GRADUATE SCHOOL
NANOTECHNOLOGY AND ENGINEERING SCIENCES
DEPARTMENT


TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY
UNIVERSITY

# PRESENCE AND DIETARY EXPOSURE ASSESSMENT OF NATAMYCIN FROM CHEESE

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**M.Sc.**

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**THESIS ADVISOR**
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**ADANA, 2023**

# DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

16/11/2023

Hatice Pınar TURAN

# ÖZET

## PEYNİRDE NATAMİSİN VARLIĞI VE DİYETLE MARUZİYETİNİN BELİRLENMESİ

Hatice Pınar TURAN
Yüksek Lisans, Nanoteknoloji ve Mühendislik Bilimleri Anabilim Dalı
Danışman: Doç. Dr. Hatice İmge OKTAY BAŞEĞMEZ
Kasım 2023, 36 sayfa

Natamisin, *Streptomyces natelensis* suşları tarafından üretilen doğal bir antimikrobiyal peptittir. Antimikrobiyal ajanlar, patojenik ve bozulmaya neden olan mikroorganizmaların büyümesini azaltmak ve önlemek için yaygın olarak kullanılmaktadır ve bunun sonucunda süt gıdalarının raf ömrü uzatılmaktadır. Natamisin peynir yüzeyine uygulanarak peynir muhafaza alanında yaygın olarak uygulanmaktadır. Natamisin genellikle küfü kontrol etmek, maya büyümesini engellemek için kullanılır. Türk Gıda Kodeksi'nde gıda katkı maddesi olarak onaylanmış ve Gıda ve İlaç İdaresi tarafından güvenli ürün (GRAS) olarak kabul edilmiştir. Natamisin, Türk Gıda Kodeksi Gıda Katkı Maddeleri Yönetmeliğine (2023) göre sert, yarı sert ve yarı yumuşak peynirlerin yüzey işlemlerinde 1mg/dm$^2$'lik yüzeyde ve 5 mm'lik derinlikte bulunmamalıdır. Toksikolojik açıdan bakıldığında, natamisin tüketimine bağlı olarak hayvanlarda gözlenen etkiler; besin alımında azalma, canlı ağırlık artışında azalma, gastrointestinal tahriş ve ishaldir. Müşterek ( FAO/WHO ) Gıda Katkı Maddeleri Uzman Komitesi (JECFA) toksikolojik çalışmalara dayanarak natamisin için izin verilen günlük alım miktarını 0.03 mg/kg va/gün olarak belirlemiştir. Bu tezde Adana ilinde toplumun kolaylıkla erişebildiği açık alanlarda satılan farklı peynir örneklerinde natamisin varlığı ve bunların tüketimi ile natamisin maruziyetinin hesaplanması amaçlanmıştır. Araştırma sonucunda peynir örneklerinin % 42.5'unun Türk Gıda Kodeksine uygun olmadığı belirlenmiştir. Ayrıca 0.001 mg/kg va/gün olarak belirlenen tahmini günlük alım miktarının, JECFA tarafından belirlenen izin verilen alım miktarının çok altında olduğu bu nedenle de peynir tüketimi ile yetişkinlerde sağlık riski oluşturmadığı sonucuna varılmıştır.

**Anahtar Kelimeler:** Natamisin, primarisin, gıda katkı maddesi, risk değerlendirme, süt ürünleri

# ABSTRACT

## PRESENCE AND DIETARY EXPOSURE ASSESSMENT OF NATAMYCIN FROM CHEESE

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Natamycin is an antifungal effect and food additive produced by strains of *Streptomyces natelensis* that prevent the growth of unwanted molds and yeast when applied to cheeses. In this case of cheese preservation, natamycin is often applied to the surface of cheese. It has been sanctioned as a food additive by the Turkish Food Codex and is accepted as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration. According to the Turkish Food Codex the regulation on food additives (2023), natamycin can be used for the surface treatment of hard, semi-hard and semi-soft cheeses with a maximum concentration of 1 mg/dm$^2$ and 5 mm outside the surface. From a toxicological perspective, the effects observed in animals following natamycin ingestion are reduced food intake, reduced rates of weight gain, gastrointestinal irritation, and diarrhea. Based on toxicological studies JECFA assigned an ADI value for natamycin as 0.3 mg/kg body weight/day In this thesis it was aimed to evaluate NA presence in cheese samples collected from Adana city in Türkiye. Moreover, dietary exposure analysis was provided using the surveillance data. As a result of the study 42.5% of the cheese samples did not comply with the Turkish Food Codex. Also EDI value was determined as 0.001 mg/kg bw/day which indicates no health risk for natamycin due to the cheese consumption for adults.

**Keywords:** Natamycin, primaricin, food additives, risk assessment, dairy products

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From the moment I started this work, I would like to extend my thanks and respect to my advisor, Assoc. Prof. Dr. Hatice İmge OKTAY BAŞEĞMEZ, who has never forsaken your help and support at every stage and who has answered all my questions with sincerity and patience.

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At last but not least, I sincerely thank Ece KAYA for helping me throughout laboratory practices.

I also wish God's mercy to all my friends and all the people I know who lost their lives in the Kahramanmaraş earthquake, who lived with my family in the process of writing my thesis.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADI | : Acceptable Daily Intake |
| CV | : Coefficient of variation |
| CNIEL | : French Dairry Interbranch Organization |
| min | : minutes |
| EC | : European Comission |
| EFSA | : European Food Safety Authority |
| FAO | : Food and Agriculture Organization |
| F | : Dilution factor |
| FCC | : Food Chemical Codex |
| FDA | : Food and Drug Administration |
| GC | : Gas Chromatography |
| GKGM | : General Directorate of Food and Control |
| GMP | : Good Manufacturing Practices |
| GRAS | :Generally Recognized as Safe |
| HPLC | : High Performance Liquid Chromatography |
| HACCP | : Hazard Analysis and Critical Control P |
| ISTD | : Internal Standard |
| IDF | :International Dairy Federation |
| LOD | : Limit of Detection |
| LOQ | : Limit of Quantification |
| ICH | : International Conference on Harmonization |
| ISO | : International Standards Organization |
| MIC | : Minimal Inhibitory Concentration |
| MFC | : Minimal Fungicidal Concentration |
| MS | : Mass Detector |
| NA | :Natamycin |
| SD | : Standard Deviation |
| TGK | : Turkish Food Codex |
| WHO | : World Heath Organization |

# LIST OF SYMBOLS

$^{o}C$ **:** Centrigate Degree

# 1. INTRODUCTION

Food preservation involves handling and treating food to prevent spoilage caused by microorganisms. Preserving the nutritional value, the texture and the flavor important for maintaining the value of food. Preservation is often done to safe to food health and the growth of mold, fungi, and another yeast and bacteria, delay fat oxidation which causes rancidity. Some preservation methods required by food, for example, High temperature processing, low and dry temperatures help preserve foods for longer periods of time. Another way of food preservation is treatments with chemicals. The word antibiotic is derived from the word "antibiotic," which literally means "against life." Antibiotics are sources of microbial substances, while "antimicrobial" refers to any substance, including synthetic compounds, capable of destroying microorganisms. Antimicrobial agents include antibacterial, antifungal, and antiviral agents. In addition to treating bacterial infections, antibiotics are used in animal, poultry and fish feed and in food preservation (De Briyne et al., 2014).

