REPUBLIC OF TÜRKİYE
DICLE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

# DETERMINATION OF CHANGES IN *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* NUMBERS DURING THE PRODUCTION AND STORAGE OF KISHK

Sara KHEDR

MASTER'S THESIS

DEPARTMENT OF FOOD HYGIENE AND TECHNOLOGY

ACADEMIC SUPERVISOR
Prof. Dr. Hüsnü Şahan GÜRAN

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**APPROVAL OF THESIS**

The master's thesis titled **"Determination of Changes in *Escherichia coli* O157:H7 and *Salmonella* Numbers During The Production and Storage of Kishk"**, prepared by Sara KHEDR, a master's student at the Department of Food Hygiene and Technology, Institute of Health Sciences, Dicle University, has been evaluated in terms of scope and scientific quality in accordance with the relevant articles of the Dicle University Postgraduate Education-Teaching and Examination Regulation and accepted as a Master's Thesis.

Advisor Prof. Dr. Hüsnü Şahan GÜRAN _____

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Date: 19/06/2025

This thesis has been approved by Dicle University Health Sciences Institute Board of Directors with the decision numbered.… dated …. /…/….

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Prof. Dr. Mahmut BALKAN
Dicle University
Director of the Institute of Health Sciences

## BEYAN

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19/06/2025

Sara KHEDR

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I declare that this thesis is my work, that I have not acted unethically in any phase from the planning to the writing of the thesis, that I have obtained all the information in this thesis within academic and ethical rules, that I have cited all the information and comments not obtained through this thesis and included these sources in the list of sources, that I have not violated any patent or copyright during the work and writing of this thesis, and that I have prepared my thesis in accordance with the Dicle University Health Sciences Institute thesis writing guide standards.

19/06/2025

Sara KHEDR

# ACKNOWLEDGMENTS

I would like to express my gratitude and respect to my esteemed supervisor, Prof. Dr. Hüsnü Şahan GÜRAN, for his invaluable support throughout my master's journey, for guiding me with his extensive knowledge, experience, and expertise, and for illuminating my way by teaching me scientific research discipline, scientific ethics, and scientific values. I also thank the esteemed faculty members of the Department of Food Hygiene and Technology for their contributions during my master's studies.

I would like to sincerely thank Lecturer Uğur UÇAR, Dr. Reşat ÇİFÇİ, and the Research Assistant Enes SEZER & Ruhal KIVRAK for their unwavering support and contributions throughout my thesis work.

I express my deep appreciation to my dear mother, Ebtisam HAMSHO, and my dear father, Moutaz RASLAN, who have always supported me at every moment of my life, who have been a source of strength, and whose great efforts were instrumental in my success. I also thank my dear brother, Dr. Munthir RASLAN, who has always guided and led me, especially in terms of some English language skills and access to different sources while writing my thesis.

I would like to convey my heartfelt gratitude to my life partner, my beloved husband Ezelldein KHEDR, for his constant support, for being by my side in every step and every struggle, and for always bringing me peace and happiness during the whole journey. I also thank my dear daughter Bella Fatma KHEDR, who has always encouraged and motivated me with her eloquent words and wonderful smile.

Special thanks to the Dicle University Scientific Research Projects Coordination (DÜBAP) that supported and funded this thesis under project number Veteriner. 24.013.

