically safe, pure and clean. Also the kind of milk plays very important role in the quality of the final product. Best milk for manufacture of Feta cheese is ewe's milk, but commonly is used a mixture of ewe's and goat's milk. According to Greek Codex Alimentarius and the standards that were promoted Feta to become a PDO cheese, goat's milk share must not exceed more than 30 %. Also the used milk must become only from the Greek areas of Macedonia, Thrace, Epirus, Thessaly, Central Greece, Peloponnese and Lesvos. Forbidden are the use of concentrated milk, the addition of milk powder or milk concentrate, milk proteins, caseinates, colorants and preservatives. Milk is filtered and standardized to 5.8-6 % fat. The main target is the production of a cheese with the best quality that means that the fat content in the cheese will be 19-43 % in dry matter. The casein and fat ratio is usually 0.7-0.8:1 and pH value must be at least 6.5 (Zerfiridis, 2001, Greek Codex Alimentarius, 2011).

2.1.2 Heat treatment of milk

Milk for Feta cheese manufacture is commonly pasteurized at 72-75 °C for 15-30 seconds (flash process) or at 63-65 °C for 30-32 minutes (holder process) (Katsiari and Voutsinas, 1994, Mallatou, et al., 1994, Kehagias, 2005, Zerfiridis, 2001, Belitz et al., 2006, Litopoulou-Tzanetaki and Tzanetakis, 2011, Moatsou and Govaris, 2011). After pasteurization treatment milk is cooled to a temperature of 32-35 °C. Then is added a saturated $CaCl_2$ solution in quantity 100 ml/100 kg of milk or 0.1-0.2 g/ kg of milk (Katsiari and Voutsinas, 1993, Zerfiridis, 2001, Greek Codex Alimentarius, 2011, Moatsou and Govaris, 2011). The addition of $CaCl_2$ is to replenish the soluble calcium and help the texture development. Calcium cations help the formation of the curd. Calcium strengthens the hydrophilic bonds that are molded between caseins in order to develop the curd (Kehagias, 2005, Belitz et al., 2006).

2.1.3 Starter cultures

Starter cultures used are a combination of selected lactic acid bacteria. Generally yogurt culture is usually used (*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus*, 1:1). It is possible to use 24 hours old yogurt for inoculation (the traditional way). From time to time these cultures were replaced by other commercial cultures capable to produce more acids and have higher acidification rate, e.g. homofermentative coci as *Lactococcus lactis* subsp. *lactis*, a *Lactococcus lactis* subsp. *cremoris*. The culture is added in a quantity of 0.5-1.0 % (v/v) and incubation takes approximately 20-30 minutes and then rennet is added (Fox, et al., 2004, Litopoulou-Tzanetaki and Tzanetakis, 2011, Moatsou and Govaris, 2011).

2.1.4 Milk coagulation

Milk coagulation takes place at temperatures from 32 °C to 35 °C. The added rennet quantity is chosen, so the curd formation could be after 45 to 50 minutes (Katsiari and Voutsinas, 1993, Fox et al., 2004, Moatsou and Govaris, 2011). Coagulation of milk traditionally is made with rennet from the abomasa of ruminants. Calf rennet is the most frequently used worldwide, while rennet made from the abomasa of unweaned lambs and kids is used restrictedly in the area of Mediterranean Sea (Moschopoulou et al., 2006, Litopoulou-Tzanetaki and Tzanetakis, 2011). In Greece the PDO Feta cheese is manufactured either industrially with calf rennet or traditionally with artisanal lamb and kid rennet (Moatsou et al., 2002, 2004, Georgala et al., 2005). The Greek artisanal rennet is in liquid form and it is prepared by the cheese manufacturers from mixed lambs‘ and kids‘ whole abomasa according to local traditional procedures. This rennet is widely used in small and medium Feta cheese industries (Moschopoulou et al., 2007).

2.1.5 Curd cutting and whey draining

The formed curd is cut cross wide in shape of cubes, sized 2-3 cm. Then the curd is allowed to rest for 5-15 minutes for partial exudation of the whey (Katsiari and Voutsinas, 1993, Moatsou and Govaris, 2011). This time must not be higher because the curd will expose a big amount of whey and the cheese texture will become hard (Zerfiridis, 2001). Subsequently the curd gradually is transferred in perforated moulds, where a big amount of whey will remove (draining by gravity without pressing application) (Litopoulou-Tzanetaki

and Tzanetakis, 2011, Moatsou and Govaris, 2011). The moulds are cylindrical when the cheese is to be packed in barrels and rectangular when intended for packaging in tin cans. The gradual curd transfer leads to the formation of small holes in almond shape, which is a typical characteristic of Feta cheese (Zerfiridis, 2001, Fox, et al., 2004).

2.1.6 Salting

When the curd is sufficiently firm the curd is removed from the mould and cut in equal pieces 23 x 11.5 cm or in pieces 11.5 x 11.5 cm. These pieces are placed on a salting-table which is sprinkled with granular salt. The cheese surface is coated with salt. After every 12 hours cheeses are turned and the surface is salted again. This operation is repeated until the cheese will contain 3.0-3.5 % salt (Litopoulou-Tzanetaki and Tzanetakis, 2011). Consequently the cheese blocks remain on the salting-table for a few days (about 5 to 15 days at 16-18 °C), to form a bacterial slime and the surficial fungi and yeast formation (McMahon et al., 2009, Moatsou and Govaris, 2011). Dry salting and slime formation are crucial for the characteristic flavor during ripening process. Before cheese packaging into containers the bacterial slime is removed by rinsing with water or brine (McMahon et al., 2009). Nowadays in large industries formation, salting and draining are mechanically performed. The curd with the help of gravity is led to the forms. The moulds are placed on a conveyor pass and led into a vessel, automatically filled with the help of gravity force, no pressing machines are used. After a period of 2 hours palettes that help shape formation are turned, for better draining of whey. Then the curd is cut into the desired size of the final product and dry salted again. The next morning cheese is layered in tin cans. The bottom of the can and each layer are salted. After 2 days the cheeses are inserted into the final consumer packaging (Fox, et al., 2004).

2.1.7 Packaging

Traditionally wooden barrels were used for Feta cheese packaging. Problems could occur with the handling of a full barrel, which weighed nearly 50 kg. Nowadays Feta cheese is packed in tin cans weighing 19 kg (net weight 16 kg). These tin cans have the main advantage that their transport is much easier and more economic. On the other hand, wooden barrels have the advantage that helps the creation of a stronger and spicier flavor (Fox, et al., 2004, Litopoulou-Tzanetaki and Tzanetakis, 2011).

2.1.8 Ripening

The cheese pieces are firmly placed into tin cans, in order to be little space between them. Brine with salt concentration 6.0-9.5 % is added into the container to fill the space between cheeses and also to cover their surface (McMahon et al., 2009, Litopoulou-Tzanetaki and Tzanetakis, 2011, Moatsou and Govaris, 2011). Typically the ratio of brine and cheese is 1:8 (v/w). Cheeses are kept at a temperature of 16-18 °C until the pH drops to 4.4-4.6 and moisture drops to a value less than 56 %. The vessel's lid is opened in order to escape gases produced during fermentation. The level of the brine is controlled because it must always cover the cheese surface. This practice i applied to the cheese that matures in wooden barrels. If the surface will not be cover with brine, this will cause surface drying, color change from white to light yellow and also is possible mold and yeast occurrence. At the end of the ripening process the tin cans are kept at 4-5 °C. Feta cheese of good quality can be kept in brine for up to one year at 2-4 °C (Fox, et al., 2004).

2.1.9 Yield and gross composition

Sheep's milk dry matter is between 18 and 20 % and the yield is expected to be nearly 25 % (Mallatou et al., 1994, Alichanidis, Polychroniadou, 1996). This means that about 4 kg of milk is needed to produce 1 kg of cheese. The yield is related to the addition of goat's milk, since is known that milk's composition varies at different times of year. According to Greek Codex Alimentarius, 2011, Feta cheese could contain maximum moisture 56 % and minimum fat in dry matter 43 %. The average value of moisture in Feta cheese is 54.6 %, fat in dry matter 49.1 %, protein 17.1 % and salt 5.3 % (of the aqueous phase). Analysis of 60 different commercial samples of Feta showed that the composition (g/100 g) was; moisture 54.2 %, fat in dry matter 50.8 %, protein 17.2 %, salt 6.3 % (Fox, et al., 2004).

2.2 Teleme cheese

Teleme cheese manufacture technology is similar to Feta cheese. Teleme cheese's manufacture is easier than Feta cheese's, because successfully can be applied to milk suspect for oxidation (sour milk) and also can be produced during summer when cheeses can easily spoil. The main advantage is that the production process is performed by direct immersing cheese blocks in brine. By this way, salt entrance into the cheese mass is more effective

and quicker. On the other hand, this is the main factor that Teleme cheese does not obtain the pleasant organoleptic properties which characterize Feta cheese (Zerfiridis, 2001).

2.2.1 Milk and milk coagulation

The casein/fat ratio after standardization was found to range between 0.73-0.78:1. After pasteurization process milk is cooled to 32 °C (Mallatou, et al., 2003, Pappa et al., 2006a, 2006b, 2007, 2008). Higher temperatures can be applied nearly 35-37 ° C to obtain harder curd. These temperatures are characterized as very high. Therefore, the latter mentioned temperature is used during winter time when the loss of initial temperature is more probable (Zerfiridis, 2001). The same starter culture could be added as is used in manufacture of Feta cheese. It is recommended to use 0.5 % (v/v) culture, containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis* subsp. *lactis* in ratio 1:3 (Pappa et al., 2006a, 2006b). According to Mallatou et al., 2003, a mixture of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, can be added. In Feta and Teleme cheese production, traditional yoghurt culture is used as starter (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) by the traditional cheesemakers (Zerfiridis, 2001, Pappa et al., 2006). While a mixture of commercial starters, including mesophilic starters, are used in industrial production (Pappa et al., 2006a, 2006b). According to Mallatou, et al., 2003, 27 ml to 100kg of milk can be added. Zerfiridis, 2001, refers that yoghurt gives the worst way of acidity during the first stages of ripening. In the first ripening stages culture acidity is necessary for curd syneresis and serves as prevention to other microflora development. The latter author also mentioned that the combination of *Lactobacillus delbrueckii* subsb. *bulgaricus* and *Lactococcus lactis* subsp. *lactis* influences the decreasing rate of pH value in the early stages of acidification. This low value is observed during pressing and salting. *Lactococcus lactis* subsp. *lactis* is more resident to antibiotics than *Streptococcus salivarius* subsp. *thermophilus* contained in yoghurt culture. Also the final product has better flavor. An appropriate time for culture addition is one hour before milk coagulation. In the milk can be added chlorophyll for discoloration. The quantity depends on manufacturer's instructions. For example 250-300 ml/ton of commercial product "Bleko" is used. The added quantity is higher in the spring when cows are fed with more green grass and their milk is richer in carotenoids. On the contrary, the added

quantity in the winter is lower because cows are fed with more dry grass. This addition is necessary to maintain the balance of green (from chlorophyll) and yellow/orange color (from carotenoids). If the chlorophyll addition is high, this will cause a negative result, which means that the cheese will gain greenish color. In case that in milk are added enzymes such as lipase, in order to improve flavour, must be firstly dissolved in water, for their better dispersion in the milk. Important also is that added enzymes must be added before rennet (chymosin). For white cheeses matured in brine a limit of 10 g per milk ton exists for lipase addition. To assist curdling of milk $CaCl_2$ solution (40 % w/v) in amount of 200-300 ml/ton of milk is added. Finally rennet is added in such quantity so the curd will be formed and cut within one hour. Mallatou, et al., 2003 refers that rennet powder can be added at a quantity of 3.0- 4.5 g/100 kg of milk. Higher rennet quantities are used when milk is pasteurized. If rennet is powder form must be first dissolved in cold water, a little amount of salt must be added and immediately used. If rennet is in solution form must be diluted in water at ratio 1:8. The addition of rennet solution must be in small batches and the stirring very gently (Zerfiridis, 2001, Pappa et al., 2006a, 2006b, Pappa et al., 2007).

2.2.2 Curd cutting and formation

After milk's coagulation, the curd is cut by a special slicer in cubes of 2 cm x 2 cm x 2 cm or 1.5 cm x 1.5 cm x 1.5 cm (Mallatou, et al., 2003, Pappa et al., 2006a, 2006b). Afterwards is left to rest for 10-15 minutes so they whey can exclude. The process is similar to that of Feta cheese, the difference is that during Teleme cheese manufacture, pressing and syneresis last longer. This is due to cow's milk contains more serum. Then the curd is formed in plastic, iron or wooden forms, in dimension 44,5 cm x 22 cm x 20,5 cm (Zerfiridis, 2001, Mallatou,et al., 2003, Pappa et al., 2006a, 2006b, Pappa et al., 2007).

2.2.3 Salting

Room temperature must be 18-20 °C and the concentration of brine in NaCl 14-18 %. The cheese's surface is salted with coarse salt. Within a few hours the brine solution is diluted to 8 % because cheese excludes whey. After a period of 6-8 hours, the brine is removed and cheeses are salted again. The salt content in brine increases to 12%. Because whey exudation continues, the brine is diluted to 10 %. The next morning, temperature in cheese's center is 20 °C. The acidity must be such that pH value will decrease below 5 and

moisture will be 62-65 %. Acidification process and salting process in the first 24 hours have crucial meaning to the quality of the final product (Zerfiridis, 2001, Mallatou,et al., 2003, Pappa et al., 2006a, 2006b, Pappa et al., 2007).

2.2.4 Ripening

When the cheese will reach a pH value below 4.8, salt of the aqueous phase 5 %, moisture 54 % is placed into a refrigerator at 4-5 °C for 2 months (Pappa et al., 2006a, 2006b). During this time the ripening process will continue. If maturation period will be higher than 2 months, temperature must be reduced to 2 °C, for a significant slowdown of proteolysis and lypolysis process (Zerfiridis, 2001). According to Greek legislation ripening in brine can last at least 15 days when pasteurized milk was used or at least 2 months when unpasteurized milk was used (Greek Codex Alimentarius, 2011).

2.3 Differences between Feta cheese and Teleme Cheese

The basic difference between these cheeses is that Feta cheese is made only from pure sheep's milk or a mixture of sheep's and goat's milk (up to 30 % goats' milk into the mixture) (Pappa et al., 2006a, 2006b). Teleme cheese can be made from various kinds of milk (cow, goat, sheep or a mixture of them), usually is used cow's milk (Mallatou et al., 2004, Mallatou and Pappa, 2005). Another very important difference appears in manufacture process, Feta cheese is produced by combined dry salting and immersion in brine, Teleme cheese is direct immersed in brine and some pressure is applied to the curds in the moulds to aid in the expulsion of the whey (Fox et al., 2000). During Feta cheese ripening a microbiological slime on the surface is developed. Feta cheese has smooth texture, soft paste, which can be easily sliced and are present small holes in the shape of almond. If are present small and large holes in big amount, cheese looks like a "sponge", this means that cheese was microbial infected by bacteria, capable producing gases during fermentation. These types of fermentation are undesirable. Feta cheese's flavor (taste and aroma) is creamy, rich, slightly acid, salty, mildly rancid and very pleasant (Zerfiridis, 2001, Fox et al., 2004). Telemes cheese has stiffer paste that is easily friable; its flavor is sour and spicy. Feta cheese's color is snowy white while Teleme cheese's color is white to slightly yellow. Generally Feta cheese production prevails on Teleme cheese production. Technology procedures should be much more strictly controlled in case of Feta cheese due to some micro-

biological risk events (e.g. dry salting on a salting-table). Teleme cheese salting takes place directly in brine so the protection is better (Zerfiridis, 2001, Mallatou and Pappa, 2005).