Dairy products, in spite of their high acidity and organic acid content, which could create an environmental that is conducive to the growth of fungus with other spoilage bacteria. Dairy product fungal deterioration is a major cause of food sustainability and financial situation(Pitt and Hocking, 2009). The existence of colonies or main on the product's surface makes it typically easier to recognize. However, fungal decomposition can also occur covertly, changing the product's flavor or texture through fungal metabolic process (Ledenbach and Marshall, 2010). This makes the product unfit for consumption. The dairy sector today uses preventative and control strategies to improve product shelf life and prevent fungal spoiling. Good manufacturing and hygiene practices(GMP), the application of the Hazard Analysis Critical Control Point (HACCP) system, and the use of air filtration or aseptic packaging are examples of preventive techniques. (Garnier et al., 2017). Food Control mechanism may include the using of heat treatment, modified atmosphere packaging, or fermentation with beneficial microorganisms (Garnier et al., 2017;; Nguyen Van Long et al., 2016). Among dairy products, cheese is very susceptible to physical, chemical and microbiological spoilage. The major reason of spoilage of cheese due to the fungi are belong to the genera *Penicillium*, *Aspergillus* and *Fusarium* (de Campos et al., 2022).

Among these chemical preservatives, the natamycin (NA) is a natural fungal effect generally used in the food industry, especially dairy products due to its wide spectrum as an antifungal agent (Kallinteri et al., 2013). NA is accepted as a food additive in more dozen countries, is classified as a GRAS compound (generally recognized as safe) by the FDA (Food and Drug Administration), and can be used as a food additive under EFSA (Resa, Jagus, & Gerschenson, 2014; European Food Security Service, 2009). It is accepted as a food additive under the code of NA

It is mainly used for surface treatment of cheese and sausages. Colorless, odorless and tasteless, with broad anti-fungal and yeast effects but not bacteria, viruses and protozoa (Resa et al., 2014). NA can bind to ergosterol in fungal cell membranes, triggering cell death. Furthermore, NA can also form micelles at relatively low solution concentrations, but they are effectively against fungal cells (Welscher & Ten Napel et al ,2008).

Natamycin is an antimicrobial produced by the aerobic fermentation of *Streptomyces* species such as *S. chattanoogensis*, *S. gilvosporeus* , *S. lydicus*, *S. natalensis (*Kallinteri et al., 2013).

According to Directive 95/2/EC and Turkish Food Codex (2023), NA is used for the surface treatment of semi-hard and semi-soft cheeses at a maximum dosage of 1 mg/dm$^2$ for the outer 5 mm of the surface, equivalent to 20 mg/kg.

In toxicological works, the following effects were observed in animals: decreased food intake, weight gain, and gastrointestinal irritation and diarrhea. Research has found that dogs are most sensitive to these effects. Based on these results, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of NA in 1968, 1976 and 2002 and founded an ADI (Acceptable Daily Intake) of 0.3 mg/kg body weight (BW)/day (EFSA, 2009).

The objective of the presence study is to evaluate NA presence in cheese samples collected from Adana city in Türkiye. Moreover, dietary exposure analysis is provided using the surveillance data.

## 1.1. General Information about Cheese

Cheesemaking began approximately 8,000 years ago and today there are more than 1,000 cheese species worldwide (Sandine & Elliker, 1970), each unique in flavor and shape.

Cheeses vary in their nutritional content. Cheese source of important nutrient . an average nutritional value is given as an example for semi-hard chese. Most cheeses are high in protein, 24.7 and 25.1 g/100 g per serving, because protein is an important ingredient for human health.(Fox et.al., 2017).

**Table 1.1.** Approximate Nutritional Components of Extra Hard Cheese (Govindasamy-Lucey et al.,2004)

| Nutrient | Extra Hard Cheese |
|---|---|
| Moisture (g/100 g) | 34.5-37.5 |
| Protein (g/100 g) | 24.7-25.1 |
| Sat Fat (g/100 g) | 32.8-33.9 |
| Calcium (mg/100 g) | 780-830 |
| Calcium (mg/g protein) | 28.24 |
| pH | 5.2-5.3 |
| Salt (g/100 g) | 1.6-2.2 |

Due to the increase of organic acids in cheese production, the pH of curd after production is between 4.5 and 5.3; such a low pH does not allow acid-containing species to survive. The optimal pH for most bacterial growth is around neutral, but growth tends to be poor at pH < 5.0. (Beuchat and Golden, 1989). The main organic acids in cheese are lactic acid, acetic acid and propionic acid. At the same cheese concentration, lactic acid is the one of the most effective microbial inhibitor. But, the concentration of lactic acid in cheese curds is always much higher than the another two acids, with the exception of Swiss cheese, which may have a higher propionic acid concentration than mature cheeses and has an affecting nutritional profile. Cheese contains protein, fat, calcium, phosphorus, potassium and vitamin B12, making of import food in a balanced diet (Fox et.al., 2017)

Cheese is very important as being one of the main calcium source in diet. The most important task of calcium is the formation keep bones and teeth strong. Regular beating of the heart, regular functioning of the blood clotting system, healthy functioning of the nerves and proper

functioning of the muscles are also possible with the help of calcium. Although it varies by age and gender; people need an average of 1000-1500 mg of calcium. In order to meet the calcium needs required for each age group, at least 2-3 portions of dairy products (such as 1-2 glasses of milk or yoghurt or 50 g of cheese) and the same amount of calcium-containing vegetables should be consumed per day (Fleming et al., 1994).

### 1.1.1. Production of cheese

Humans began to take advantage of milk through domestication of animals about 4,000 years ago, and it is believed that the date when cheese first came out was between 8,000 and 9,000 BC. According to various sources, there are between 2,000 and 4,000 kinds of cheese in the World (Mikkelsen, 2000). World cheese production by years is given in Table 1.1.1. and by countries is given in Table 1.1.1. According to IDF.(International Dairy Federation) data, world cheese production is estimated to increase by 2% in 2020 to approximately 24 million tonnes. (Processed cheeses are not included in the calculation for the purposes of preemptive calculation). The data on cheese production concern only cheeses produced from cow's milk, which represent only 90% of total production, while many countries do not include cheeses from other species not included in national statistics (sheep, goat) in the calculation of domestic and farm-level cheeses (Besse et al., 2006).