Sara KHEDR
DİYARBAKIR-2025

***To my dear parents,***
***Ebtisam and Moutaz,***

***My husband Ezelldein, &***
***My daughter Bella Fatma….***

**TABLE OF CONTENTS**

## INDEX OF ABBREVIATIONS AND SYMBOLS

**AFM1**: Aflatoxin M1

**CAGR**: Compound Annual Growth Rate

**CDC**: Centers for Disease Control and Prevention

**CFU**: Colony-Forming Units

***E. coli***: *Escherichia coli*

**EEA**: European Economic Area

**EFSA**: European Food Safety Authority

**EHEC:** Enterohaemorrhagic *E. coli*

**EPS**: Exopolysaccharides

**EU**: European Union

**FAO**: Food and Agriculture Organization

**FDA**: Food and Drug Administration

**GIT**: Gastrointestinal Tract

**GMPs**: Good Manufacturing Practices

**HUS:** Hemolytic Uremic Syndrome

**HLYs**: Healthy Life Years

**IPEC**: Intestinal Pathogenic *E.coli*

**LAB**: Lactic Acid Bacteria

**LSD test**: Fisher's Least Significant Difference test

**MaO**: Ministry of Agriculture

**MENA**: Middle East and North Africa

**MSs**: Member State

**Non-MSs**: Non-Member State

**NORS**: National Outbreak Reporting System

**NTS**: Non-Typhoidal strain / Non-Typhoidal Salmonella

**PW**: Peptone Water

**SAS**: Statistical Analysis System

**SEK**: Süt Endüstrisi Kurumu (Milk Industry Institution)

**SEs**: Staphylococcal Enterotoxins

**SFP**: Staphylococcal Food Poisoning

**SO**: Salmonella Outbreaks

**SSSS**: Staphylococcal Scalded Skin Syndrome

**SSSI**: Skin and Soft Tissue Infection

**STEC**: Shiga Toxin-Producing *Escherichia coli*.

**TSS**: Toxic Shock Syndrome

**TTP:** Thrombotic Thrombocytopenic Purpura

**UK**: United Kingdom

**USDA-FSIS**: U.S. Department of Agriculture's Food Safety and Inspection Service

**UTIs**: Urinary Tract Infections

**VTs**: Verotoxins

**WHO**: World Health Organization

# INDEX OF FIGURES

**Page No**

# INDEX OF TABLES

**Page No**

**Kişk Üretimi ve Muhafazası Sırasında *E. coli* O157:H7 ve *Salmonella* Sayısında Meydana Gelen Değişimlerin Belirlenmesi**

**Öğrencinin Adı ve Soyadı:** Sara KHEDR

**Danışmanı:** Prof. Dr. Hüsnü Şahan GÜRAN

**Anabilim Dalı:** Besin/Gıda Hijyeni ve Teknolojisi

## 1. ÖZETLER

### 1.1 Türkçe Özet

**Amaç:** Bu tez çalışmasında kişk üretimi ve muhafazası sırasında *E. coli* O157:H7 ve *Salmonella*'nın hayatta kalma yeteneklerinin belirlenmesi amaçlandı.

**Gereç ve Yöntem:** Kişk yapımı için bulgur, yoğurt, süzme yoğurt ve tuz yerel bir zincir marketten temin edildi. Hazırlanan kişk karışımı, *E. coli* O157:H7 ve *Salmonella* ile kontamine edildikten sonra 35 $^{o}$C'de 48 saat fermente edildi ve ardından 53°C'de 4 gün bekletilerek kurutuldu. Kurutma işlemini takiben kişk örnekleri 4°C ve 24°C olmak üzere iki farklı sıcaklıkta 36 gün steril polietilen torbalarda muhafazaya alınarak belirli günlerde mikrobiyolojik [*E. coli* O157:H7, *Salmonella*, toplam mezofilik aerobik bakteri (TMAB), toplam psikrofilik aerobik bakteri (TPAB), laktik asit bakterileri (LAB), koliform, *Staphylococcus-Micrococcus* spp., maya ve küf] ve kimyasal [pH, asittlik ve kuru madde] analizler gerçekleştirildi.