3 BIOCHEMISTRY OF CHEESE RIPENING

Cheeses coagulated with rennet (chymosin) can mature for a period from 2 weeks up to 2 years, or more. During ripening the characteristic flavor and texture are developed. Ripening process involves changes in cheese microflora, like death and lysis of the starter culture cells and the development of non-starter microflora and in many cheeses the growth of secondary microflora. The metabolic activity of secondary microflora often is dominant on the development of flavor and in some cases on texture development (e.g. white mould cheeses). During cheese ripening texture often becomes softer, because of the hydrolysis of the casein matrix, changes in the ability of the curd binding water and pH changes. Also during ripening due to the production of a wide range of sapid compounds by biochemical pathways, flavor is developed. The biochemistry of cheese ripening is very complex. Biochemical reactions which occur in cheese during ripening are grouped in three major categories; (1) metabolism of lactose and lactic acid, (2) lipolysis and (3) proteolysis (Katsiari et al., 2000a, 200b, Hayaloglu et al., 2003, Fox et al., 2004, Mallatou et al., 2004, Mallatou and Pappa, 2005).

3.1 Metabolism of lactose and lactic acid

As is known cheeses are fermented dairy products and the lactose metabolism to lactate is essential in all cheese varieties. Cheese curd contains a low level of residual lactose which rapidly early is metabolized to lactate. Lactate catabolism probably occurs in all cheeses. The pathway which through lactose is metabolized depends on the type of starter culture (McSweeney and Sousa, 2000). The final stage of glykolysis of lactose is the conversion pyruvate to lactate, catalyzed by lactate dehydrogenase (LDL). The type of LDL in the cell D- or L -LDL, (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus* is D-, *Lactococcus*, *Streptococcus salivarius* subsp. *thermophilus* are L-, *Lactobacillus helveticus* is D/L-) The final product of glykolysis is lactate. During this process 1 mol of lactose is converted to 4 mol of lactate with the production of 4 mole of ATP too. The metabolism of lactose to lactate is essentially complete at the end of manufacture or at the early stages of ripening. Most of lactose in milk is lost in the whey and the amount which remains in the cheese curd is rapidly metabolized after draining. The activity of the starter culture is reduced in the end of manufacture, or often because of the low pH in combination with high NaCl content and the lack of fermentable carbohydrate. Fresh cheese curd contains very low level of lactose,

which in some cases can be reduced to trace levels within one month of ripening, due to the activity of starter culture or by the action of non-starter lactic acid bacteria. Lactate contributes to cheese flavor. Also is an important substance for many reactions which can have a positive or a negative effect to cheese ripening. Lactate can be metabolized to acetate and CO_2 by some non-starter lactic acid bacteria (Fox et al., 2004).

3.2 Lipolysis

Lipolysis is an important biochemical event occurring during cheese ripening. Free fatty acids are known as precursors of catabolic reactions, which produce compounds that are volatile and contribute to flavor, such as methyl ketones, alkanones, lactones, esters and secondary alcohols (Collins et al., 2003). Milk fat is essential for the flavor development of all ripened cheeses. Lipids presence in cheese can undergo hydrolytic or oxidative degradation. The latter does not occur to a significant extent in cheese because of its low redox potential (-250 mV) and the presence of natural antioxidants. Lipolytic agents in cheese can be lipolytic enzymes naturally found in milk (milk lipase), rennet (pregastric esterases) and microflora (Collins et al., 2003, Fox et al., 2004, Kehagias, 2005, Georgala et al., 2005). Milk contains an indigenous lipoprotein lipase (LPL), which contributes to lipolysis in cheese during ripening. Lipoprotein lipase activity is of more significance in cheeses made from raw milk than in that made from pasteurized milk due to that the enzyme is extensively inactivated by pasteurization treatment. As was mentioned above fatty acids have a direct impact on the flavor of many cheese varieties. In particular, C_4-C_{10} acids are strongly flavored. Levels of fatty acids vary considerably between cheese varieties. In addition to their direct role in cheese flavor, fatty acids are important precursors for the production of other volatile flavor compounds during ripening**.** Fatty acid esters are produced by reaction of fatty acids with an alcohol; ethylesters are most common in cheese. Thioesters are formed by reaction of a fatty acid with a thiol compound formed via the catabolism of sulphur containing amino acids. Fatty acid lactones are cyclic compounds formed by the intramolecular esterification of hydroxyacids; y- and δ-lactones contribute to the flavor of a number of cheese varieties (Collins et al., 2003, Fox et al., 2004, Bontinis et al., 2011).

3.3 Proteolysis

Proteolysis is the principal, most complex and the most important biochemical event occurring during the maturation of the majority of ripened cheese varieties. Proteolysis directly affects the development of the desired texture, aroma and intensity of background flavor of most matured cheeses (Katsiari et al., 2000a, 2000b, Fox et al., 2004, Mallatou et al., 2004, 2005, Belitz et al., 2006). Proteolysis is very important for cheese texture by hydrolyzing the para-casein matrix which gives cheese its structure and by increasing the water-binding capacity of the curd. Proteolysis may indirectly affect texture by increasing pH through the production of NH_3 following amino acid catabolism (Katsiari et al., 2000a, 2000b, Fox et al., 2004). Peptides may have a direct impact on cheese flavor. Some peptides are bitter if proteolysis is not well balanced, leading to the accumulation of an excess of hydrophobic peptides of intermediate size (Katsiari et al., 2000a, 2000b, Fox et al., 2004). The major role of proteolysis in cheese flavor is in the production of amino acids which act as precursors for a range of catabolic reactions which produce many important volatile flavour compounds (Katsiari et al., 2000a, 2000b, Fox et al., 2004, Mallatou et al., 2004, 2005, Belitz et al, 2006). In most cheese varieties, the initial hydrolysis of caseins is caused by the coagulant and to a lesser extent by plasmin and perhaps somatic cell proteinases (e.g. cathepsin D) which result in the formation of large (water-insoluble) and intermediate-sized (water-soluble) peptides which are subsequently hydrolyzed by the coagulant and enzymes from the starter and non-starter flora of the cheese. The production of small peptides and amino acids is caused by the action of microbial proteinases and peptidases, respectively. Chymosin is the principal proteinase (88-94 %) in traditional calf rennets, the remainder is pepsin. Although, the principal role of the coagulant in cheese making is to coagulate milk, some activity is retained in the curd, depending on factors such as coagulant type, cooking temperature and pH at drainage and contributes to proteolysis in many varieties. Plasmin is the dominant indigenous proteinase in milk and is produced from its inactive precursor, plasminogen, by a system of plasminogen activators. Milk contains somatic (white blood) cells, which contain lysosomes, which in turn, contain many proteolytic enzymes. Lactic acid bacteria possess very comprehensive proteolytic systems. Non-starter lactic acid bacteria are present initially at low numbers. Their activity appears to be a supplement to the proteolytic action of the starter. In many cheese varieties a secondary microflora (secondary starter) is added intentionally and/or encouraged to grow by environmental conditions and

has a diverse range of functions, depending on the organisms used (Hayaloglu et al., 2003). The final products of proteolysis are amino acids, the concentration of which depends on the cheese variety. Amino acids contribute directly to cheese flavor as some amino acids can taste sweet (e.g. Gly, Ser, Thr, Ala, Pro), sour (e.g. His, Glu, Asp) or bitter (e.g. Arg, Met, Val, Leu, Phe, Tyr, Ile, Trp). It is now generally believed that the principal role of proteolysis in the production of flavor compounds is the liberation of amino acids as precursors for a complex series of catabolic reactions that produce many important volatile flavor compounds (Katsiari et al., 2000a, 2000b, Hayaloglu et al., 2003, Fox et al., 2004, Mallatou et al., 2004, 2005, Bontinis et al., 2011). Amino acid catabolism appears to precede via two major pathways; transaminase action and elimination reactions. Transaminases catalyze the transfer of the α-amino group from an amino acid to an α-keto acid (usually α-ketoglutarate) with the production of the corresponding amino acid and an α-keto acid corresponding to the amino acid substrate. The second pathway which is initiated by elimination reactions is particularly important in the production of volatile sulphur compounds from the side chain of methionine. In addition decarboxylases remove the carboxylic acid group of amino acids to produce amines, some of which have physiological effects. Decarboxylases may also act on a-keto acids to produce aldehydes, which in turn may be oxidized to carboxylic acids or reduced to primary alcohols. The a-amino group of amino acids may be removed by the action of deaminases, with the formation of a carboxylic acid and ammonia. In addition, the side chains of amino acids may be degraded by the action of various lysases.

3.3.1 Biogenic amines

Biogenic amines are basic nitrogenous compounds with low molecular weight formed by microbial decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Silla-Santos, 1996, Valsamaki et al., 2000, Innocente et al., 2007, Buňková et al., 2009, 2010, Pachlová et al., 2011, Buňka et al., 2012). Several of them play very important roles in many human physiological functions. Consumption of food containing high concentrations of biogenic amines (>100 mg/kg) may cause toxic or some deleterious effects. The presence of biogenic amines is highly related with food spoilage and also influences the food safety and quality (Silla Santos, 1996, Valsamaki et al., 2000, Buňková et al., 2009, 2010, Tao Tang et al., 2011). Biogenic amines in food and beverages are formed

by the enzymes of the raw material or are generated by microbial decarboxylation of amino acids (Silla-Santos, 1996). Biogenic amines can be expected in all foods containing proteins or free amino acids and are subject to conditions enabling microbial and biochemical activity. The prerequisites for biogenic amine formation by microorganisms are; (1) availability of free amino acids, (2) presence of decarboxylase-positive microorganisms, (3) conditions that allow bacterial growth, decarboxylase synthesis and activity. Biogenic amines are present in fermented foods; such is beer, cheese, aged meat, wine and fishery products. Also can be found in vegetables, fruits, nuts and chocolate (Silla-Santos, 1996, Tao Tang et al., 2011). The chemical structure of biogenic amines can be; aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine), heterocyclic (histamine, tryptamine). The precursors of the main biogenic amines involved in food poisoning are;

histidine → histamine,

tyrosine → tyramine,

hydroxytryptophane → serotonin,

tryptophane → tryptamine,

lysine → cadaverine,

ornithine → putrescine,

arginine → spermine/spermidine,

phenylalanine → phenylethylamine.

Cheeses belong among foodstuff in which enzymatic and microbial activity cause the formation of amino acids and biogenic amines, due to their high content in protein (Silla-Santos, 1996, Buňka et al., 2012). In fact during cheese ripening the degradation of casein is leading to the accumulation of free amino acids that can be converted into biogenic amines by the activity of bacterial decarboxylases (Innocente et al., 2007). Cheeses belong to the most common sources of biogenic amines. The main biogenic amines found in cheese are histamine, tyramine, tryptamine, putrescine and cadaverine. After fish cheese is the next most commonly implicated food associated with histamine poisoning (Silla Santos, 1996, Valsamaki et al., 2000, Buňková et al., 2010). Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual. An intake of >40 mg BA per meal has been considered potentially toxic. As not all amines

are equally toxic, histamine, tyramine and phenylethylamine are of concern (Shalaby, 1996). Spanjer and van Roode (1991) suggested that the sum of tyramine, histamine, putrescine and cadaverine should not exceed of 900 mg/kg cheese. For the moment, no legal upper limit for biogenic amines in cheese has been established. The levels of histamine in food were restricted by the European Union to 100 mg/kg (Valsamaki et al., 2000, Buňková et al., 2009, 2010, Tao Tang et al., 2011).

4 RHEOLOGICAL PARAMETERS OF CHEESE

Texture is one the most important parameters of cheese, which can determinate its identity and greatly affects consumer preference (Raphaelides, et al., 1995, Antoniou, 2000). Cheese texture is very variable but the factors that affect it are the same for all kinds of cheese. Protein, fat and water content significantly affect rheological properties. Cheese structure is made of protein (casein) matrix, fat globules and water is included in this network (Prentice, 1993). Other factors affecting the texture of cheese are the type of used milk, ripening/storage temperature, starter culture, and salt concentration (Pappas et al., 1996, Prasad and Alvarez, 1999, Kandarakis, et al., 2001, Tsigos, et al. 2003).

The most widely used method for studying mechanical properties of cheese is the unaxial compression force. The probe (plate) is applied to the sample and needed force is recorded. The method is suitable for studying rheological parameters of ripened cheeses and was applied at French cheeses, Cheddar cheese, Gouda cheese and Parmesan cheese etc. (Spangler et al., 1990, Noel, et al., 1996, Antoniou, et al., 2000). Application of instrumental texture profile analysis with two cycle compression is used in food analysis (Pappa, et all., 2006). Instron and the TA.XT*Plus* Texture Analyzer are devices used worldwide, very valuable, used for study of food texture and they allow the study of different types of cheese (Raphaelides, et al., 1995).

Feta cheese has short, firm, smooth texture and easily can break into pieces when is compressed, so data on its rheological properties are limited. Hardness (N) varies from 26.478 to 68.647, force to fracture (N) from 14.710 to 23.536 and compression to fracture (N) from 183.384 to 210.843 (Katsiari, et al., 1997, Kandarakis, et al., 2001).

As was above mentioned Teleme cheese is white brined cheese matured in brine and its ripening period can be from 15 days to 2 months. In the first stage of cheese making, after curdling the Teleme cheese has soft texture, due to its high content in moisture. During the first month of ripening the texture becomes firmer. This happens due to the loss of moisture, which makes the structure more compact. After the first month Teleme cheese becomes progressively softer due to proteolysis. Teleme cheese becomes more fragile after the first month of ripening, due to the cleavage of some of the peptic bonds during proteolysis. Also elasticity increases during the first month of ripening. Teleme cheese is usually crumbly. Strength and firmness are large due to casein framework. Another parameter that

affects the cheese texture is the pH. Cheeses with higher pH are softer than those with lower pH (Creamer and Olson, 1982). Close to isoelectric point of caseins (pH≈4.6) these proteins are held together with strong ionic and also hydrophobic forces. Water fills the empty spaces. With a higher pH, caseins become more negatively charged and due to ionic repulsion they can bind more amount of water. Cheeses with higher pH show the behavior of concentrated protein emulsions, whereas cheeses with lower pH have the form of porous masses of casein (Raphaelides, et al., 1995).

II. ANALYSIS

5 MASTER THESIS OBJECTIVES

This current master thesis had two main targets. The first one was the study of manufacture technology and properties characterization of Greek cheeses ripened in brine. The second main target was the chemical analysis including analysis of pH, dry matter, NaCl, free amino acids, biogenic amine contents and performance of texture profile analysis in commercial and laboratory manufactured Greek type white cheeses ripened in brine.

Procedure for objectives achievement:

- Create a synopsis of Greek cheeses ripened in brine,
- Describe the technological manufacture process of Greek cheeses ripened in brine,
- Characterize selected properties of Greek cheeses ripened in brine.

To handle the practical part of this work it was necessary to accomplish these specific objectives:

- Optimize the laboratory manufacture process of cheeses ripened in brine,
- With laboratory optimized manufacture process manufacture cheeses ripened in brine with and without the addition of decarboxylase positive lactococci,
- Subject the samples to analysis of pH, dry matter, NaCl, free amino acid and biogenic amine contents and perform texture profile analysis,
- Compare the results with commercially produced cheeses from the area of Greece,

6 MATERIALS AND METHODS

6.1 Experimental design

The experimental part obtained three basic steps. The first step included the chemical analysis of four commercial samples of Greek white brine cheeses. Two of the samples were Feta cheese (*Feta Dodoni, Feta G.A.L.P.O. traditional*) and the other two were Teleme cheese (*Choriatiko, Livadi*). The chemical analysis included analysis of pH values, dry matter, NaCl contents, free amino acid and biogenic amine contents also texture profile analysis was performed. The analyes of pH, dry matter, NaCl contents in each sample were done in triplicate. For the determination of free amino acid and biogenic amine contents, each of the simultaneously obtained samples was lyophilized twice and each extract was analyzed three times. The texture profile analysis to each sample was done in duplicate. All the analyses were performed at freshly unpacked cheese samples and at cheese samples that were kept at 2-4 °C for a period of 14 days.