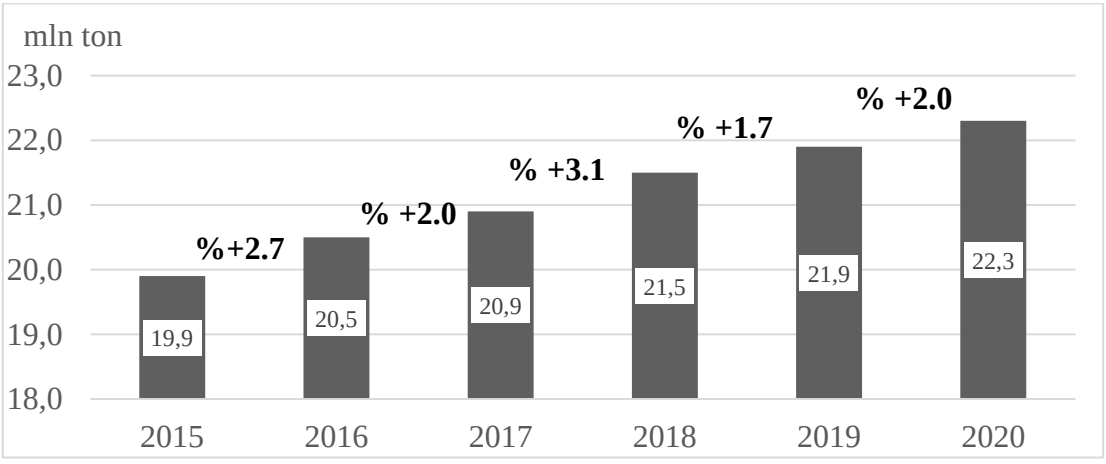


**Figure 1.1.1.** Production of Cheese in the World

Cheese production increases in EU-28 1.9% in 2020. Cheese production is expected to decline due to the decline in consumption due to COVID-19 in the same year, while the special storage aid applied by the European Commission in many Member States has been preceded by production shortages.Cheese production increased in Germany (+2.5%) and Italy (+1.2) in the same year, while production declined in France depending on consumption (-%1.5) (Besse et al., 2006).

**Table 1.1.2.** Cheese Production Leading Countries in World

| Country | 2020 | 2019-2020 (Change, %) |
|---|---|---|
| **EU-28** | 9,9 | +1,9 |
| **ABD** | 6,0 | +0,9 |
| **BRASIL** | 1,1 | +2,8 |
| **TÜRKİYE** | 0,8 | +8,6 |
| **CANADA** | 0,5 | +1,4 |
| **MEXICO** | 0,5 | +2,2 |
| **EGYPT** | 0,5 | +6,8 |

Source: CNIEL,IDF(International Dairy Federation) national committees, national statistics, 2021

The total cheese production in Türkiye, which consists mostly of cheese derived from cow's milk, has decreased by 0.45% in 2021 to 763 thousand tonnes compared to the previous year as it can be seen in Table 1.1.3 (Besse et al., 2006).

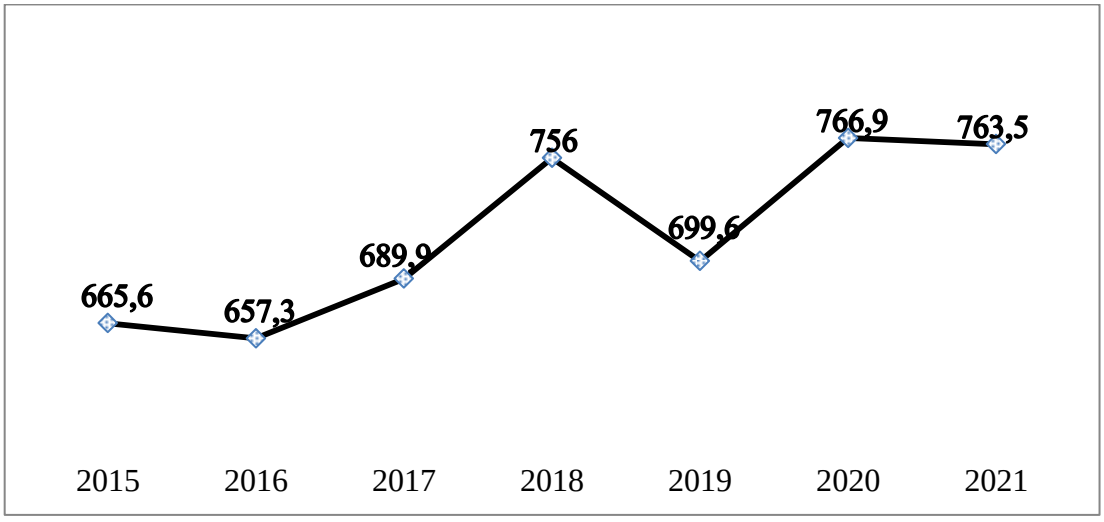


**Figure 1.1.2.** Cheese Production in Türkiye (Thousand Tonnes)(Source: CNIEL,IDF national committees, national statistics, 2021)

Cheese is broadly divided into yogurt cheese, rennet cheese, natural cheese, and processed cheese. Fresh cheeses and cream cheese are occured acidification directly. Most of cheeses, use of rennet (an enzyme) moreover a starter culture to coagulate the milk. "Natural cheese" is the industry name for cheese made directly from milk. Processed cheese is made from natural cheese and is cooked with other ingredients to change the texture, meltability, and extend shelf life. The thalli component of cheese is milk. Cheese is made from cow's milk, goat's milk, sheep's milk, buffalo milk, or a mixture of these milks. The type of coagulant used depends on the type of cheese desired. Rennet cheese uses cheese produced by a microbial bioprocess known as calf rennet, or more commonly known as rennet. Calcium chloride is sometimes added to cheese to improve the coagulation properties of milk. (Szabolcs et al., 2014).

Cultures are added to supply or improved the characteristic flavor and texture of cheese. The cultures that make cheese are called as lactic acid bacteria (LAB) so their main thalli of energy source from lactose in milk and their main metabolite is lactic acid. Different bacterial cultures give cheese different flavor and textural characteristics. Use a starter culture privately in the cheesemaking process to aid coagulation by decreasing the pH before adding rennet. The metabolism of the starter culture helps produce the requested flavor and prevents the enlargement of spoilage-causing microorganisms and pathogens. A typical starter bacterium is *Lactococcus lactis* subsp. Lactic acid or milk fat, *Streptococcus salivarius* subsp. *Lactobacillus thermophilus, Lactobacillus delbrueckii* subsp. *Lactobacillus bulgaricus* and *Lactobacillus helveticus.* Common additional cultures added during the manufacturing process include *Lactobacillus casei* and *Lactobacillus plantarum*, which are used to add flavor to cheddar cheese, or *Propionibacterium geudeensis*, which is used to form Swiss cheese eyes. Yeast and mold are used in some cheeses, giving some cheeses their unique colors and flavors. Torula yeast is used in the mash to mature brick cheese and Mozzarella cheese. Examples of molds include *Penicillium camembert* in camembert and brie cheeses, and *Penicillium roquefortii* in blue cheese (Desmasures, 2014). Depending on the cheese, spices may be added. Common ingredients include herbs, spices, paprika, paprika, horseradish and port wine. Although cheese production processes vary depend on cheese types, essential steps are the same.

### 1.1.2. Essential steps of cheese process

Cheese can be extensively divided into yoghurt or rennet cheese and natural or processed cheese (Walstra et al., 2005).

- **Milk clothes:** This is accomplished by an acid or an enzyme (or both). The enzymes are involved in the removal of caseinomacropeptides "hairs" from k-casein: the following paracasein is mixed with aggregates. The acid normally formed from lactose is dissolved in the colloidal calcium phosphate of the micelles by lactic acid bacteria and neutralizes the charge of the resulting particles, which then aggregate. This accumulation creates a space-filling network around the whey and fat micelles.

- **Removal of whey:** The gel formed tends to work spontaneously. A gel is formed by removing the whey from the cheese. Curd is formed by breaking the gel into pieces.The curd obtained accounts for 10% to 30% of the original milk volume. The drier the curds, the harder the cheese and the longer it will last.