**Bulgular:** Kişk üretimi sırasında fermantasyonun 24. saatinde *E. coli* O157:H7'nin tespit edilebilir limitin altına (<10 cfu/g) düştüğü, *Salmonella*'nın ise var/yok analizi sonucunda tamamen öldüğü saptandı. Fermantasyonun 48. saatinde ise *E. coli* O157:H7'nin var/yok analizi sonucunda öldüğü belirlendi. Toplam mezofilik aerobik bakteri (TMAB) sayısı, özellikle 4°C'de olmak üzere, üretim ve muhafaza süresince azalma görüldü. Laktik asit bakterileri (LAB) ve toplam psikrofilik aerobik bakteriler (TPAB) fermantasyon sırasında anlamlı şekilde artış göstermiş, ancak 24°C'de muhafaza sürecinde azalma eğilimi gösterdiği saptandı ($P<0,05$). Koliform bakteriler, maya ve küfler, kurutma aşamasının ardından muhafaza süresince tespit edilebilir sınırların altında belirlendi. Kişk üretimi ve muhafazası sırasında *Micrococcus-*

*Staphylococcus* spp. ise hiçbir örnekte saptanmadı. Muhafazanın başlangıcından sonuna kadar geçen sürede özellikle 24°C'de muhafaza edilen örneklerde pH değerinde belirgin bir düşüş asitlik düzeylerinde ise artış saptanırken kuru madde oranında hafif artışın olduğu tespit edildi.

**Sonuç:** Bu çalışma sonucunda, kişk üretiminin *E. coli* O157:H7 ve *Salmonella*'nın yaşam kabiliyeti üzerinde önemli bir azaltıcı (~ 6-9 log) etki gösterdiği belirlenmiştir. Bu bağlamda, kişk'in söz konusu patojenlere karşı mikrobiyolojik açıdan güvenli bir ürün olarak değerlendirilebileceği düşünülmektedir. Ancak, bu varsayımın doğrulanabilmesi için vahşi tip (wild-type) suşların canlılıklarının araştırıldığı ileri çalışmaların gerçekleştirilmesi gerekmektedir.

**Anahtar sözcükler:** *E. coli* O157:H7, kişk, muhafaza, *Salmonella,* yaşam kabiliyeti.

**Determination of Changes in *Escherichia coli* O157:H7 and *Salmonella* Numbers During the Production and Storage of Kishk**

**Student's Name:** Sara KHEDR

**Advisor:** Prof. Dr. Hüsnü Şahan GÜRAN

**Department:** Food Hygiene and Technology

**1.2 Abstract**

**Aim:** This thesis aimed to determine the survival capabilities of *Escherichia coli* O157:H7 and *Salmonella* during the production and storage of kishk.

**Materials and Methods**: Bulgur, yogurt, strained yogurt, and salt used in the preparation of kishk were procured from a local chain market. The prepared kishk mixture was artificially contaminated with *E. coli* O157:H7 and *Salmonella*, then subjected to fermentation at 35 °C for 48 hours, followed by drying at 53°C for four days. After the drying process, the kishk samples were stored at two different temperatures (4°C and 24°C) in sterile polyethylene bags for 36 days. Microbiological analyses (*E. coli* O157:H7, *Salmonella*, total mesophilic aerobic bacteria (TMAB), total psychrophilic aerobic bacteria (TPAB), lactic acid bacteria (LAB), coliforms, *Staphylococcus-Micrococcus* spp., yeasts, and molds), and chemical analyses (pH, titratable acidity, and dry matter content) were conducted at predetermined intervals.

**Results:** During kishk production, *Escherichia coli* O157:H7 levels dropped below the detectable limit (<10 cfu/g) by the 24th hour of fermentation, while *Salmonella* was found to be completely eliminated based on presence/absence analysis. By the 48th hour of fermentation, *E. coli* O157:H7 was also no longer detectable by the presence/absence test. The total mesophilic aerobic bacteria (TMAB) count showed a decreasing trend throughout production and storage, particularly at 4°C. Lactic acid bacteria (LAB) and total psychrophilic aerobic bacteria (TPAB) increased significantly during fermentation but demonstrated a tendency to decline during storage at 24°C ($P < 0.05$). Coliforms, yeasts, and molds were found to be below detectable limits following the drying stage and throughout the storage period. *Micrococcus-Staphylococcus* spp. were not detected in any sample during the

entire production and storage phases. Over the course of storage, especially in samples stored at 24°C, a marked decrease in pH was observed, accompanied by an increase in acidity levels and a slight rise in dry matter content.