The second experimental step included the optimization of the manufacture technology of cheese similar to Greek Teleme cheese under laboratory conditions and their chemical analysis. The chemical analyses of pH, dry matter, NaCl contents and the texture profile analysis were performed at cheese samples before ripening and after 28 days and 56 days of ripening. The analysis of pH, dry matter, NaCl contents in each sample was done in triplicate. The determination of free amino acid and biogenic amine content were performed at cheese samples which underwent ripening of 28 days and 56 days. For the determination of free amino acid and biogenic amine contents, each of the simultaneously obtained samples was lyophilized twice and each extract was analyzed three times. The texture profile analysis to each sample was done in duplicate.

The third step included the manufacture of four series of cheese similar to Greek Teleme cheese and their chemical analysis. Each series obtained a pair of cheese samples with and without the addition of decarboxylase positive lactococci. The chemical analyses were done parallel to each pair of all four series. The chemical analyses of pH, dry matter, NaCl contents were performed at the cheese samples before ripening and after 28 days and 56 days of ripening. The analysis of pH, dry matter, NaCl contents in each sample was done in triplicate. The determination of free amino acid and biogenic amine contents were per-

formed at cheese samples which underwent ripening of 28 days and 56 days. For the determination of free amino acid and biogenic amine contents, each of the simultaneously obtained samples was lyophilized twice and each extract was analyzed three times. The texture profile analysis to each sample was done in duplicate.

6.1.1 Characteristics of commercial cheese samples

Two of the examined cheeses were Feta cheese and two were Teleme cheese. The analyzed Feta cheeses were *"Feta Dodoni"* (Dodoni agricultural industry of Epirus, Greece) with minimum moisture 43 %, minimum dry matter 44 %, production date was 05.04.2011, consumption date was until 05.04.2012 and *"Feta G.A.L.P.O. traditional"* (Tyras A.E., Trikala, Thessaly, Greece) with maximum moisture 56 %, minimum fat in dry matter 48 %, production date was 09.05.2011, consumption date was until 15.04.2012. The examined Teleme cheeses were *"Livadi"* (white cheese matured in brine) (Dodoni agricultural industry of Epirus, Greece) with maximum moisture 58 %, fat in dry matter minimum 47 %, production date was 23.07.2011, consumption date was until 15.04.2012 and *"Choriatiko"* (white cheese in brine) (A.E. Olympos, Greece) with maximum moisture 58 %, fat in dry matter minimum 47 %, production date was 16.07.2011, consumption date was until 23.03.2012.

6.2 Cheese making

6.2.1 Cheese making technique without the addition of decarboxylase positive lactococci

White brine cheese similar to Greek Teleme cheese was manufactured under laboratory conditions from 4 l of fresh pasteurized cow's milk. Commercial cow's milk was used of the Czech OLMA a.s. dairy industry. A mixture of whole milk under the brand "*SELSKE"* and half-fat milk was used in ratio 1:1. By mixing those milks the fat content in milk was standardized to 2.5 %.

(a) (b)

Fig. 1: (a) whole milk with 3.5 % fat content, (b) half-fat milk with fat content 1.5 % (www.olma.cz).

The standardized milk was heated to 32 °C because it was kept in a refrigerator with temperature 2-4 °C. After heating a mixture of cultures was added. The mixture contained classic yogurt culture and basic mesophilic cheese making culture in ratio 1:1. To assist curdling of the milk $CaCl_2$ solution (37 % w/v) was added at a ratio 2.0 ml/4 l of cow's milk. Then the milk was allowed to relax for a period of 30 minutes. After this period rennet (LAKTOCHYM with power 1:53 000) was added, in quantity 0.274 ml/4 l of milk to obtain coagulation time 55 minutes. After coagulation the formed curd was cut cross-wise into cubes of 2 cm x 2 cm x 2 cm. Then the curd was stirred for a period of 50 minutes. After the curd was transferred to special rectangular plastic moulds for whey exudation and shape formation. Every 30 minutes the cheese was turned over and for more effective whey exudation a counterweight weighting 1 kg for 1 minute was applied on the cheese. This phase was repeated three times. Then the cheese was left under constant temperature (20 ± 2°C) and moisture conditions overnight. The next day the cheese was placed into brine with salt concentration 18 % w/v. The ripening period was 2 months under standard temperature (10 ± 2 °C).

6.2.2 Cheese making technique with the addition of decarboxylase positive lactococci

Under laboratory conditions from 4 l of fresh pasteurized cow's milk were manufactured cheese samples similar to Greek Teleme cheese. Commercial milk was used from OLMA

a.s. Czech dairy industry. A mixture of whole milk (SELSKE) and half milk in ratio 1:1 was used. The fat content in the milk was standardized to 2.5 %. Then followed the heating of the milk was to 34 °C because it was stored in a refrigerator at 2-4 °C. After heating were added to the milk classic yogurt culture and basic mesophilic culture in ratio 1:1. To assist curdling of the milk $CaCl_2$ solution (37 % w/v) was added at a ratio 2.0 ml/4 l of cow's milk. Then the milk was allowed to relax for a period of 30 minutes. After this period rennet (LAKTOCHYM with power 1:53 000) was added, in quantity 0.274 ml/4 l of milk to obtain coagulation time 55 minutes. Parallel with the rennet addition was added culture decarboxylasa positive lactococci containing strains of *Lactococcus lactis* subsp. *lactis*. The incubation was performed on petri bowl which contained nutrient substrate M17 with 0.5 % lactose. For the cheese manufacture of the first and second series (I, II) was done a dilution of 10^{-6} and the number of the counted microorganisms was $2.0 . 10^{9}$ CFU/ml of broth (series I), and $1.28 . 10^{10}$ CFU/ml of broth (series II). For the third and fourth manufacture series (III, IV) was performed dilution of 10^{-8}. The number of the counted microorganisms was $1.1 . 10^{11}$ CFU/ml of broth (series III) and $1.1 . 10^{11}$ CFU/ml of broth (series IV). The incubation took place at 30 °C and the counting was done after 24 hours. The formed curd was cut cross-wise into cubes of 2 cm x 2 cm x 2 cm. Then the curd was stirred for a time period of 50 minutes. Then curd was transferred to special rectangular plastic moulds for whey exudation and shape formation. Every 30 minutes the cheese was turned over and for more effective whey exudation a counterweight weighting 1 kg for 1 minute was applied on the cheese. This phase was repeated three times. Then the cheese was left under constant temperature (20 ± 2°C) and moisture conditions overnight. The next day the cheese was placed into brine with salt concentration 18 % w/v. The ripening period was 2 months under standard (10 ± 2 °C).

6.2.3 Preparation of the yogurt culture

For the yogurt culture-preparation were used 40 ml of milk pasteurized at 95 °C for 2-3 minutes. Then the milk was cooled to 43 °C and vaccinated with 0.2 g/ 40 ml of dried culture *"Lactoflora® jogurtová"* (MILCOM a.s., Prague, Czech Republic) and it was well stirred. The cultivation took place in a thermostat at 23°C for 16-20 hours.

6.2.4 Preparation of the basic mesophilic culture

For the basic mesophilic cheese making culture 40 ml of milk were pasteurized at 95 °C for 2-3 minutes. Then the milk was cooled to 25 °C. To the milk were vaccinated 0.2 g/ 40 ml dried culture of "Lactoflora® smetanová" (MILCOM a.s., Prague, Czech Republic) and it was well stirred. The cultivation took place in a thermostat at 23 °C for 16-20 hours.

6.3 Chemical analysis

For the chemical analysis accomplishment of the cheese samples were done analysis of pH, dry matter, NaCl, free amino acid and biogenic amine contents and texture profile analysis was performed.

6.3.1 Dry matter analysis

The dry matter content was determined gravimetrically based on the loss of volatiles, including free water associated with heating to 105 ± 2 °C until the mass remains constant according to ISO 5534 (2004). The method does not remove molecular bond water. Metallic weighting dishes which contained sea sand and a glass rod were weighted on an analytical weight (A&D GH-200 EC) and the mass (W_p) was recorded accurately to four decimal places. The balance was tarred with the metallic weighting dish on it. Then approximately into the weighting dish were transferred 3 g of cheese sample (W_s). Then the samples were placed into a forced air drying oven pre-heated at 105 °C ± 2 °C until the weight remained constant. Afterwards the weighting dishes were placed into a desiccator to cool. Finally the dishes were weighted and the dry matter (W_d) was recorded accurately to four decimal places. The dry matter content as percent (%) was calculated according to the following equation:

$$DMC = \frac{Wd - Wp}{Ws} \; X\; 100\%$$

Where:

DMC: is the dry matter content as percent (%).

W_d: is the weight of the cheese sample including the weighting dish, the sea sand and the glass rod, after drying.

W_p: is the weight of the weighting dish, the sea sand the glass rod.

W_s: is the weight of the cheese sample including the weighting dish, the sea sand and the glass rod, before drying.

6.3.2 Analysis of pH values

In chemistry pH is a measure of the acidity or alkalinity of an aqueous solution at a specified temperature usually 20 °C or 25°C. It is measured on a continuous scale from 0 to 14. The pH is defined as a negative decimal logarithm of the hydrogen ion activity in a solution, given by the following equation:

$$pH = - \log [H^+]$$

For the pH measurement of the cheese samples was used a calibrated pH-meter (pH Spear for food testing, Eutech Instruments).

6.3.3 Sodium chloride analysis

The sodium chloride content was determined argentinometrically according to Mohr's method. The Mohr titration is a direct titration method for chloride anion quantization. The chloride containing sample solution is titrated with a standard solution of silver nitrate. After the silver from the silver nitrate has complexed with all the available chloride in the sample, the silver reacts with chromate that has been added in the sample, to form an orange colored solid, silver chromate. The volume of silver used to react with the chlorine is used to calculate the sodium content of the sample.

Chemicals:

- Potassium chloride (KCl)
- Potassium chromate (K_2CrO_4)
- Silver nitrate ($AgNO_3$)
- Distilled water

Supplies:

- Beakers, 250 ml
- Buret, 25 ml
- Brown bottle, 500 ml
- Erlenmeyer flasks
- Magnetic stir bars
- Pippete bulb or pump
- Spatulas
- Weighting paper and boats
- Volumetric pipette, 1 ml

Equipment:

- Analytical balance (A&D GH-200 EC)
- Magnetic stir plate

For the standardization of 0.1 M $AgNO_3$ a quantity of 400 ml 0.1 M $AgNO_3$ were transferred to a brown bottle. This solution was standardized and then used to titrate the cheese samples. A buret was filled with this $AgNO_3$ solution. Then the primary standard (NaCl, Mr=58.454 mol^{-1}) solution was prepared in triplicate. Accurately to four decimal places were weighted about 0.5845 g NaCl and placed into Erlenmeyer flasks. Then was added

distilled water and 2-3 drops of potassium chromate (K_2CrO_4) solution as indicator. A magnetic stir bar was placed in each flask with the NaCl solution and the beaker was placed on a magnetic stir plate below the buret for titration. The $AgNO_3$ solution in the buret was used to titrate the NaCl solutions to the appearance of a pale orange color. Then the volume of $AgNO_3$ was recorded.

For the determination of NaCl content 1 g of cheese was accurately weighted in triplicate into 250 ml beakers. Then about 50 ml of warm distilled water were added (50-55 °C) into each beaker. The solution was agitated using a glass stirring rod. Then 1 ml of potassium chromate (K_2CrO_4) as indicator was added. Finally each solution was titrated with standardized $AgNO_3$ solution to the first visible pale red-brown color that persisted for 1 minute. The volume of the titrant was recorded (Nielsen, 2003).

6.3.4 Texture analysis

The texture analysis was performed using a TA.TX.*Plus* Texture analyzer (Stable Micro Systems, Surrey, UK).

Fig. 2: TA.TX.*Plus* Texture analyzer (www.stablemicrosystems.com).

The cheese samples were prepared to the shape of cylinder with diameter of 25 mm and a height of 15 mm. A cylindrical probe (P/50) was used for compression of the cheese sam-

ples at the speed of 1 mm/s. The software used for sample analysis and evaluation was the Exponent Lite software.

6.3.5 Biogenic amine content determination

Four series of cheese samples were manufactured and each series obtained cheese sample with and without decarboxylase positive lactococci. The cheese samples were submitted to biogenic amine content determination. Cheese samples weighting 20 g were frozen at -80 °C and lyophilized by ALPHA 1-4 LSC. After lyophilization 1 g of cheese sample weighted on an analytical balance (A&D GH-200 EC) was placed into 15 ml centrifuge plastic tubes and 10 ml perchloric acid ($HClO_4$ 0.6 M, 70-72 %) were added. The mixture had been shaking (laboratory shaker LT2) for 30 minutes at room temperature and centrifuged (EBA 21 centrifuge) for 20 minutes at 6,000 r.p.m. The content was transfused into a 25 ml volumetric flask. Another 7 ml of perchloric acid ($HClO_4$ 0.6 M, 70-72 %) were added into the plastic tubes containing the cheese samples. The mixture had been shaking for 20 minutes at room temperature and centrifuged for 20 minutes at 6,000 r.p.m.. The content was transfused into a 25 ml volumetric flask. Again 7 ml of perchloric acid ($HClO_4$ 0.6 M, 70-72%) were added into the plastic tubes containing the cheese samples. The mixture had been shaking for 20 minutes at room temperature and centrifuged for 20 minutes at 6,000 r.p.m. The content again was transfused into a 25 ml volumetric flask and the flask was filled with perchloric acid ($HClO_4$ 0.6 M, 70-72 %) to the mark. Then followed filtration was through filtration paper. Then 1 ml of the sample was placed into the derivatization dish and 100 μl of internal standard (histamine, approx. 97 %, 2-phenethylamine, tyramine 99 %, putrescine dihydrochloride, cadaverine, agnatine sulfate, spermidine, spermine, tryptamine, 1,7-diaminoheptane) in concentration 500 mg l^{-1} were added. Then followed the addition of 1.5 ml carbonate buffer with pH 11.1-11.2. Also 2 ml of freshly prepared solution of dansyl chloride (BioReagent, suitable for amino acid labeling, powder and chunks, ≥99 %) in concentration 5 g/l in acetone were added. The dish was well sealed and had been shaking for 20 hours at room temperature without the effect of light. Then 200 μl of L-Proline solution were added. The mixture had been shaking for another 1 hour. 3 ml of heptanes (CHROMASOLV®, for HPLC, ≥99 %) were added and the mixture had been shaking manually for 3 minutes. Into a vial was placed 1 ml of the heptane layer. Evaporation to dry followed at temperature 60 °C under a stream of air. The dry evaporator

was diluted with 1.5 ml of acetonitrile (CHROMASOLV® Plus, for HPLC, ≥99.9 %). The sample was filtrated through a syringe filter with porosity 0.22 μm and injected into the chromatography system. The analysis was performed using an ion-exchange chromatogram. A HPLC system (binary pump LabAlliance, USA, autosampler LabAlliance, USA, column with thermostat; UV/VIS DAD detector (λ = 254 nm); a degasser 1260 Infinity, Agilent Technologies) was used for the determination of biogenic amines. Each of the simultaneously obtained samples was lyophilized twice and each extract was analyzed three times. The determination of biogenic amine content in the cheese samples was performed after 28 days and 56 days of ripening.