- **Acid production during the manufacturing process:** This is as a lactic acid bacteria convert lactose into lactic acid. The pH of curd and cheese affects parameters such as syneresis, consistency and ripeness of cheese.

- **Salting:** Cheese generally contains 1% to 4% added NaCl. This does not use with certain types of fresh cheese, such as curd. Salt affects the shelf life, flavor and texture of cheese, either directly or by affecting ripening.

- **Merge the curd grains into a coherent loaf:.** Additionally, cheese can have a rind to protect the inside. Pressing promotes melting of the curd and creates a closed crust.

- **Ripening:** Ripening in cheese always in its dynamism due to biochemical and enzymatic reactions occurring in the mass, regardless of the type of cheese. Proteolysis has been the most widely studied subject on the concepts of maturity, studied in three major chapters: proteolysis, lipolysis and glycolysis. The role of protein, a key element in the milk to cheese conversion mechanism, in cheese maturation remains equally important. The methods used to determine the maturity of

cheese are also being updated according to scientific and technological developments. Cheese ripening is a complex process including various microbial and biochemical changes in the curd, resulting in each cheese's unique flavor and texture. In addition to coagulants, proteases derived from lactic acid bacteria, pepsin, and plasmin are also involved in post-coagulation ripening and subsequent flavor development of cheese. This ensures that conditions during cheese storage and handling allow for the required maturation. Ripening is the main factor that determines the typical taste and texture of each cheese. To obtain this, cheese needs to be stored under suitable conditions for varying periods of time. Storage conditions vary greatly depending on the type of cheese. Various ripening methods can be seen in Table 1.1.4.

**Table 1.1.4.** Ripening Method of Cheeses According Ripening Situation and Ripening Day

| **Ripening** | | **Minimum Ripening Time** | |
|---|---|---|---|
| **Ripening Method** | **Ripening State** | **Mass>1.5 kg** | **Mass<l ,5 kg** |
| **Unripened** | Fresh | - | |
| **Ripened** | Ripened | 90 | 45 |
| **Ripened with mold cultures** | Ripened | 90 | 45 |
| **Ripened in brine** | Ripened | 90 | 90 |

Different methods of speeding up cheese ripening include:

- Add exogenous enzymes
- Increase LAB enzyme levels by using a weakened starter or increasing lysis rate
- Use additional crops
- Genetic modification of starter cultures
- Use high hydrostatic pressure
- Increase ripening temperature.

### 1.1.3. Cheese classification

Cheeses are classified by moisture-hardness content; moisture and ripening; ripenig situation and ripening day; ripenig and dry matter and the ratio of salt and moisture for Turkish cheeses (Cambaztepe et al., 2009). According to cheese texture and hardness, cheese can be sort out as extra-hard, hard, semi-hard, semi-soft and soft cheese.

- **Extra-Hard Cheese:** Extra-hard is a term used in the cheese world to describe a group of different cheeses that are hard and crispy. This type of cheese becomes very hard because they contain very little fat and moisture (Hinrichs, 2001).

- **Hard Cheese:** Hard cheese is made by removing most of the whey from the curd and applying pressure to it. The cheese then develops its own edible rind, sometimes with a waxy rind (Hinrichs, 2001).

- **Semi-Hard Cheese:** This low-moisture cheese type includes some of the most popular and hardest cheeses. These cheeses are matured for a shorter period of time than hard cheeses and then placed in a wax rind.which helps prevent unwanted mold and maintains the correct moisture content for semi-hard cheeses (Hinrichs, 2001).

- **Semi-Soft Cheese**: The biggest difference between semi-soft cheese and soft cheese is that semi-soft cheese usually has a rind that, while softer and edible, is due to mold that forms during the brief aging process. The flavor profile of semi-soft cheese can range from buttery and salty to richer and earthier, depending on how long it has been matured (Hinrichs, 2001).

- **Soft Cheese**: This type of cheese is sometimes called "fresh" cheese because the cheese has not yet been aged. Soft cheeses have a higher moisture content and have no rind. The soft cheese category offers a variety of flavors, from mild to complex, making it an interesting cheese texture category worth trying (Hinrichs, 2001).

Water activity ($a_w$) is without delay rational to the moisture content of cheese and invertedly proportional to the amount of sodium chloride and other molecular weight compounds (Esteban & Marcos, 1989). In the cheese making process, $a_w$ is about 0.99, which is good for the growth of and activity of the starter culture. Still, the prevailing $a_w$ values during whey dehydration, salting and maturation (0.917–0.988) (Rüegg & Blanc, 1981 ) are well below the optimum requirements of the starter bacteria. Therefore, $a_w$ can help control their digestion activity and proliferation (Brown, 1976).

Lactic acid bacteria always have exalted $a_w$ minimal than other cheese bacteria. *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Propionibacterium frederi* subsp. *shermanii* were 0.93, >0.98, >0.96, and 0.96, respectively (Weber & Ramet, 1987). During cheese ripening, the aw value decreases due to water loss due to evaporation, salinity, breakdown of proteins into unıts( peptides and amino acids, and hydrolysis of triglycerides )into glycerol and fatty acids. Hydrolysis of each peptide or ester bond requires a water molecule. Important degradation of protein in cheese reduces free water content during ripening (Cogan, 2000).

### 1.2. General Information about Natamycin

Natamycin was discovered in 1958 by Struyk at the Gist-Brocades Research Laboratory from *S. natalensis* cultures filtrates in South Africa (Stark, 2003). Natamycin (primaricin) is an antimicrobial produced by the aerobic fermentation of *Streptomyces* species such as *S. chattanoogensis, S. gilvosporeus, lydicus* (Kallinteri et al., 2013).

In the EU it is considered a natural preservative (EEC 235) as it is generally recognized as safe (GRAS). Natamycin is typically used at concentrations of 1 to 10 ppm. It is particularly effective against yeast and mold, but less effective against bacteria, protozoa and viruses. It is used in dairy products to prevent spoilage caused by fungi, especially cheese. Most molds are prevented by 0.5 to 6 μg/mL of NA, but some molds require 10 to 25 μg/mL. Most yeasts are prevented by NA at concentrations between 1.0 and 5.0 μg/mL (Stark et al., 2003).

In laboratory studies, natamycin was toxic and no allergic reactions were found. Insoluble or slightly soluble in water and oil. It is reported to be soluble and 90% excreted in feces after

ingestion (Ersan & Kurdal, 2005). Natamycin can be stored as a chemical dry powder for several years with a little loss of activity. High pH, chlorine, oxidants and heavy metals affect stability. NA is amphoteric bacause it is a macrolide (Stark et al., 2003).

Commercially, NA is produced by fermenting a *Streptomyces* culture and consist of carbon source and a fermentable nitrogen source. The carbon source is starch or molasses, and corn steep liquor, soybean meal, and casein serve as fermentable nitrogen sources (Medina et al., 2007).