6.3.6 Free amino acids content determination

Four series of cheese samples were manufactured and each series obtained cheese sample with and without decarboxylase positive lactococci. The cheese samples were submitted to free amino acids content determination. Cheese samples weighting 20 g were frozen at -80 °C and lyophilized by ALPHA 1-4 LSC. After lyophilization 1 g of cheese sample weighted on an analytical balance (A&D GH-200 EC) was placed into 15 ml plastic tubes and 5 ml of lithium-citrate buffer were added too. The mixture had been shaking (laboratory shaker LT2) for 45 minutes at room temperature and centrifuged (EBA 21 centrifuge) for 15 minutes at 6,000 r.p.m. The content then was transfused into eppendorfs (two from each sample). Then the mixture had been centrifuged (EBA 21 centrifuge) for 15 minutes at 15,000 r.p.m.. Then followed filtration through filter with porosity 0.45 μm was applied. The Amino Acid Analyzer AAA 400 (Ingos, Prague Czech Republic) was used for the determination of free amino acid content.

7 RESULTS AND DISCUSSION

7.1 Chemical analysis

7.1.1 First cheese making attempt

During the first cheese making attempt white brine cheese similar to Greek Teleme cheese was manufactured under laboratory conditions from 3 l of fresh pasteurized cow's milk. Commercial cow's milk was used of the Czech OLMA a.s. dairy industry. A mixture of whole milk under the brand *"SELSKE"* and half-fat milk was used in ratio 1:1. By mixing those milks the fat content in milk was standardized to 2.5 %. The standardized milk was heated to 37 °C because it was kept in a refrigerator with temperature 2-4 °C. After heating a mixture of cultures was added. The mixture contained classic yogurt culture and basic mesophilic cheese making culture in ratio 1:1. To assist curdling of the milk $CaCl_2$ solution (37 % w/v) was added at a ratio 1.5 ml/3 l of cow's milk. Then the milk was allowed to relax for a period of 30 minutes. After this period rennet (LAKTOCHYM with power 1:5000) was added in quantity 2.2 ml/3 l to obtain coagulation time of 30 minutes. After coagulation the formed curd was cut cross-wise into cubes of 2 cm x 2 cm x 2 cm. Then the curd was agitated for a period of 30 minutes. The result was that small fragments of curd appeared and the cheese grain was totally destroyed. The attempt was characterized as unsuccessful.

7.1.2 Second cheese making attempt

At the second cheese making attempt were applied some changes in the manufacture technology. The heating temperature of milk was reduced from 37 °C to 32 °C and the coagulation time increased from 30 to 55 minutes. Otherwise the cheese making technology was the same as is described above (first cheese making attempt). The result of the applied changes was that the cheese drain after cutting and agitating was smaller and small fragments of curd appeared too but in lower quantity. The attempt was characterized as successful because the curd was compact. After the curd was transferred to special rectangular plastic moulds for whey exudation and shape formation. Every 30 minutes the cheese was turned over for whey exudation and this was repeated three times. Then the cheese was left at room temperature 10 ± 2 °C. The next day the cheese was placed into brine with salt

concentration 18% w/v. The ripening period was 2 months under standard temperature and moisture conditions.

7.1.3 Second cheese making attempt

During the third cheese making attempt the heating temperature of milk was increased from 32 °C to 34 °C and after curd cutting the curd was allowed to rest for 5 minutes for partial exudation of the whey. Otherwise the manufacture process was the same as is described above (first and second cheese making attempt). The attempt was characterized also as successful.

7.2 Dry matter analysis

7.2.1 Determination of dry matter contents of Greek commercial cheeses

The dry matter analysis reshly unpacked commercial Teleme cheese *"Choriatiko"* was 45.87 ± 0.08 % and of *"Livadi"* Teleme cheese was 41.13 ± 0.31 %. After 14 days of storage at 2-4 °C the dry matter content of *"Choriatiko"* Teleme cheese slightly decreased to 43.29 ± 0.29 % and the dry matter content of *"Livadi"* Teleme cheese presented a negligible increase to 41.99 ± 0.50 %. The dry matter analysis of freshly unpacked commercial *"G.A.L.P.O. Feta"* cheese determined to be 45.06 ± 0.28 % and of the *"Dodoni Feta"* cheese was 43.96 ± 0.17 %. After a period of 14 days of storage at 2-4 °C the dry matter content of *"G.A.L.P.O. Feta"* cheese decreased to 42.29 ± 0.14 % and the dry matter content of *"Dodoni Feta"* cheese presented a small decrease to 44.88 ± 0.30 %. The results of the dry matter analysis of the freshly unpacked commercial Greek cheeses were that all the cheese samples maintained the Greek legislation standards. The small changes of the dry matter content values after 14 days of storage at 2-4 °C may be due to signs of secondary contamination that showed the commercial cheese samples. These signs were, not pleasant aroma and the brine had a colloidal texture.

7.2.2 Determination of dry matter contents of manufactured cheese series

The analysis of dry matter content of the manufacture series I without the addition of decarboxylase positive lactococci showed a dry matter value 47.72 ± 0.80 % at the beginning of the ripening process (day 1). After 28 days of ripening under constant temperature and

moisture conditions the dry matter content significantly increased to 50.87 ± 0.04 % and after 56 days of ripening under constant temperature and moisture conditions the dry matter increased to 51.60 ± 1.61 %. The same upward trend presented and the manufactured cheese samples of series 1 with the addition of decaroxylase positive lactococci. The dry matter at the beginning of the ripening process (day 0) was 44.46 ± 0.58 %, after 28 days of ripening under constant temperature the dry matter increased to 49.56 ± 0.22 % and after 56 days of ripening the under constant temperature the dry matter slightly increased to 50.83 ± 0.27 %.

The analysis of the dry matter of the manufacture series II without the addition of decarboxylase positive lactococci appeared to have the same upward trend which had the cheese samples of the manufacture series I. Specifically the dry matter content at the beginning of the ripening (day 1) was 40.35 ± 0.28 %, after 28 days of ripening under constant temperature and moisture conditions the dry matter increased to 42.17 ± 1.14 % and after 56 days of ripening the under constant temperature and moisture conditions the dry matter slightly increased to 43.02 ± 1.93 %. The cheese samples with the addition of decarboxylase positive lactococci showed an upward trend too. The dry matter content at the beginning of the ripening was found to be 37.86 ± 0.32 %, after 28 days of ripening under constant temperature and moisture conditions increased to 43.13 ± 0.40 % and after 56 days of ripening the value presented a negligible increase to 43.98 ± 1.22 %.

The dry matter analysis of the cheese samples of the manufacture series III presented a noteworthy difference in comparison with all the manufacture series (I, II, IV). In all cheese samples, with and without the addition of decarboxylase positive lactococci can be observed an upward trend of the dry matter from 1 days to 28 days of ripening under constant temperature, but the dry matter content from 28 days to 56 days significantly decreased. The determined values for the cheese samples without the addition of decarboxylase positive lactococci were; 43.80 ± 3.54 % (1 days of ripening), 48.02 ± 0.60 % (28 days of ripening) and 40.24 ± 0.37 % (56 days of ripening). The determined values for the cheese samples with the addition of decarboxylase positive lactococci were; 42.96 ± 1.58 % (1 days of ripening), 45.59 ± 0.38 % (28 days of ripening) and 39.85 ± 0.45 % (56 days of ripening). The observed downward trend of the dry matter from 28 to 56 days of ripening under constant temperature could be probably explained by the presence of secondary contaminating microflora which caused the spoilage of the cheese samples. During the sen-

sory-visual observation of the cheese samples were observed a very unpleasant aroma and the brine appeared to have a blur, probably occurred from the contamination of secondary microflora.

The analysis of the dry matter of the manufacture series IV without the addition of decarboxylase positive lactococci presented an upward trend during the whole experiment period. The determined value of the dry matter at the beginning of the ripening process under constant temperature and moisture conditions (1 days of ripening) was 38.21 ± 0.53 %, after 28 days of ripening the value obviously increased to 47.70 ± 1.22 % and after 56 days of ripening increased to 50.51 ± 1.03 %. The same trend presented and the cheese samples with the addition of decarboxylase positive lactococci. The dry matter content at the beginning of the ripening under constant temperature and moisture conditions (1 days of ripening) was found to be 39.54 ± 0.55 %, after 28 days of ripening the value increased to 43.87 ± 0.87 % and after 56 days of ripening the value increased to 50.51 ± 0.99 %.

7.3 Analysis of pH values

7.3.1 pH values of Greek commercial cheeses

The analysis of the pH values of all the commercial samples of Teleme and Feta cheese showed that after a storage period of 14 days at 2-4 °C the pH decreased. The freshly unpacked *"Choriatiko"* Teleme cheese presented a reduction from 4.91 ± 0.17 to 4.35 ± 0.02 after 14 days of storage at 2-4 °C and the *"Livadi"* Teleme cheese a reduction from 4.87 ± 0.26 to 4.21± 0.06. The freshly unpacked *"G.A.L.P.O Feta"* cheese showed a reduction from 5.68 ± 0.09 to 4.56 ± 0.25 after 14 days of storage at 2-4 °C and the *"Dodoni Feta"* cheese from 5.33 ± 0.37 to 4.12 ± 0.11. The decrease of the pH values was probably due to secondary contamination while the cheese samples were kept at 2-4 °C for 14 days. According to Moatsou and Govaris, 2011, can be concluded that the Teleme cheese is more acid than the Feta cheese. This was obviously shown by the pH values of the freshly unpacked Teleme and Feta cheese. The lactic acid that accumulates (i.e. from the milk and the starter culture) resulted in the high acidity of Teleme cheese. No microbiological analysis was performed to none of the commercial cheese samples.

7.3.2 pH values of manufactured cheese series

The analysis of the pH values of all the manufacture series with and without the addition of the decarboxylase positive lactococci presented the same trend during the whole experimenal period of 56 days. The pH values of the cheese manufactured series without the addition of the decarboxylase positive lactococci from 1 days to 28 days of ripening under constant temperature and moisture conditions presented a slight increase from 4.69 ± 0.24 to 4.81 ± 0.27 and from 28 days to 56 days of ripening under constant temperature and moisture conditions presented a decrease from 4.81 ± 0.27 to 4.51 ± 0.21. The pH values of the cheese manufactured series with the addition of the decarboxylase positive lactococci from 1 days to 28 days of ripening under constant temperature and moisture conditions presented a slight increase from 4.55 ± 0.09 to 4.64 ± 0.09 and from 28 days to 56 days of ripening under constant temperature and moisture conditions presented a decrease from 4.64 ± 0.09 to 4.38 ± 0.42. Exception to this phenomenon was the manufacture series III, where was observed a decrease of the pH values from 1 days to 28 days of ripening under constant temperature and moisture conditions and from 28 days to 56 days of ripening under constant temperature and moisture conditions was observed an increase. A possible explanation of this phenomenon could be the presence of secondary microflora which caused the spoilage of the cheese samples. According to Buňková et al., 2010, Pachlová et al., 2012 the pH increase of the cheese samples could be due to the formation of alkaline substances (ammonia, ketones and aldehydes) and due to degradation of lactic acid into other compounds. Also according to McMahon et al., 2009 and Pachlová et al., 2012 at the very first days of ripening the pH values decrease because of the fast rate growth of lactic acid bacteria (either starter or nonstarter bacteria). This decreasing phenomenon was not observed in our experiment probably because the 28 days of ripening are quite a long period of ripening. The latter author also referred that the decrease of the pH in the first days of ripening could be caused by the microbial fermentation of lactose leading to the formation of lactic acid. The observed pH increase from 1 to 28 days of ripening could be due to the same reason mentioned above, the formation of alkaline compounds and the degradation of lactic acid (Buňková et al., 2010, Pachlová et al., 2012). The exact reason why the pH from 28 to 56 days decreased was not found in literature. One possible explanation could be the production of acidic compounds from the contamination of secondary micro-

flora. Also must be noticed that no microbiological analysis was done to the cheese samples.

Another important observation was that all the manufactured series with the addition of decarboxylase positive lactococci had lower pH (more acid) than the manufactured series without the addition of decarboxylase positive lactococci. This could be due to the addition of the broth which contained strains of *Lactococcus lactis* subsp. *lactis* capable of producing biogenic amines and the fermentation of lactose to lactic acid was more intense.

7.4 Sodium chloride contents

7.4.1 Sodium chloride contents of Greek commercial cheeses

The analysis of sodium chloride contents of freshly unpacked *"Choriatiko"* Teleme cheese was found to be 4.98 ± 0.01 % and after 14 days of storage increased to 5.17 ± 0.02 %. The sodium chloride content of the freshly unpacked *"Livadi"* Teleme cheese was 5.01 ± 0.01 % and after 14 days of storage at 2-4 °C increased to 5.21 ± 0.01 %. The sodium chloride content of the freshly unpacked *"G.A.L.P.O. Feta"* cheese was 4.75 ± 0.01 % and after 14 days of storage at 2-4 °C increased to 4.87 ± 0.01 %. The sodium chloride content of the freshly unpacked *"Dodoni Feta"* cheese was 4.69 ± 0.01 % and after 14 days of storage at 2-4 °C increased to 4.82 ± 0.01 %. The slight sodium chloride reduction which was observed in all commercial cheese samples could be due to that during brining the salt is absorbed into the cheeses with a concomitant decrease in the concentration of salt in the brine (McMahon et al., 2009). Another noteworthy observation is that Teleme cheeses had a higher salt content than the Feta cheeses. The higher salt level of Teleme cheese is due to a different salting technique used during manufacture technology. Teleme cheese is salted by immersion in brine immediately after draining of the curd, while Feta cheeses surface is sprinkled with coarse salt.

7.4.2 Sodium chloride contents of manufactured cheese series

The analysis of the sodium chloride contents of the cheese manufacture series I ripening in brine under constant temperature and moisture conditions without the addition of decarboxylase positive lactococci was found to be 8.76 ± 0.01 % after 28 days of ripening. The sodium chloride content increased to 9.69 ± 0.01 % after 56 days of ripening. A similar

increase had shown the cheese samples with the addition of decarboxylase positive lactococci, from 9.41 ± 0.01 % after 28 days, to 10.82 ± 0.01 % after 56 days of ripening.

The sodium chloride contents at the cheese manufacture series II without the addition of decarboxylase positive lactococci was found to be 9.91 ± 0.01 % at 28 days of ripening in brine under constant temperature and moisture conditions. After a ripening period of 56 days the sodium chloride content increased to 10.39 ± 0.03 %. The cheese samples with the addition of decarboxylase positive lactococci had sodium chloride content 9.51 ± 0.01 % at 28 days of ripening in brine under constant temperature. After a ripening period of 56 days the sodium chloride content increased to 10.57 ± 0.01 %.

The sodium chloride contents at the cheese manufacture series III without the addition of decarboxylase positive lactococci was found to be 8.78 ± 0.01 % at 28 days of ripening in brine under constant temperature and moisture conditions. After a ripening period of 56 days the sodium chloride content increased to 10.77 ± 0.02 %. A similar increase had shown the cheese samples with the addition of decarboxylase positive lactococci, from 8.81 ± 0.01 % after 28 days, to 10.89 ± 0.01 % after 56 days of ripening in brine under constant temperature.

The determination of the sodium chloride contents of the cheese manufacture series IV ripening in brine under constant temperature without the addition of decarboxylase positive lactococci was found to be 8.85 ± 0.01 % after 28 days of ripening. The sodium chloride content increased to 10.37 ± 0.01 % after 56 days of ripening. A similar increase had shown the cheese samples with the addition of decarboxylase positive lactococci, from 9.68 ± 0.01 % after 28 days, to 10.65 ± 0.01 % after 56 days of ripening in brine under constant temperature and moisture conditions.