### 1.2.1. Chemical and physical properties of natamycin

Natamycin macrolide is characterized by a a double carbon bond on the ring. Natamycin's closed formula and molecular weight are $C_{33}H_{47}NO_{13}$ and 665.73 g/mol, respectively (Ersan & Kurdal, 2005). Pure produced NA is colourless, odorless and unpleasant. For commercial purposes, NA is a crystalline powder of white or creamy white color. The antifungal agent has purified and has a characteristic odor and a melting point of 180 °C. The compound can be soluble in ethyl acetate, n-butanol, acetone, ethanol, methanol, and 10% isopropanol, however natamycin can not soluble in water, petroleum ether, hexane, and benzene. Factors affecting its stability, acidity (pH), temperature, light, oxidants and heavy metals (Shahan et al., 2004).

### 1.2.2. Food applications of natamycin

Natamycin is a natural fungus commonly used in the food processing industry, particularly for producing dairy products due to its wide spectrum as an antifungal effect. NA is commonly using as a biopreservative in the surface treatment of hard, semi-hard and soft cheese and sausage products. NA also generally acts as an fungustatic preservative on other food producing products like yoghurt, khoa, sausages, juices, wines, etc. (Kallinteri et al., 2013).

The mold growth of the surface in cheese can be an important factor in limiting the shelf life of cheese. Not only growth of mold, but also occurence of mycotoxins is a risk in cheese. Many types of cheese are ripened for several months in ripening chambers at temperatures ranging from 10°C to 12°C, which is very favorable to mold growth. There are many ways of

NA usage during cheese production. NA can be applied by spraying, brushing or dipping, or it can be added to a plastic coating and then dipped either brushed onto the cheese. If addition of NA can be conducted by soaking, it is recommended to add 10% salt to the solution to prevent bacterial growth. When NA is applied as a dipping solution or spray, it tends to settle unless stirred or agitated. Modified formulations containing food-grade thickeners can be used to both prevent NA from settling and improve adhesion to product surfaces (Stark, 1999). Mixtures in combination with anti-caking agents such as cellulose powder are also available. Moreover, a new method of applying NA to cheese surfaces via electrostatic coating has been proposed (Elayedath & Barringer, 2001). NA is approved by the U.S. Food and Drug Administration (FDA) for use in all standardized cheeses for which antifungal agents are approved.

### 1.2.3. Antimicrobial activity of natamycin

Natamycin has a protective effect all yeasts and molds, however not against bacteria, protozoa, and viruses. Natamycin is so beneficial because it does not interfere with the fermentation or ripening process.

Polyene macrolides such as NA complexed with ergosterol, which is to import in fungus cell membranes structure. The irreversible binding of NA and ergosterol damage membranes, increases cell permeability, and causes cell death. Because cell membrane of viruses,bacteria and protozoa does not contain ergosterol, explains why these microorganisms are resistant to NA (Hamilton-Miller, 1974). Inhibitory effects of polyene macrolides on glycolysis and respiration have been demonstrated, but these are thought to be secondary to membrane effects. Some wild-type fungi are less sensitive to NA, probably due to lower ergosterol content in their membranes. Examples include *P. discolor, Verticilium cinnabarinum,* and *Botrytis cinerea.* Most molds are inhibited at concentrations of 0.5-6 μg/mL, but some species require 10-25 μg/mL. Most yeasts are prevented at concentrations between 1.0 and 5.0 μg/mL (Delves Brought et al., 2005).

The minimum inhibitory concentration of NA against various yeast strains is given in Table 1.2.3. The minimum inhibitory concentration of NA on mycelial growth of *Botrytis cinerea* is 5.0 to 10.0 $mgL^{-1}$, and the minimum inhibitory concentration on spore germination is 0.7 to

1.0 $mgL^{-1}$ .Minimum inhibitory concentration NA againts *A. carbonarius* growth and ochratoxin production was determined as 50-100 ng/mL (Medina et al., 2007). In case of *Yarrowia lipolytica* 20 ppm of NA was showed antimycotic effect (Resa et al., 2014).

**Table 1.2.3.** MIC Value (Minimal Inhibitory Concentration) of NA against Some Yeast Strains

| Yeast Strains | MIC Value(mg/ml) |
|---|---|
| American ale (*S.cerevisiae*) | <0.003 |
| Belgian ale (*S.cerevisiae*) | 0.006 |
| English ale (*S.cerevisiae*) | <0.003 |
| German wheat (*S.cerevisiae*) | <0.003 |
| Czech lager (*S.pastoriyanus*) | 0.006 |
| Cider yeast (*S. bayanus*) | 0.024 |
| *Candida albicans* | 0.19 |

### 1.2.4. Toxicology of natamycin

Polyene macrolide antibacterial agents are most toxic by the intravenous route and least toxic by the oral route. Apparently, NA at doses up to 500 mg/day is not absorbed from the human intestine after 7 days of administration (Brik, 1981). The information is available on the sorption, distribution, excretion, or metabolism of natamycin in humans. Levels of NA detected in the blood of human subjects after ingestion of 500 mg of NA were less than 1 mg/L (Anonymous, 1968).

In a study NA was administered orally to 10 patients with fungal diseases. Doses given to patients varied from 25 to 1000 mg/person/day for 20 to 180 days. Therapeutic doses of 200 mg/person/day and above may cause loss of appetite, nausea, and vomiting. Flatulence has been reported at concentrations of 50 mg/person/day. Only 1 of 10 patients taking 25-75 mg/person/day alone for 70 days reported no adverse treatment effects. Even though NA has a structural genotoxicity alert because the molecule contains an epoxide ring, it has no evidence related to genotoxicity of NA (Newcomer et al., 1960).

In animal toxicology studies, the following effects were observed: reduced food intake, reduced rate of weight gain, gastrointestinal irritation and diarrhea. Dogs are the species most sensitive to these effects (EFSA,2009). Based on three animal toxicity studies, a no observed adverse effect level (NOAEL) of 45 mg/kg bw/day was assumed for rats and a NOAEL value of 12 mg/kg body weight/day was assumed for dogs. Transient diarrhea and mild weight loss were observed (EFSA, 2009).

There are two long-term studies conducted on animals, a two-chronic toxicity study in rats and a two-year chronic toxicity study in dogs. In the rat study, reduced food intake and growth rate were observed only in the highest dose group. The data showed no significant differences in the number and type of tumors in any of the NA-treated groups compared with untreated control animals. The NOAEL for this study was assumed to be 22.4 mg/kg body weight/day. In studies with dogs, the highest food concentrations resulted in obesity in the animals. The NOAEL for this study was assumed to be 6.25 mg/kg body weight/day (EFSA,2009). The $LD_{50}$ values of NA after intarvenous, oral, introperitonal and subcutaneous administration is given in Table 1.2.4.

**Table 1.2.4** Acute Toxicity Of Different Polyene Antimicrobials

| | **$LD_{50}$ (mg/kg)** | | | |
|---|---|---|---|---|
| | **Intravenous** | **Subcutaneous** | **Intraperitoneal** | **Oral** |
| Amphotericin B | 4-6 6 | — | 28.0 | >8000 |
| Candie idin | — | 160-280 | 2.1-7.0 | 90-400 |
| NA | 5-10 | 5000* | 250* | 1500* |
| Nystatin | 3 | 24 | 8-14 | >3500 |

*Rats

Source: Adapted from Hamilton-Miller (1973)

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of NA in 1968, 1976 and 2002 and established an ADI of 0.3 mg/kg body weight ( bw)/day due to the toxicological studies conducted so far.