During brining the salt is absorbed into the cheeses with a concomitant decrease in the concentration of salt in the brine. This net movement of Na^+ and Cl^- ions occurs because of the differences in osmotic pressure between cheese and brine (McMahon et al., 2009). The used brine for the experiment had a concentration of 18 % (w/v) NaCl. The salt concentration in the brined cheeses was influenced by the salt content of the brine. This could explain the high salt content which had all the manufactured cheeses. A remarkable proposal for future work would be the decrease of the NaCl content of the brine.

7.5 Free amino acids

7.5.1 Sodium chloride contents of manufactured cheese series

The free amino acid contents in four commercial Greek cheeses were determined. Two of the Greek cheeses were Feta cheese and two were Teleme cheese. The determination of free amino acids was applied at cheese samples that were unpacked, lyophilized and stored at -80 °C until the analysis day and at cheese samples that were unpacked, kept at 2-4 °C for a period of 14 days, then were lyophilized and stored at -80 °C until the day that analysis was performed. Free amino acids are considered as precursors of biogenic amines (Silla Santos, 1996). The total content of the determined free amino acids is presented the Fig. 3 below.

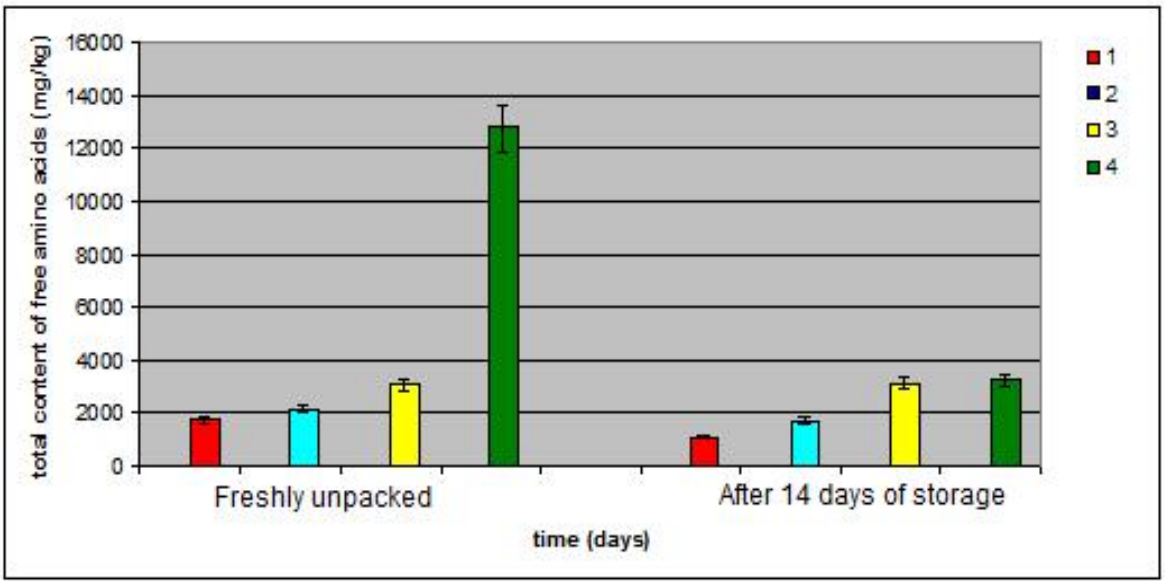

Fig. 3: Total contents of free amino acid in commercial Greek cheeses that were freshly unpacked and stored at 2-4 °C for 14 days. 1: "Choriatiko", 2: "Livadi", 3: "G.A.L.P.O. Feta", 4: "Dodoni Feta"

The results of the analysis of the free amino acid contents showed that at all commercial cheese samples the total content slightly decreased. Exception was the *"Dodoni Feta"* where the decrease was very intense, almost triple. This phenomenon can be combined with the intense biogenic amine production of the *"Dodoni Feta"* during the storage of 14 days at 2-4 °C as is shown at Fig. 5.

7.5.2 Free amino acid contents of manufactured cheese series

The determination of free amino acids was applied at cheese manufactured series (I-IV) samples that were lyophilized and stored at -80 °C until the analysis day. The result of the analysis was that all manufactured series cheese samples with and without the addition of decarboxylase positive lactococci presented the same increasing trend of the total free amino acid contents (Fig. 4), although the content was non-standard. Exception was the cheese manufactured series IV with and without the addition of decarboxylase positive lactococci because the total free amino acid contents decreased from 28 to 56 days of ripening. A probable explanation of this phenomenon could be their conversion to biogenic amines, as is shown at Fig. 11. The individual manufactured series were a lot different to the total content of free amino acids. More emphasis should be given to the standardness of the cheese manufacture process. The total free amino acid contents are shown at the Fig. 4 below.

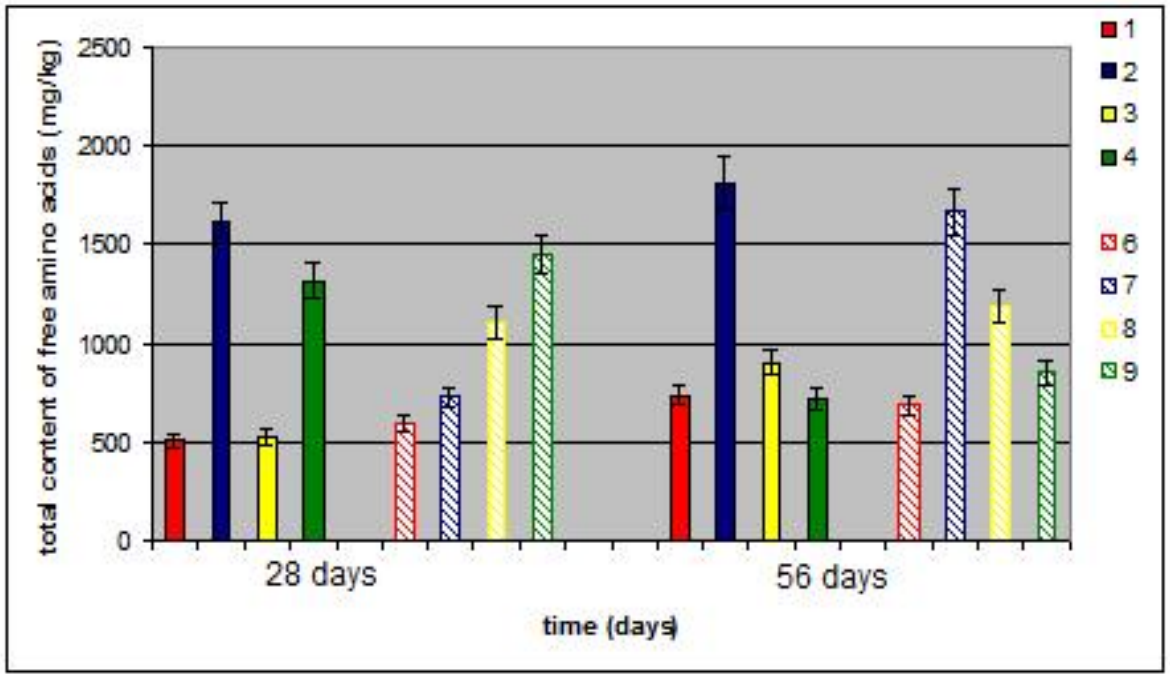

Fig. 4: Total contents of free amino acid of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with addition decarboxylase positive lactococci

Since free amino acid release is attributed to the action of microbial peptidases we could assume that aminopeptidases of the starter microorganism were probably responsible for the massive production of free amino acids during the first 28 days of ripening. Lactococcal peptidases are intracellular and their action indicates cell lysis (Valsamaki et al., 2000). In the present experiment and in the experiment that provided Valsamaki et al., 2000, the very high salt content and the low pH of the curd may create favorable conditions for cell lysis. Although probably the high rate of free amino acid production during the first 28 days resulted from the cell lysis of the starter microorganisms. Also according to Valsamaki et al., 2000, the role of non-starter lactic acid bacteria should not be ignored. In our experiment no microbiological analyses were performed, it was possible that secondary microbial contamination occurred. Tzanetakis and Litopoulou-Tzanetaki, 1992 referred that high counts of lactic acid bacteria and lactobacilli were present in the cheese curd, which increased significantly during two weeks of ripening. The presence of this native microflora is usually desirable because it contributes significantly to the development of characteristic flavor. It was also possible that non-starter lactic acid bacteria together with the starter microorganisms contributed to the formation of free amino acids. A general conclution was that the content of free amino acid of all manufactured cheese samples was considerably affected by the ripening time. The total amino acid contents increased with ripening time. Similar results were found by Pappa and Sotirakoglou, 2008.

7.6 Biogenic amine content

7.6.1 Biogenic amine contents of Greek commercial cheeses

The biogenic amine content in four commercial Greek cheeses was determined. Two of the Greek cheeses were Feta cheese and two were Teleme cheese. The determination of biogenic amines was applied at cheese samples that were unpacked, lyophilized and stored at -80 °C until the analysis day and at cheese samples that were unpacked, kept at 2-4 °C for a period of 14 days, then were lyophilized and stored at -80 °C until the day that analysis took place. The biogenic amines that were determined in all the commercial cheese samples of Feta and Teleme cheese were: putrescine, cadaverine, histamine tyramine, spermidine, spermine. One the most abundant biogenic amine in food histamine (Pachlová et al., 2012) was detected in the commercial cheese samples. Silla Santos (1996) and Val-

samaki et al, (2000) referred that after fish, cheese is the most commonly implicated food associated with histamine poisoning. The determined level of histamine in commercial freshly unpacked cheese samples was found to be 1.3-6.9 mg/kg and after storage at 2-4 °C for a period of 14 days increased to 1.6-16.2 mg/kg. This level is much lower than that which was found from Valsamaki et al. (2000). Noteworthy is that cadaverine, a biogenic amine which was identified as potentiator of toxic effects of other amines (Valsamaki et al., 2000, Buňková et al., 2010) was detected in two of the samples, *"Dodoni Feta"* and *"Choriatiko"* Teleme cheese. Phenylethylamine was not detected in none of the cheese samples except the *"Dodoni Feta"* cheese which was freshly unpacked and stored at 2-4°C for a period of 14 days.

The total biogenic amine contents of commercial Greek cheese samples are presented at Fig. 5. The total biogenic amine content of freshly unpacked *"Feta G.A.L.P.O. traditional"* was 119 mg/kg and *"Feta Dodoni"* 249.9 mg/kg. After 14 days of storage at 2-4°C the total biogenic amine content of *"Feta G.A.L.P.O. traditional"* showed an increase to 169.5 mg/kg and the total biogenic amine content of *"Feta Dodoni"* a significant increase to 562.4 mg/kg. The total content of biogenic amines of freshly unpacked *"Choriatiko"* Teleme cheese was found to be 88.2 mg/kg and of the freshly unpacked *"Livadi"* Teleme cheese was detected 63.8 mg/kg. After 14 days of storage at 2-4°C the total biogenic amine content of *"Choriatiko"* Teleme cheese presented an increase to 203.8 mg/kg and of the *"Livadi"* Teleme cheese an increase to 163.3 mg/kg. According to Spanjer and Roode (1991), the sum of tyramine, histamine, putrescine and cadaverine should not exceed of 900 mg/kg cheese. Silla Santos (1996) suggested that the biogenic amine content in food should not be more than 1000 mg/kg because is considered dangerous for health. Pursuant to this was the determination of total biogenic amine contents in all commercial Feta cheese and Teleme cheese because did not exceeded this certain limit.

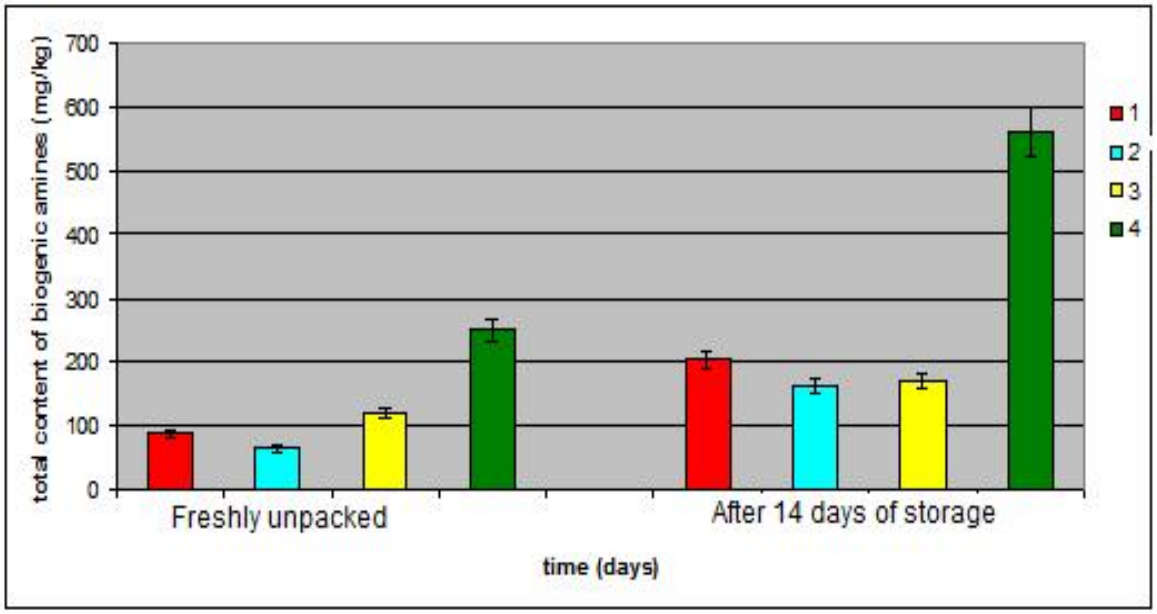

Fig. 5: Total contents of biogenic amine of Greek commercial cheese samples that were freshly unpacked and stored at 2-4 °C for 14 days. 1: "Choriatiko", 2: "Livadi", 3: "G.A.L.P.O. Feta", 4: "Dodoni Feta"

The major biogenic amines which were determined at freshly unpacked *"Feta G.A.L.P.O. traditional"* were putrescine (63.4 mg/kg), tyramine (24 mg/kg), spermine (16.3 mg/kg). At freshly unpacked *"Feta Dodoni"* were putrescine (136.8 mg/kg), tyramine (60.4 mg/kg) and spermidine (33 mg/kg). After 14 days of storage at 2-4 °C the major determined biogenic amines at *"Feta G.A.L.P.O. traditional"* were putrescine (115.6 mg/kg), tyramine (24.1 mg/kg) and spermidine (15.4 mg/kg). At *"Feta Dodoni"* were putrescine (246.1 mg/kg), tyramine (240.1 mg/kg) and spermidine (35 mg/kg). The major biogenic amines which were detected at freshly unpacked *"Choriatiko"* Teleme cheese were tyramine (39.6 mg/kg), putrescine (30.7 mg/kg), histamine (6.9 mg/kg) and spermidine (6.8 mg/kg). At freshly unpacked *"Livadi"* Teleme cheese were putrescine (34.2 mg/kg), tyramine (12.3 mg/kg) and spermine (7 mg/kg). After 14 days of storage at 2-4°C the major biogenic amine detected at *"Choriatiko"* Teleme cheese were putrescine (130.3 mg/kg), tyramine (45.5 mg/kg) and histamine (16.2 mg/kg). The major biogenic amines detected at *"Livadi"* Teleme cheese were putrescine (85.9 mg/kg), tyramine (57.1 mg/kg) and spermidine/spermine (7.4 mg/kg).

At all freshly unpacked commercial Feta cheese samples the main biogenic amine which was determined was putrescine and remained the main determined biogenic amine after 14 days of storage at 2-4 °C. Another biogenic amine which was determined in all commercial cheese samples at high levels was tyramine. The tyramine contents had shown an upward trend with the passage of 14 days of storage. Noteworthy is the significant growth of tyramine at *"Dodoni Feta"*, which is almost triple.

Putrscine generally was the major biogenic amine which was found almost in all commercial Feta and Teleme cheese samples. In our experiment the putrescine levels were found to be quite high. Similar putrescine levels were found by Valsamaki et al. (2000) when mixtures of lactobacilli were used. Tyramine after putrescine was the biogenic amine which was determined in greater concentration in all commercial Teleme and Feta cheese samples. But after 14 days of storage at Feta cheese samples tyramine became the main biogenic amine. Spermidine, spermine, cadaverine and histamine were found at very low levels. Gennerally the contents of spermidine, spermine, cadaverine and histamine have an increasing trend with the passage of the 14 days of storage, but sometimes they remained almost constant or had shown a slight reduction.

7.6.2 Biogenic amine contents of manufactured cheese series

The determination of biogenic amines was applied at cheese manufactured series (I-IV) samples that were lyophilized and stored at -80 °C until the analysis day. The biogenic amines that were determined in all cheese samples were: putrescine, tyramine, spermidine, spermine. Histamine, one of the most commonly implicated biogenic amine with food poisoning (Silla Santos, 1996, Valsamaki et al., 2000, Pachlová et al., 2012), was not determined in none of the cheese samples. Phenylethylamine, tryptamine, cadaverine were not detected at the cheese manufactured series without the addition of decarboxylase positive lactococci at 28 days of ripening in brine. At the cheese manufactured series in which had been added with decarboxylase positive lactococci and had been ripening for 28 days was determined a small amount of phenylethylamine (4.6 mg/kg) only at series III. Otherwise cadaverine and tryptamine were not detected too. The analysis that was performed at the cheese manufactured series without the addition of decarboxylase positive lacococci and had been ripen for 56 days showed that no cadaverine and histamine were found. But a small amount of phenylethylamine (2.5 mg/kg) was determined only at series III. Otherwise

none of the rest manufactured series contained phenylethylamine. The analysis of the cheese manufactured series with the addition of decarboxylase positive lactococci and had been ripening for 56 days showed that none of the samples contained phenylethylamine, cadaverine and histamine. Tryptamine was determined in all manufactured cheese series, although tryptamine was not found in manufactured cheese series that had been ripening for 28 days. The absence of cadaverine in all cheese manufactured series may be explained either by a lack of strains with lysine-decarboxylating activity or most probably by the inhibition of this activity by the unfavourable environment of white brined cheeses (Valsamaki et al., 2000).

At the 28th day of ripening the determination of biogenic amine contents of the cheese manufactured series samples without the addition of decarboxylase positive lacococci showed that the major biogenic amines were putrescine (14.5-83.7 mg/kg), tyramine (2.2-11.2 mg/kg), spermidine (1.5-6.8 mg/kg) and spermine (1.0-8.7 mg/kg). The cheese manufacture series samples with the addition of decarboxylase positive lacococci had as major biogenic amines putrescine (23.4-69.7 mg/kg), tyramine (24.6-48 mg/kg), spermidine (2.2-4.4 mg/kg) and spermidine (4.2-5.6 mg/kg).

At the 56th day of ripening the determination of biogenic amine contents of the cheese manufactured series samples without the addition of decarboxylase positive lacococci showed that the major biogenic amines were putrescine (53.3-130.8 mg/kg), tyramine (2.5-88.1 mg/kg), spermidine (3.0-5.0 mg/kg) and spermine (6.1-16.3 mg/kg). The cheese manufacture series samples with the addition of decarboxylase positive lacococci had as major biogenic amines putrescine (46.3-125.6 mg/kg), tyramine (24.9-114.6mg/kg), spermidine (2.5-6.7mg/kg) and spermidine (6.7-8.9 mg/kg).

Putrescine as is shown at Fig. 6, generally was the major biogenic amine which was found almost in all cheese manufactured series samples. Putrescine has less pharmacological activity that the aromatic amines but probably is a potentiator of their toxicity (Joosten, 1988). Also putrescine may be indicator of extended protein degradation and spoilage. *Enterobacteriaceae* are possibly responsible for putrescine build up (Valsamaki et al., 2000). In our experiment the putrescine levels were found to be quite high. Similar putrescine levels were found by Valsamaki et al., 2000 when mixtures of lactobacilli were used. This was confiremed in our experiment because as decarboxylase positive lactococci were used strains of *Lactococcus lactis* subsp. *lactis*.

Tyramine as is presented at Fig. 7 was the second biogenic amine which was determined in high levels after putrescine in all manufacture cheese series samples. Joosten and Northolt (1987) reported that some strains of *Lactobacillus brevis* can produce high amounts of tyramine. But the presence of *Lactobacillus brevis* in the cheese manufactured samples which were analyzed cannot be confirmed. *Lactobacillus delbrueckii* subsb. *bulgaricus* was found to have in vitro decarboxylating activity on tyrosine (Silla Santos, 1996, Valsamaki et al., 2000). The presence of some strains of this microorganism in the examined manufactured cheese samples is certain, since is known that yogurt culture was used as starter culture.

At Fig. 6, 7, 8, 9 and 10 are presented the changes in the amounts of biogenic amines during ripening. Also in generally can be said that the contents of all the determined biogenic amines presented a significant increase from 28 days to 56 days of ripening under constant temperature and moisture conditions. Also can be noticed the absence of tryptamine at the 28th day of ripening.

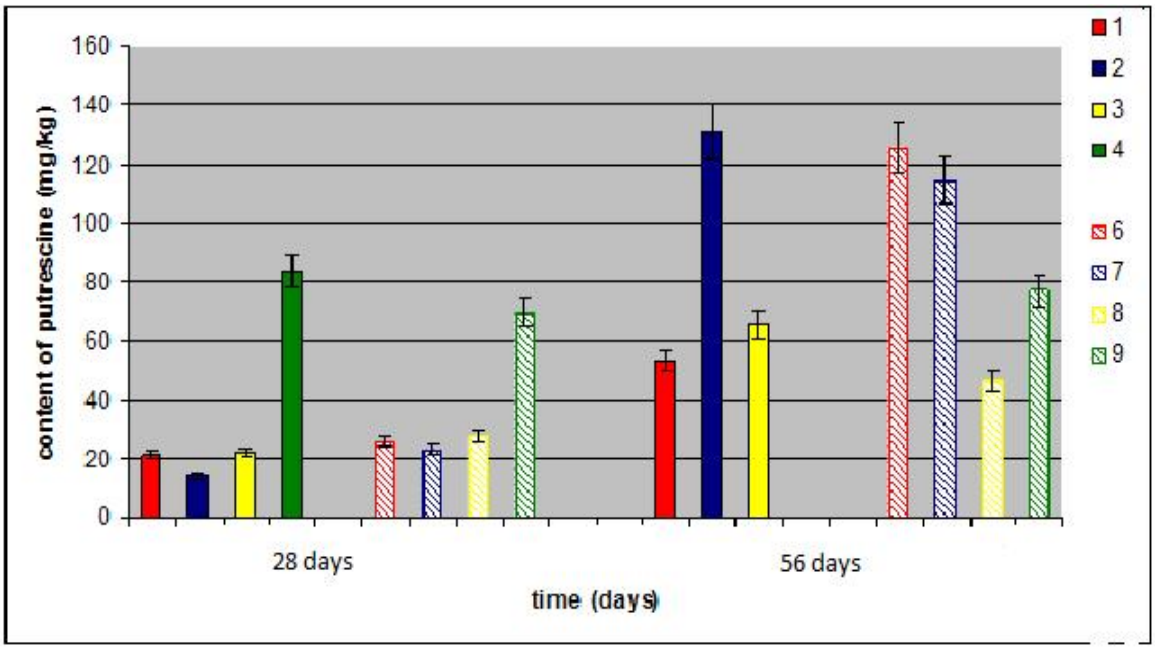

Fig. 6: Contents of putrescine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

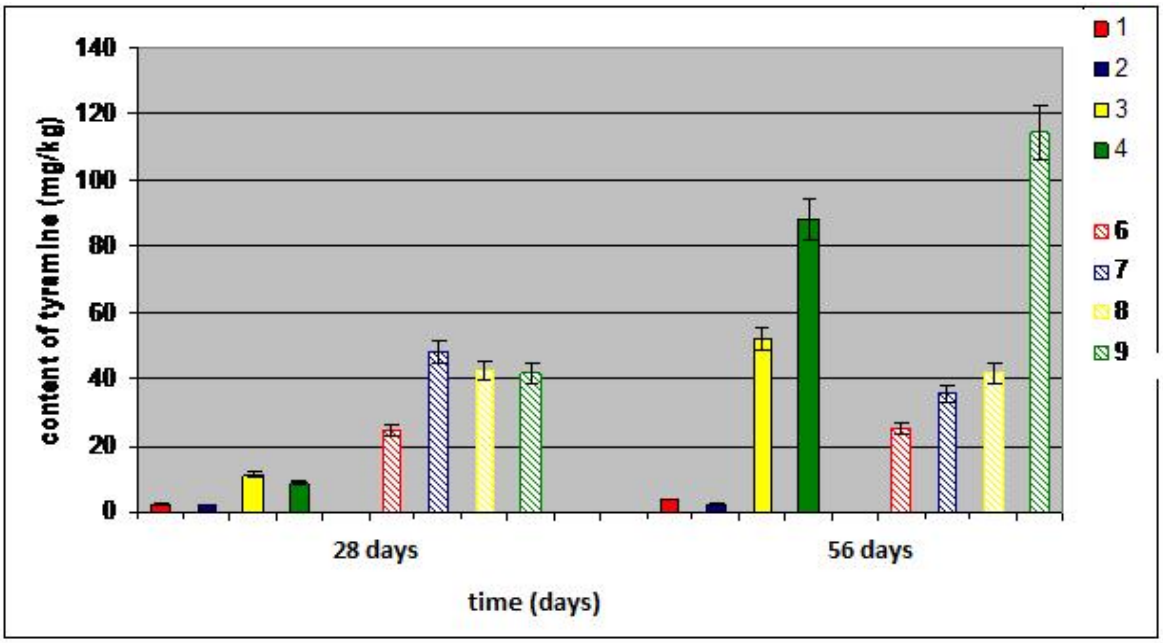

Fig. 7: Contents of tyramine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

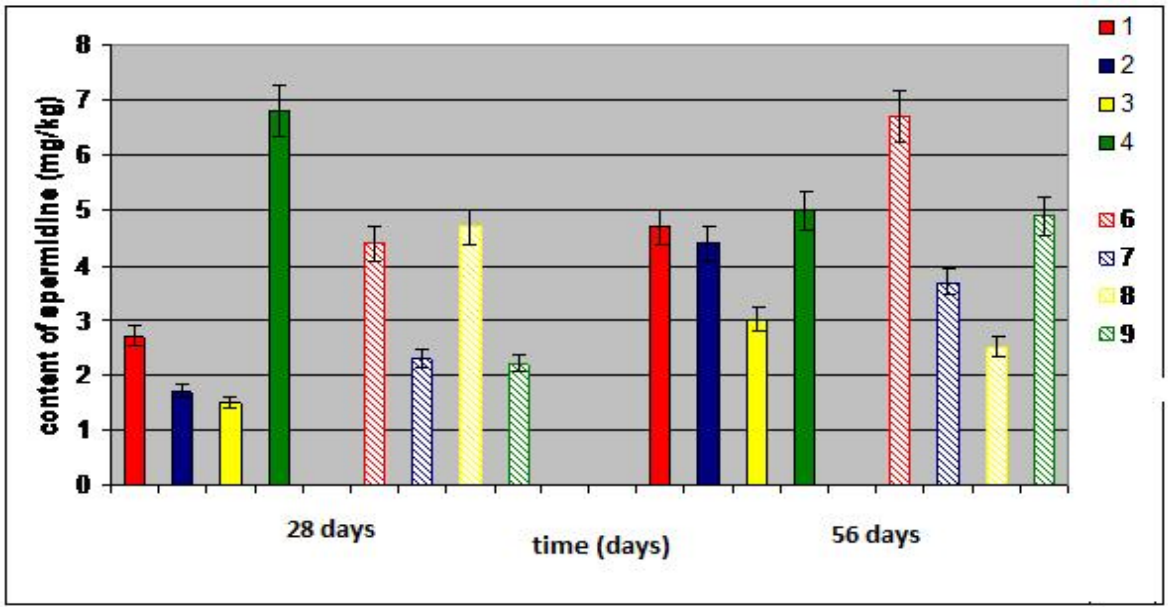

Fig. 8: Contents of spermidine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

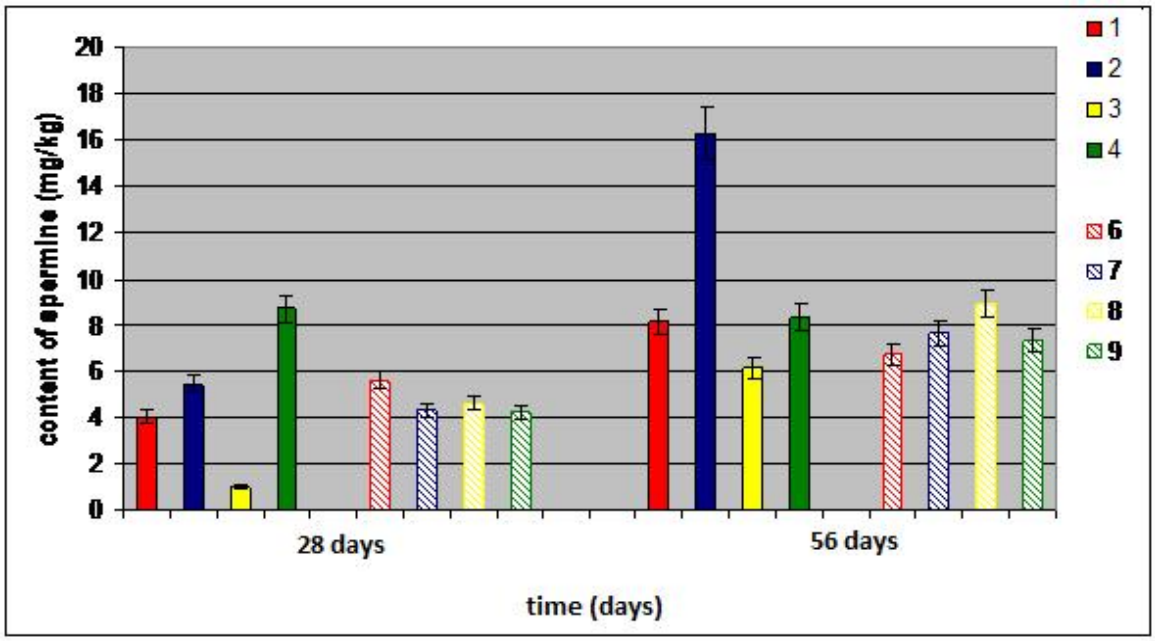

Fig. 9: Contents spermine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

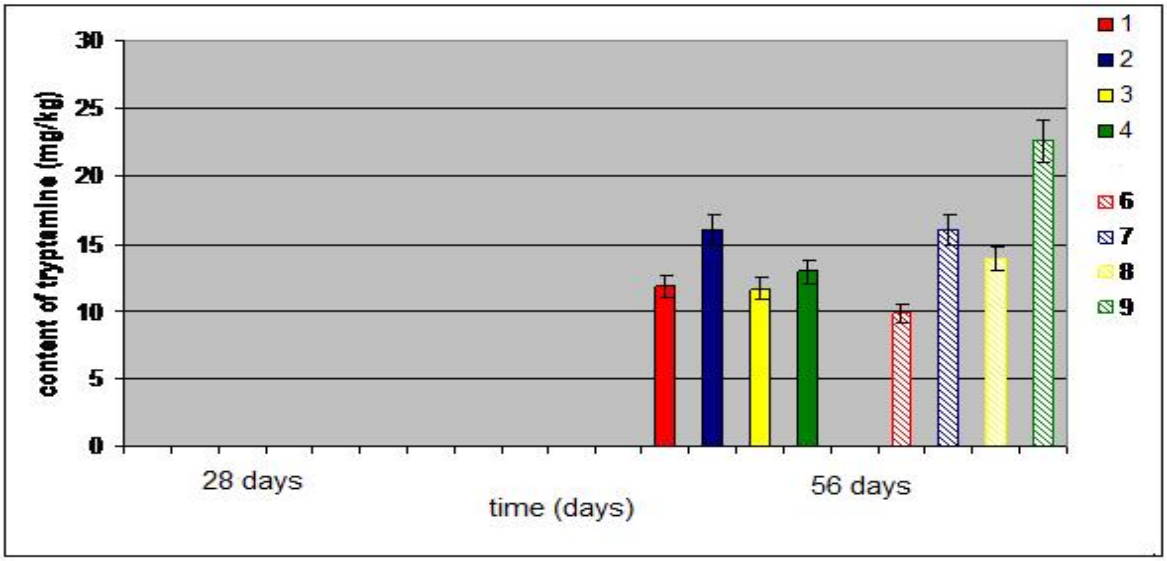

Fig. 10: Contents tryptamine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