### 1.2.5. Regulatory status of natamycin

In the European Union (EU), the maximum allowable degree of NA on the surface of hard, semi-hard and semi-soft cheese is 1 mg/dm$^2$ of surface, and NA must not be present deeper than 5 mm (Delves et al.,2005). In Türkiye, NA can be used for external surface applications of uncut/unsliced hard, semi-hard and semi-soft cheeses with a limit of 1 mg/dm$^2$ on the surface (should not be at a depth of 5 mm) (TGK 1.2.4 Regulation, 2023). NA usage in foods can vary from country to country, and also depending on types of food.

## 1.3. Risk Analysis

Food naturally, intentionally or unintentionally, contains various desirable substances such as nutrients, additives and some other ingredients such as fiber, or undesirable substances such as natural toxins, pesticide residues, mycotoxins. As with all of these substances, levels that are too high or sometimes too low can pose some risks. Therefore, reliable estimates of consumption are needed (Kroes at al., 2002).

Formally, risk is not simply a measure of the likelihood or probability that something will go wrong (that is, an adverse event will occur). It also measures the severity of a problem when it occurs. In situations where large numbers of people have been or are likely to be exposed to relatively small amounts of chemicals that have been identified as hazardous to health, regulatory action is required, but only under relatively intense conditions of exposure (Morris, 2013). Risk analysis activities consist of three main components; risk assessment (scientific advice and information analysis), risk management (supervision and control) and risk communication.

Risk assessment is effective for identifying and evaluating potential risks to human health from exposure to various contaminants. Risk assessment is the scientific evaluation of potential adverse health effects resulting from human exposure to hazardous substances or situations (Kroes et al., 2002). It uses toxicological data on the chemicals people are exposed to and predicts likely risk levels. A four-step flow chart developed by the Research Council of the National Academies is commonly used for this purpose (National Research Council, 1983). The first step is "hazard detection," which involves identifying the health problems caused by the contaminants. Hazard Identification: “What are the consequences of exposure

to this contaminant?" It answers questions such as this and assesses the strength of the evidence supporting the definition. The second step in the flowchart is when a factor is identified as a hazard. A "dose-response assessment" describes the likelihood and magnitude of health effects based on the amount and circumstances of exposure to a contaminant. "Exposure assessment" is the third step and estimates the duration, frequency and intensity of exposure. Finally, "risk characterization" is the step that communicates the expert's assessment. Decisions about the existence of risks and how to manage them. The first two steps are specific and objective for each chemical; the last two steps depend on the risk assessor and the exposure scenario. Epidemiologists, toxicologists, chemists, medical researchers and engineers are involved in all stages of the risk assessment process (Faustman and Omenn, 2008).

During the hazard identification step, the toxicity of the chemical must be assessed. To evaluate the chronic toxicity of chemicals, information from four categories of studies is used: structure-activity relationships, in vitro or short-term studies, in vivo animal bioassays, and information from human epidemiological studies (Faustman and Omenn, 2008). Exposure assessment is a qualitative and/or quantitative assessment of possible ingestion of a chemical through food and, if applicable, other sources of affected (WHO, 1997). Exposure assessment inidentifying the populations that may be affected from the target substance, identifying the likely routes of exposure, and estimating the magnitude, time, and expose of doses that people may take posssesion of as a result of exposure. The final step in risk characterization including integration of information from the first three steps to produce qualitative and quantitative estimates of the probability that individuals exposed to the substance in a given population will suffer harm related to the substance. Determination of the exposure to additives in diets is important both in terms of risk analysis and the realization of legal regulations to protect public health.

In vivo studies should determine the major toxic effects of the test compound and the exposure levels that do not cause deleterious impacts  termed as " NOAEL". The Acceptable Daily Intake (ADI) can then be obtained from the NOAEL using indefiniteness effects (sometimes called procure effects) that take in account the uncertainty in extrapolating results from animals to humans and between humans in order to account individual differences. The ADI is an estimate of the amount of a compound that can be ingested each day over a lifetime

without posing a significant health risk (theoretically there is zero risk). Typically, no quantitative risk assessment is performed to determine FDI; the risk is considered low and negligible from a public health perspective (Kroes et al., 2002).

Typically, one of two methods is used to combine or integrate data to obtain exposure estimates. The point estimates (deterministic modelling) and the probabilistic analyses. In the context of exposure estimation, the term "point estimate" refers to a method of matching a fixed food consumption value (e.g. average or high consumption value) (usually) to a fixed residue/concentration value (e.g. medium or high consumption value) . Value).High consumption value) match. High consumption value) times high consumption value). Probabilistic methods form probability distributions that use the data distribution of the variable rather than point values. Probabilistic methods create almost all possible scenarios to obtain a probabilistic description of the population under study. Probabilistic methods require the risk assessor to fit a distribution to the input parameters. For this reason, it neccessarries more expertise and time than deterministic methods (Faustman and Omenn, 2008).

# 2. MATERIAL AND METHODS

## 2.1. Reagents and Chemicals

Natamycin standards (≥95%), HPLC grade methanol and glacial acetic acid were obtained from Sigma Aldrich (St. Louis, USA). The water used in the experiments was purified with a Millipore Direct-Q 3 UV Ultrapure (type 1) water of purification system (MerckAG, Darmstadt, Germany).

## 2.2. Sampling and Sample Preparation

A total of 40 cheese samples will be purchased from supermarkets and open bazaars in Adana city in Turkey. The samples will be transported to the laboratory in a cooling bag and stock for to 2−3 days at 4°C before NA analysis. The determination of NA is based on removing the cheese from the environment by mass freezing after methanol extraction and reading it on the HPLC device.

The samples were prepared according to ISO+9233-2 (2018). Firstly, the cheese samples were cut vertically into 3 cm slices. From these slices, 5 mm sections were cut into pieces between 20-40 $cm^2$ and recorded in the surface $cm^2$ regions and also its mass is determined as gram.

After cutting cheese samples, 10 g ± 0,01 g sample was weighed and 100 mL of methanol added into the conical flask. The contents of the conical flask were stirred with a magnetic stirrer for 90 minutes. At the end of the stirring 50 ml water added and the conical flask placed in the freezer mmediately in laboratory. Approximately 60 min later extracts will be filtrated using filter paper and 45 µm syringe filters.

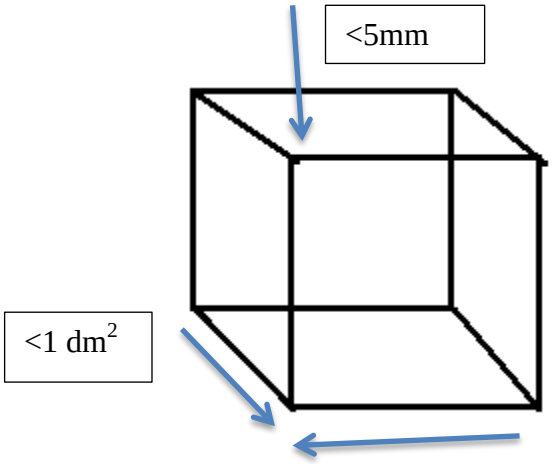


Figure 2.3 Sampling from Cheeses

### 2.3. HPLC Analysis

20 µL from the filtrate was taken and injected into the high pressure liquid chromatography device equipped with UV dedector set at 303 nm. Chromatographic separation was achieved using C18 (250 mm x 4.6 mm id., 5 µm particle size) column. The mobile phase consisted of Methanol :Water: Acetic acid - 12:8:1 (v/v). The column has well-kept at room temperature and the flow rate was 1 mL/min.