The evolution of the total biogenic amine contents during ripening is presented in Fig. 11. Can be observed the changes in the total amount of biogenic amines of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci, which had been ripening for 28 and 56 days under constant temperature. The result was that the level of the determined biogenic amines significantly increased from 28 to 56 days of ripening. At cheese manufactured series without the addition of decarboxylase positive lactococci the level of biogenic amines almost doubled or tripled. At cheese manufactured series with the addition of decarboxylase positive lactococci the level of biogenic amines almost doubled, except series III where the level did not increase significantly. At the 28th day of ripening the total amount of biogenic amines at cheese manufactured series with the addition of decarboxylase lactococci was significantly higher in comparison with the cheese manufactured series without the addition of decarboxylase positive lactococci.

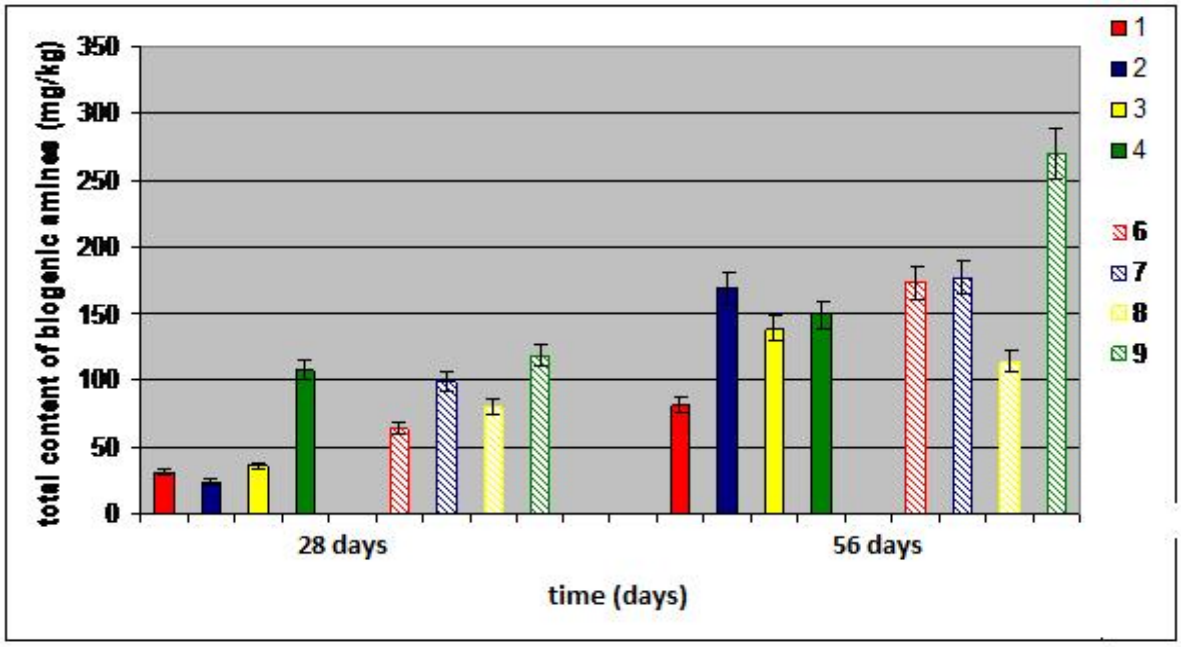

Fig. 11: Total contents of biogenic amine of cheese manufactured series (I-IV) with and without the addition of decarboxylase positive lactococci at 28 and 56 days of ripening. 1, 2, 3, 4: cheese samples without decarboxylase positive lactococci and 6, 7, 8, 9: cheese samples with decarboxylase positive lactococci

In conclusion could be said that the total biogenic amine content was low. Probably the characteristic features of the manufactured cheeses (low pH, very high salt content, ripening and storage in brine, not extended proteolysis) did not create an environment favorable

for biogenic amine accumulation (Valsamaki et al., 2000). The total levels of biogenic amines did not reach a level similar to that which is reported suspect for food poisoning.

7.7 Texture profile analysis

7.7.1 Texture profile analysis of Greek commercial cheeses

The texture profile analysis of four commercial Greek cheeses was performed. Two of the Greek cheeses were Feta cheese and two were Teleme cheese. The results of the analysis were expressed by the terms of hardness, cohesiveness and adhesiveness. In Fig. 12, 13, 14 are illustrated the changes of hardness, cohesiveness and adhesiveness between the commercial Greek cheese samples. All the commercial cheese samples had shown the same decreasing trend.

Fig. 12: Changes of hardness between Greek commercial cheese samples which were freshly unpacked and stored at 2-4 °C for 14 days. 1: "Choriatiko", 2: "Livadi", 3: "G.A.L.P.O. Feta", 4: "Dodoni Feta"

The analysis also showed that the commercial Teleme cheeses were harder than the commercial Feta cheese. The harder of the Teleme cheeses was *"Livadi"*. Between Feta cheeses there were no significant changes of hardness, although *"G.A.L.P.O. Feta"* was slightly

harder. The differences of hardness between Teleme and Feta cheeses may be due to different rates of proteolysis and manufacture techniques (Raphaelides et al., 1996). The observed decrease of the hardness (or softening) may be explained by two processes: (1) α_{s1}-casein breakdown by rennet and (2) a rise in pH caused probably by secondary contaminating microflora (Antoniou et al., 2000). The second reason also might have caused the decrease of the adhesiveness and cohesiveness of the cheese samples as is shown at Fig. 13 and 14.

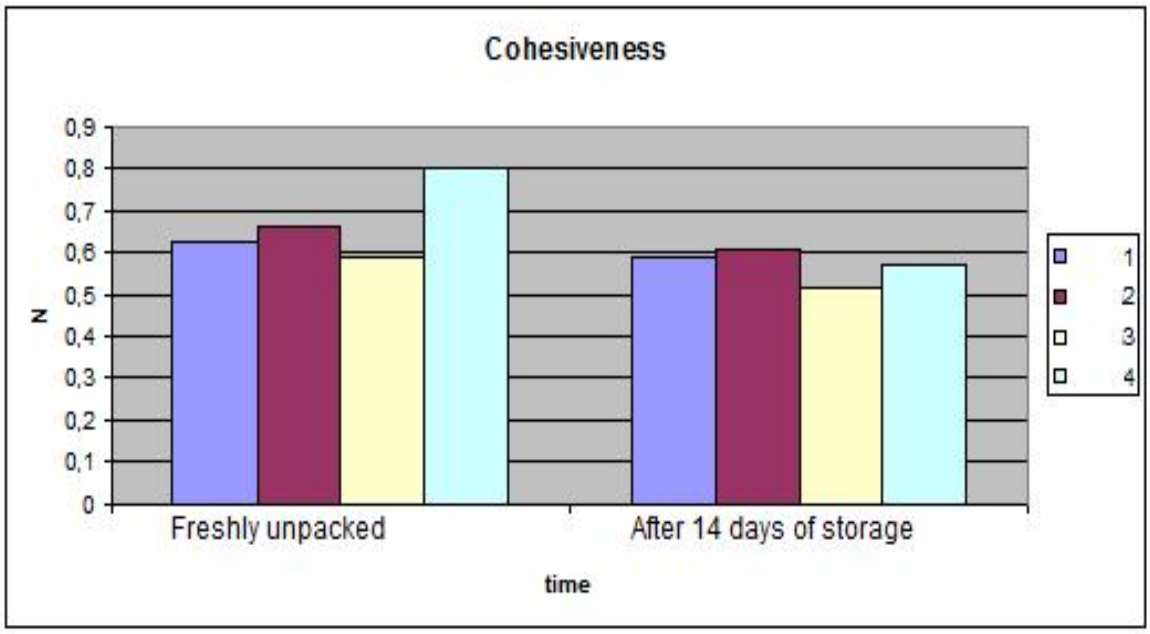

Fig. 13: Changes of cohesiveness between Greek commercial cheese samples which were freshly unpacked and stored at 2-4 °C for 14 days. 1: "Choriatiko", 2: "Livadi", 3: "G.A.L.P.O. Feta", 4: "Dodoni Feta"

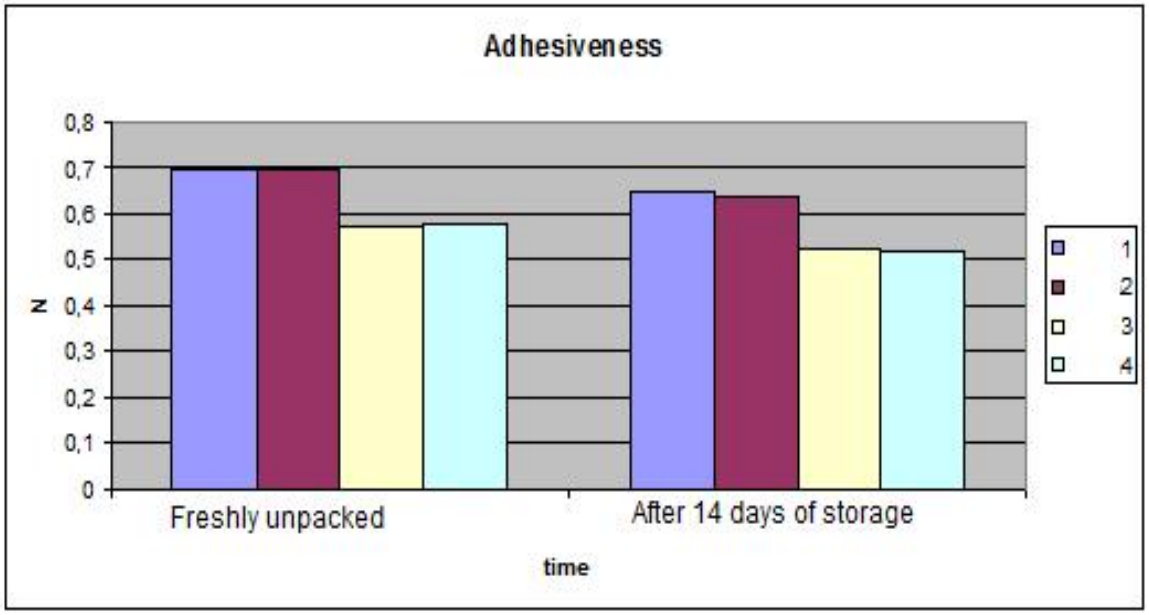

Fig. 14: Changes of adhesiveness between Greek commercial cheese samples which were freshly unpacked and stored at 2-4 °C for 14 days. 1: "Choriatiko", 2: "Livadi", 3: "G.A.L.P.O. Feta", 4: "Dodoni Feta"

7.7.2 Texture profile analysis of manufactured cheese series

The texture profile analysis was applied at cheese manufactured series (I-IV) samples with and without the addition of decarboxulase positive lactococci, immediately after curd draining, after 28 days of ripening and 56 days of ripening. The results of the analysis were expressed by the terms of hardness, cohesiveness and adhesiveness. The changes of hardness of the cheese manufactured series with and without the addition of decarboxylase positive lactococci during the experimental period of 56 days is shown at Fig. 15 and 16.

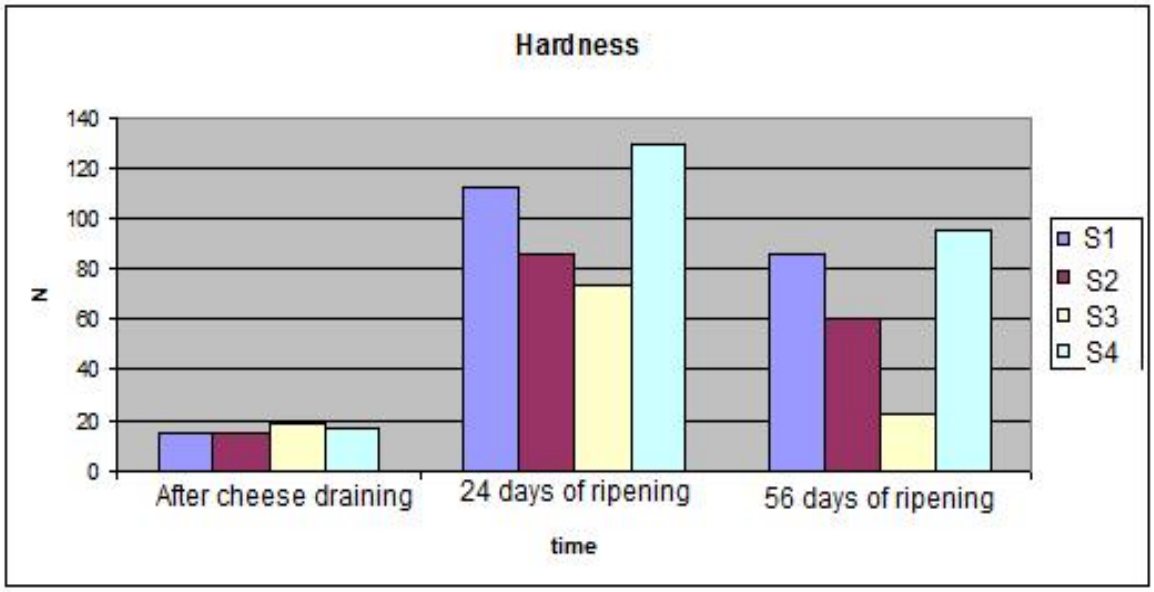

Fig. 15: Changes of hardness of cheese manufactured series (S1, S2, S3, S4) without the addition of decarboxylase positive lactococci after curd draining , after 28 days of ripening and 56 days of ripening

All the manufactured series (I-IV) samples with and without the addition of decarboxulase positive lactococci, have shown the same trend. From 1 days of ripening to 28 days of ripening was observed an increase of the hardness, but from 28 days of ripening was observed a decrease of the hardness. The manufactured cheese samples of all series after curdling had a soft texture because of the high moisture content (Raphaelides et al., 1996). During the first 28 days of ripening all the manufactured cheese series became much more firm. This was due to the moisture loss which made the structure more compact. After 56 days of ripening the texture of all manufactured series cheese samples became progressively softer due to proteolysis (Raphaelides et al., 1996). According to Antoniou et al., 2000, the decrease of the hardness (or softening) may be explained by two main processes: (1) α_{s1}-casein breakdown by rennet and (2) a rise in pH caused probably by secondary contaminating microflora. Comparing the Fig. 15 and Fig. 16, the changes of hardness are a little bit more intensive at the cheese manufactured series without the addition of decarboxylase positive lactococci. This may be due to different rates of proteolysis.