### 2.4. Risk Analysis

In this thesis, only the ingestion route was considered to evaluate the exposure associated with the occurrence of NA in cheese. To calculate risk, long-term daily intakes are used and expressed in mg/kg/day.Estimated Daily Intake calculation was conducted using Equation 1.

$EDI=(\sum c) \times (c \times N^{-1}D^{-1}K^{-1})$ **Equation 1.**

$\sum c$=The sum of NA the concentration in analysed samples.

c=is the yearly intake estimated per Turkish inhabitant

N= Total number of analysed samples.

D=is the number of days a year

K=is the mean body weight

Final step of risk assessment which is risk characterization conducted according to hazard quotient.

Non-carcinogenic risks from exposure to NA through ingestion routes were calculated using the following Equation 2..

$HQ = EDI/RfD$ **Equation 2**

HQ= hazard quotient, unitless

EDI= Chronic daily intake of NA from ingestion of cheese, mg/kg-day.

RfD= Reference dose for oral exposure, mg/kg-day

# 3. RESULTS AND DISCUSSION

## 3.1. Natamycin Presence in Cheese Samples

Natamycin quantification was carried out using HPLC in 40 cheese hard and semi-hard cheese samples and the result are shown in Table 3.1. As it can be seen from the Table NA was detected in 23 of 40 cheese samples. The presence of NA was found withing reach 1.5 mg/kg-10.4 mg/kg in 40 cheese samples analyzed, with an average of 5.32 mg/kg. According to the results, it was determined that out of the 40 cheese samples 42.5% of them were determined not to comply the Turkish food codex. The limit of detection (LOD) and limit of quantification (LOQ) of natamycin in HPLC method was obtained as 0.03 mg /kg and 0.1 mg /kg, individually.

**Table 3.1** Presence of NA in Cheese Samples

| Sample No | NATA Concentration(mg/kg) | Sample No | NATA Concentration(mg/kg) |
|---|---|---|---|
| 1 | <0.1 | 21 | 6,7 |
| 2 | <0.1 | 22 | <0.1 |
| 3 | 6,73 | 23 | <0.1 |
| 4 | 1,6 | 24 | 4,2 |
| 5 | 4,67 | 25 | <0.1 |
| 6 | 5,1 | 26 | 5,9 |
| 7 | <0.1 | 27 | <0.1 |
| 8 | 8,3 | 28 | <0.1 |
| 9 | <0.1 | 29 | 4,15 |
| 10 | 3,4 | 30 | <0,1 |
| 11 | 10,4 | 31 | <0,1 |
| 12 | 9,8 | 32 | <0,1 |
| 13 | 1,5 | 33 | <0,1 |
| 14 | 3,8 | 34 | 4,4 |
| 15 | <0,1 | 35 | <0,1 |
| 16 | <0,1 | 36 | <0.1 |
| 17 | <0,1 | 37 | 2,2 |
| 18 | <0,1 | 38 | <0,1 |
| 19 | <0,1 | 39 | <0,1 |
| 20 | <0,1 | 40 | 7,7 |

Esfandiari et al. (2013) tested the natamycin scope in 39 Iranian yogurt drink (Doogh) samples and found that the natamycin content in 4 Doogh samples was 1.2~3.6mg/L, exceeding the allowable value of Iranian standards. Koçak et al. (2013), surveyed 20 yoghurt samples NA is 8.94 mg/kg and range 1.03 to 16.5 mg/kg. Bilgic et al. (2016), surveyed 28 Turkish yoghurt samples 2.15 mg/kg and range 1.15 to 4.89 mg/kg Karalomlu et al. (2017), surveyed 46 cheese samples natamycin is 1.66 mg/kg and range <LOQ 1.66. Şanli et al (2022), surveyed 14 ayran 17 yoghurt and 12 kashar cheeses collected Natamycin is 0.745 mg/kg and range of 0.280-1.725 mg/kg and also developed a capillary zone electrophoresis (CZE) method based on monitoring concentrations using non-toxic chemicals of NA. In the Turkish Food Codex- Food Additives Standarts (2023), there is no NA limits foods other than cheese, these results means that yoghurt and ayran samples did not comply with the Codex. Various studies conducted on NA presence in food can be seen in literature (Table 4). Deficient results for natamycin in cheese was also noticed by Mologhani et al., (2016) and Paseiro-Cerrato et al. (2013). These results suggests that monitoring programmes should be considered for natamycin in different types of food.

**Table 4.** Natamycin presence in various foods

| Sample type and number | Number of positive samples | Natamycin concentration (average, mg/kg) | Natamycin concentration (range, mg/kg) | Analysis Method | References |
|---|---|---|---|---|---|
| 20 yoghurt samples | 5 | 8.94 | 1.03 -16.5 | RP-HPLC method | Koçak, 2013 |
| 28 Turkish yoghurt samples | 8 | 2.15 | 1.15 -4.89 | RP-HPLC method | Bilgic Alkaya & Karalomlu, 2016 |
| 46 Cheese Samples | 1 | 1.66 | ≤LOQ-1.66 | RP-HPLC | Karalomlu & Bilgiç Alkaya, 2017 |
| 14 ayran, 17 yoghurt,12 kashar cheese collected from local markets | 4 kashar cheese | 0.745 | 0.280 -1.725 | LC-ESI-MS | Şanli et al., 2022 |
| 195 samples of 15 brands and 5 types of commercial cheeses and yoghurts | 45 yoghurts and 19 cheeses | 6.154 (yoghurts)<br>4.014 (cheeses) | 4.355-66.462 (yoghurts)<br>5.249-158.025 (cheeses) | RP-HPLC | Mazdeh et al., 2017 |
| 10 wine samples | - | - | - | UHPLC-MS/MS | Bernardi et al., 2017 |
| Pastries<br>Cheeses<br>Beverages | - | - | - | Capillary Zone Electrophoresis | Li et al., 2020 |
| 40 Cheese samples | 4 | 3.65 mg/dm$^2$ | 1.67-6.3 mg/dm$^2$ | LS-MS-MS | Radicevic et al., 2019 |
| Ripened cheese (10 samples)<br>Fresh cheese (50 samples)<br>Pasta filata cheese (18 samples) | - | 0.66<br>6.22<br><LOD<br>1.41<br><LOD | 0.37-1.42 (surface)<br>0.74-13.00 (internal)<br>< LOD<br>1.25-4.05<br>< LOD | LC-MS-MS | Molognoni et al., 2016 |
| 26 cheese samples, 7 sausage samples, 12 wine samples | 4 cheese samples | - | 0.42-5.9 | HPLC-DAD | Paseiro-Cerrato et al., 2013 |

In order to prevent from the use of preservatives such as NA in cheese, productin of milk production should be carried out in a sterilized environmental and transported in a cold chain logistic system. In addition, good manufacturing practices should be implemented to effectively control environmental conditions during processing, storage and distribution. Governments should develop monitoring and control programs for the presence of preservatives in cheese and inform consumers about the shelf life of food products related to the presence of preservatives.

### 3.2. Dietary Exposure Assesment of Natamycin

According to TUIK (2022), daily cheese consumption per women (15+aged) 38.2 kg and daily cheese consumption per men (15+aged) is 39.8 kg. The average of daily cheese consumption is 39±33,28 grams/day. Moreover, according to TUIK (2022), the average body weight of women is 68.6 kg and the average body weight of men is 78.9 kg. The average body weight of both genders is 73.7 kg.

As a result average NA presence was determined as 5.32 mg/kg in this study. When calculating the mean NA concentration, samples with having NA below LOQ levels, was accepted as contaminated with LOQ levels as a worst case scenerio. According to Equation 1, EDI value is calculated as 0.001 mg/kg bw/day. This value is well below the ADI value (0.03 mg/kg bw/day) determined by JECFA.

EDI= 92.85/40=2.32 mg/kg
2.32 mg/kg * 0.0398 per day = 0.0923 mg/kg per day
0.0923 (mg/kg per day)/73.7 kg=0.001 mg/kg bw/day

Moreover Hazard quotient was calculated according to Equation 2. HQ value was determined as 0.0033 it can be seen in below. HQ value lower than 0.5, means that there is no risk for non-carcinogenic substances.

HQ=0.001 /0.3
=0.0033

Risk characterization of Non-carcinogenic Risk

HQ < 0.5 No Concern

0.5 ≤ HQ < 2 Concern

HQ ≥ 2 Significant

Both results, EDI and HQ values demonstrate that, there is no health risk due to the NA exposure from cheese comsumption for adults in Türkiye.The estimated dietary exposures to NA in cheese are 0.04 mg/kg body weight/day in the United Kingdom and 0.03 mg/kg body weight/day in children, and 0.02 mg/kg body weight/day and 0.02 mg in children in Germany. Kilograms of body weight per day for UK children and 0.025 mg/kg of body weight per day for German adults. Children's NA intake from dried sausages was estimated to be higher at 0.04 and 0.03 mg/kg body weight. For adults in the UK and Germany, the values were 0.006 and 0.02 mg/kg body weight, respectively. These results is comparable with our EDI result which is 0.001 mg/kg bw/day.

Despite some predictions seen after exposure to preservatives have been produced, the huge part of them use maximum permissible levels rather than actual levels to calculate exposure (for summaries, see Saba et al. 1992, Di Novi 1999, Petersen 1999). Other assessments use gravimetric or disappearance methods, in which the total amount of preservatives produced or imported by a country is distributed equally among its population (Penttila¨1996, Di Novi 1999, Petersen 1999). When dietary exposure is estimated using actual food preservative levels and realistic model diets, the average estimate is generally much lower than the ADI value (van Dokkum et al., 1982; Mareschi et al., 1992; Marro 1996; Jálon et al., 1997). , Yin et al. 2003, New Zealand Food Safety Authority 2005).

Long-term animal and human studies from JECFA used to determine ADI. The expert panel concluded that the recommended concentrations of NA do not pose a safety risk when used only surface treatment for semi-hard and semi-soft cheeses rinds.The expert group concluded that there is no reason to worry about the emergence of antimicrobial resistance. The petitioners submitted exposure estimates based on the 2002 JECFA assessment. The calculations are based on North American consumption of "a wider range of cheeses and meats", the amounts are sometimes higher than what is currently allowed in the EU. The average potential dietary exposure for UK consumers is 0.014 mg/kg body weight (bw) per day.while the mean potential dietary exposure for German consumers and children over 10

years was 0.015 and 0.01 mg/kg bw per day. Heavy users (97.5%), these estimates were 0.041, 0.051, and 0.031 mg/kg body weight/day, respectively. The expert panel noticed that JECFA reassessed exposure to NA at its 67th meeting in 2006 (JECFA, 2007). Precise predictions of dietary exposure are also based on personal consumption surveys in the UK and Germany, focusing on children aged 1.5 to 4.5 and 4 to 10 years, respectively (Gregory et al., 1995, 2000; Heseker et al., 1994). Children usually have higher dietary intakes relative to body weight than adults and therefore represent the population with the highest potential exposure to NA per kilogram of body weight. Exposure estimates for cheese at high concentrations (97.5%) were reported separately for consumers only (assuming a use of 40 mg/kg) and chopped sausages for example salami and other dry sausages (assuming a use of 20 mg /kilogram). The expert panel used this information to determine estimated exposures based on currently authorized use levels in the EU (equivalent to 20 mg/kg in cheese and raw sausages). NA is used as a food additive.

EFSA Journal 2009;7(12):1412 12 mg/kg) and ground sausage products such as salami and another dry sausages (consumption 20 mg/kg). The expert panel used this information to determine predicted exposures based on currently authorized use levels of the substance (equivalent to 20 mg/kg in cheese and raw sausages).

The maximum potential exposure to NA is 97.5. Due to the higher intake of cheese (assuming only the rind is treated with NA), the daily intake is less than 0.1 mg/kg body weight for kids and less than 0.05 mg/kg body weight for over of 15 ages) and dry sausage

For heavy users (97.5%), these predictions were 0.041, 0.051, and 0.031 mg/kg body weight/day, respectively.Available information on the metabolism of NA suggests that it is poorly absorbed from the gastrointestinal tract, remains unchanged, or is rapidly excreted in the feces as a degradation product.

In animal toxicology studies, effects were observed such as reduced food intake, reduced weight gain, gastrointestinal irritation and diarrhea. Dogs are the species most susceptible effects. Based on these results, the Joint FAO/WHO Expert Committee on Food Additives observed the safety of NA in 1968, 1976 and 2002 and recommended a value of 0.3 mg/kg body weight/day (EFSA, 2009).

# 4. CONCLUSION

In this thesis, presence of natamycin in cheese samples sold in Adana was determined. Moreover based on these suveillance results, dietary exposure assessment was conducted.

The presence of NA was found among the 40 cheese samples analyzed, its content varied from 1.5 mg/kg to 10.4 mg/kg, with an average value of 5.32 mg/kg. A total of 23 cheese samples did not comply with the Turkish Food Code.

The exposure assessment was done using deterministic approaches. The average dietary exposure estimates are well below the respective acceptable daily intakes. Based on data from this study, exposure to NA through cheese consumption does not pose a health risk.

On the other hand, there are some limitations of this study. First of all, limited number of samples were analyzed. Also bioavailability is under question. The consumption data was limited, not allowing to differentiate cheese types in the Turkish population. Normally, NA enters our body through other food sources. Since we only use cheese as a basis, it has created another limitation.

# 5. RECOMMENDATION

In this thesis, the presence of NA in cheese was investigated and its effects on health were stated. It is possible that we will get NA into our body not only from cheese but also from other permitted foods. In addition, since NA is allowed to be used within certain limits, it is important to use it within those limits and apply it correctly according to international and country regulations. But, there is strong request for "does not contain preservative" foods as humans seek more "natural" (without chemical preservatives) and "preservative-free" foods. Less processed food. Thus, industry organizations are seeking effective solvings to again chemical preservatives while maintaining the accent shelf life of food products.

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