Fig. 16: Changes of hardness of cheese manufactured series (S1Lc, S2Lc, S3Lc, S4Lc) with the addition of decarboxylase positive lactococci after curd draining , after 28 days of ripening and 56 days of ripening

The changes of cohesiveness of the cheese manufactured series with and without the addition of decarboxylase positive lactococci are presented at Fig. 17 and Fig. 18.

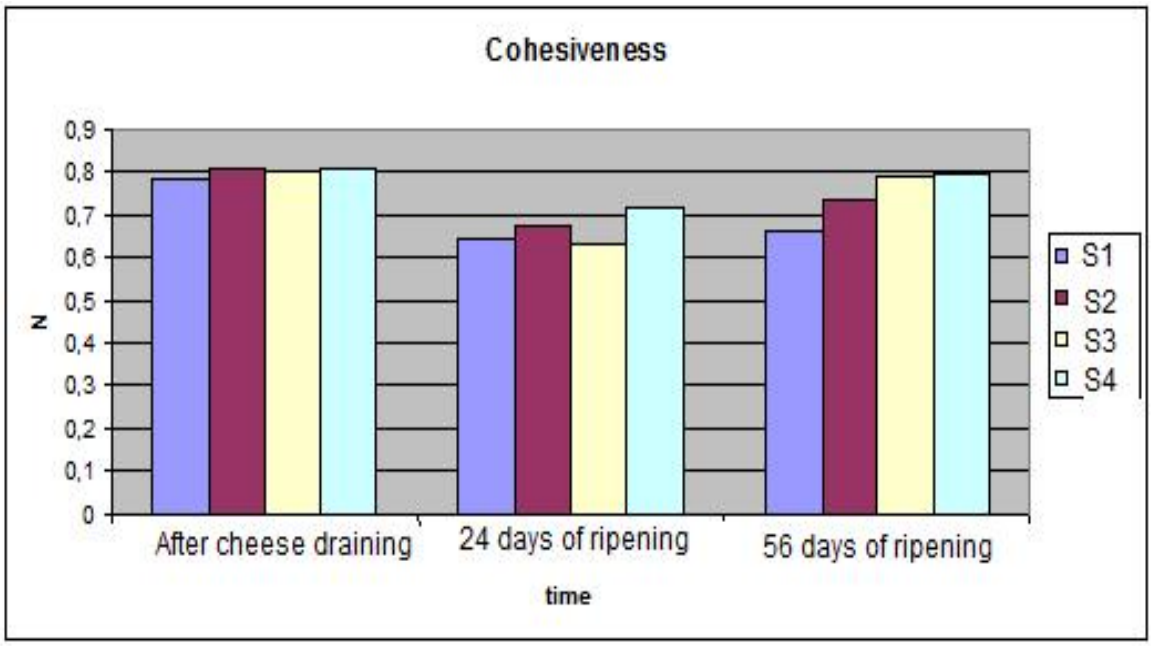

Fig. 17: Changes of cohesiveness of cheese manufactured series (S1, S2, S3, S4) with the addition of decarboxylase positive lactococci after curd draining , after 28 days of ripening and 56 days of ripening

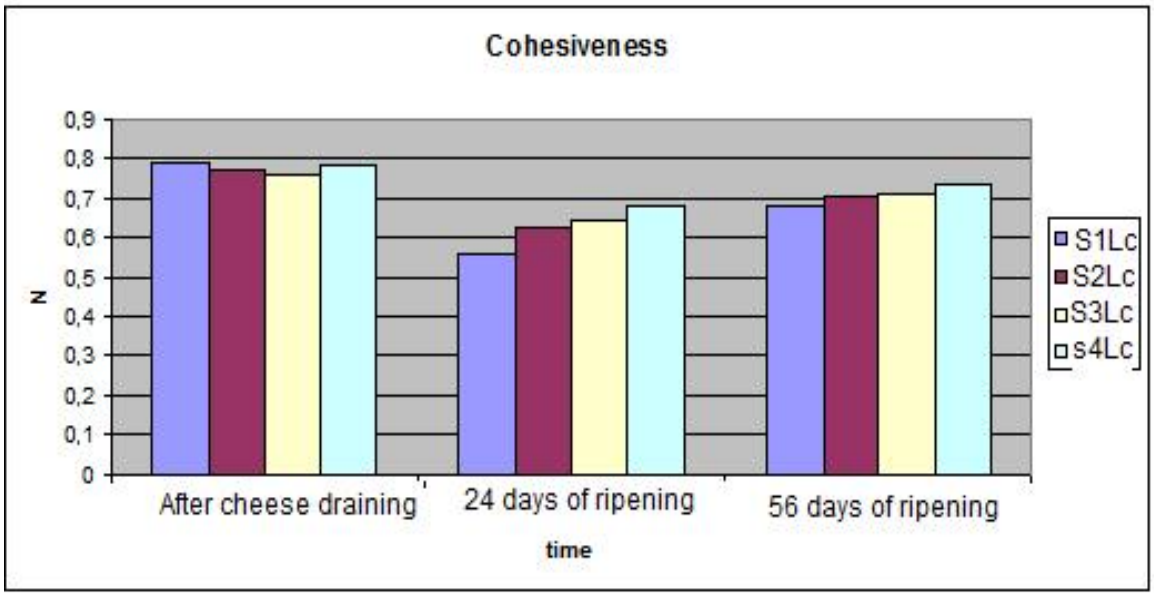

Fig. 18: Changes of cohesiveness of cheese manufactured series (S1Lc, S2Lc, S3Lc, S4Lc) with the addition of decarboxylase positive lactococci after curd draining, after 28 days of ripening and 56 days of ripening

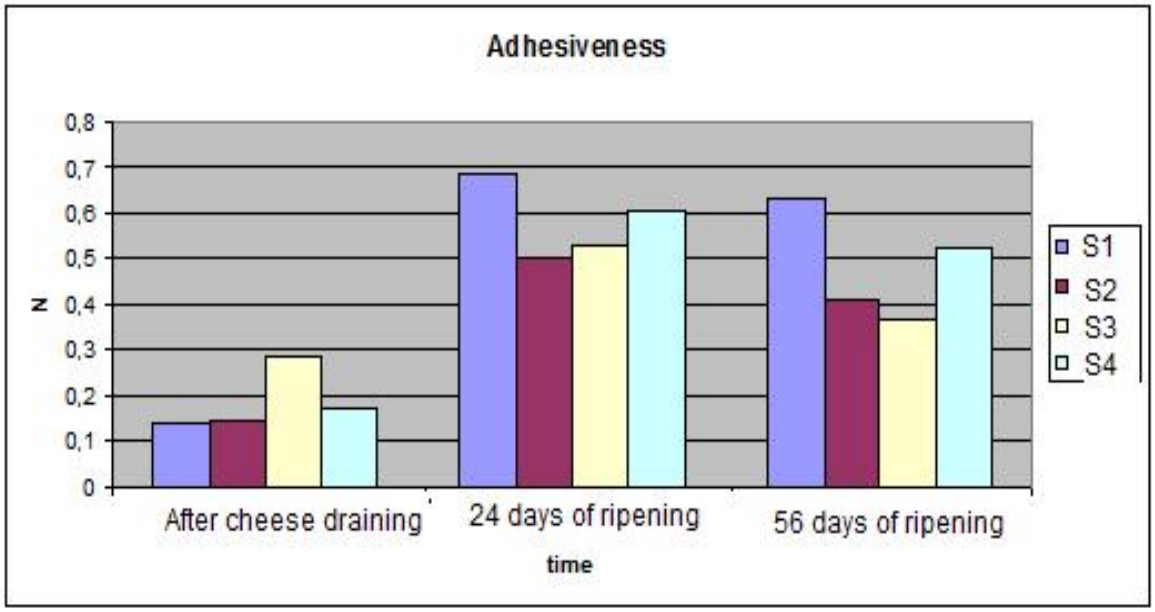

Fig. 19: Changes of adhesiveness of cheese manufactured series (S1, S2, S3, S4) with the addition of decarboxylase positive lactococci after curd draining, after 28 days of ripening and 56 days of ripening

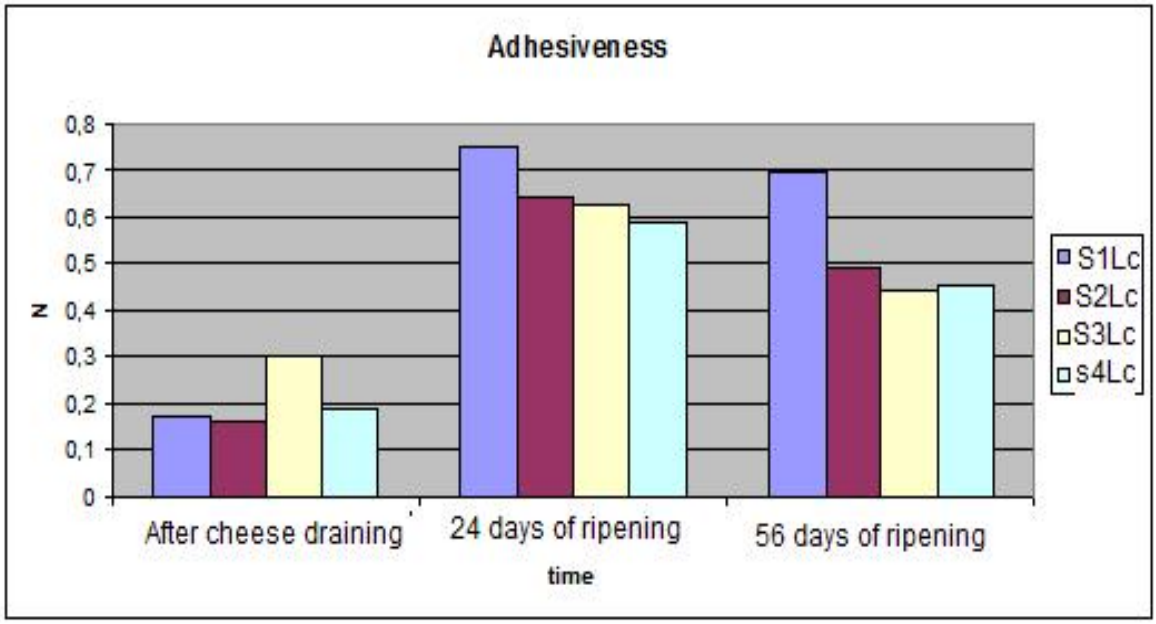

Fig. 20: Changes of adhesiveness of cheese manufactured series (S1Lc, S2Lc, S3Lc, S4Lc) with the addition of decarboxylase positive lactococci after curd draining, after 28 days of ripening and 56 days of ripening

In conclusion can be said that at all cheese manufactured series with and without the addition of decarboxylase positive lactococci had shown the same trend at the changes in hardness, cohesiveness and adhesiveness (Fig. 15, 16, 17, 19, 19, 20). From the end of curdling to the first 28 days of ripening was observed an increase of all the examined parameters. From 28 days to 56 days was observed a decrease. This phenomenon was probably occurred by different rates of protreolysis or by microbial secondary contamination or by their combination.

CONCLUSION

The optimized cheese manufacture technique was characterized as successful because the manufactured cheeses had similar properties (dry matter, pH, NaCl) to those that are manufactured in the area of Greece. The results of the analysis of dry matter content and pH value were pursuant to Greek food legislation. The NaCl contents were quite high. All the biogenic amines reported to be formed in cheese were found in the analyzed cheese samples. The periods that the determinations of biogenic amines were done were 28 days and 56 days of ripening at the manufactured cheese samples. The determination of biogenic amine contents in the commercial Greek cheeses was applied to freshly unpacked cheeses and at cheeses that were kept at 2-4°C for 14 days. The biogenic amine production may be explained by the abundance of the substrate (free amino acids) possibly formed from non-starter lactic acid bacteria together with the starter microorganisms. Probably the characteristic features of all the examined cheese samples (low pH, very high salt content, ripening and storage in brine, not extended proteolysis) did not create an environment favorable for biogenic amine accumulation. This probably could explain that the total levels of biogenic amines did not reach a level similar to that which is reported suspect for food poisoning. However secondary microbial contamination is avoidable by strict use of hygiene in both raw material and manufacturing environment.

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RESOURCES OF GREEK PDO CHEESES PICTURES

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LIST OF ABBREVIATIONS

LDL	Lactate dehydrogenase.
LPL	Lipoprotein lipase.
BA	Biogenic amine.
M 17	Terzaghi's agar for the determination of lactic acid bacteria
ATP	Adenosine triphosphate
ISO	International organization for standardization
EL.STAT.	Hellenic statistical authority
Gly	Glycine
Ser	Serine
Thr	Threonine
Ala	Alanine
Pro	Proline
His	Histidine
Glu	Glutamine
Asp	Asparagine
Arg	Arginine
Met	Methionine
Val	Valine
Leu	Leucine
Phe	Phenylethylamine
Tyr	Tyrosine
Trp	Tryptophane

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APPENDIX P I: PICTURES OF GREEK PDO CHEESES

Fig. 1: Feta cheese [1].

Fig. 2: Teleme cheese [2].

Fig. 3 and 4: Kalathaki Limnou cheese [3].

Fig. 5: Sfela cheese [4].

Fig. 6: Batzos cheese [5].

Fig. 7: Kefalotyri cheese [6].

Fig. 8: Kefalograviera cheese [7].

Fig. 9: Ladotyri Mytilinis cheese [8].

Fig. 10: Formaella Parnassus cheese [4].

Fig. 11: Graviera Kritis cheese [9].

Fig. 12: Graviera Agrafon cheese [10].

Fig. 13: Kasseri cheese [6].

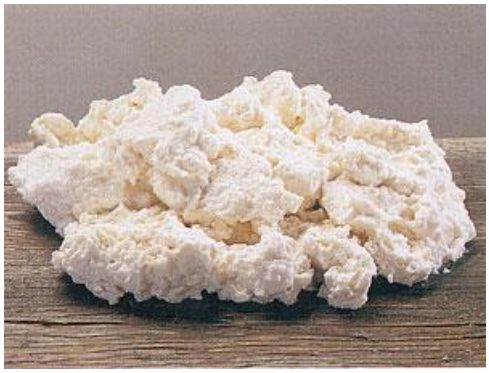

Fig. 14: Anevato cheese [4].

Fig. 15: Galotyri cheese [7].

Fig. 16: Katiki Domokou cheese [11].

Fig 17: Pichtogalo Chanion cheese [11].

Fig. 18: Xinotyri cheese [7].

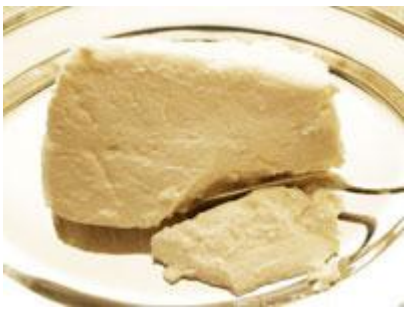

Fig. 19: Touloumotyri cheese [12].

Fig. 20: Myzithra cheese [9].

Fig. 21: Manouri cheese [10].

Fig. 22: Ksinomyzithra Kritis cheese [13].