**Conclusion:** As a result of this study, kishk production was found to have a significant inhibitory effect (~ 6-9 logs) on the viability of *Escherichia coli* O157:H7 and *Salmonella*. In this context, kishk may be considered a microbiologically safe product with respect to these pathogens. However, to validate this assumption, further studies investigating the survival of wild-type strains are necessary.

**Keywords**: *E. coli* O157:H7, kishk, *Salmonella*, storage, survival ability.

## 2. INTRODUCTION AND AIM OF THE STUDY

Fermentation, one of the oldest and most cost-effective methods of food processing globally, is defined as a technique that utilizes the growth and metabolic activities of microorganisms to preserve food. This low-cost process requires relatively minimal energy input, making it a primary strategy for food production in various traditional cultures (1,2).

Fermented foods, such as kishk, have been integral to the human diet for centuries, particularly in economically disadvantaged regions like Africa and Southeast Asia. This is because of the health benefits and the long preservation opportunities it offers. As per the documentation, more than 25-30% of the global diets consist of fermented products, and over 3,500 fermented foods and beverages based on fruits, vegetables, and dairy are produced worldwide. Most of these products are made in homes or small-scale industries in Asian and African countries (3-8).

The international market of fermented foods is too vast, as stated by the Cognitive Market Research, the market size of such products reached USD 584 658 20 million in 2024. Of this, the Middle East and African regions dominated the market and accounted for approximately 2% of the global earnings, with a market size of USD 11 690 billion in 2024. This region is expected to expand at a compound annual growth rate (CAGR) of 5,9% from 2024 to 2031. (https://www.cognitivemarketresearch.com/fermented-foods-market-report/ Access date: 09 May 2025). However, the global fermented dairy market was valued at about USD 300 billion in 2023 and is expected to grow at 5,6% CAGR to reach over USD 435 billion by 2031. (https://www.databridgemarketresearch.com/reports/global-fermented-milk-market/ Access date: 09 May 2025). Solely, yogurt is among the most widely produced and consumed fermented dairy products globally, with its market value surpassing 1813 billion U.S. dollars in 2024. This figure will rise to approximately 237 billion U.S. dollars by 2028. (https://www.statista.com/statistics/870893/global-yogurt-market-value-forecast/ Access date: 09 May 2025). In Türkiye, fermented products and probiotic-rich foods are deeply embedded in the Turkish culture and traditions as well. The annual consumption of fermented foods per capita is estimated to be 30-40 kg per person. The fermented dairy sector in Türkiye sees over 100 million metric tons of production, with

a large share attributed to yogurt and cheese. As the consumption of yogurt alone, the most consumed in Türkiye, is estimated to be between 17-30 kg per person annually. The global demand for fermented dairy products continues to rise in light of their ability to promote gut health and microbiome and reduce the risk of heart diseases and cancer (6,7).

Kishk is a highly nutritious traditional fermented product made from bulgur, salt, and yogurt. It traces its origins back to the era of the Pharaohs and continues to be widely consumed and prepared with care across countries in the Middle East, Mediterranean, and Indian subcontinent, in various regional forms. The production of kishk may vary between countries due to differences in the ingredients used and the methods of preparation. Consequently, the chemical, physical, nutritional, and sensory characteristics of the final product exhibit regional variations across different parts of the world (3). To this day, kishk has gained global recognition and development, appreciated for its nutritional value, distinctive flavor, non-hygroscopic properties, and extended shelf life (ranging from 1 to 3 years) (3). Kishk has a significant presence in the Middle East and North Africa (MENA region), particularly in Lebanon, Syria, Egypt, Jordan, and Palestine, where it is more commonly consumed as 0,5 - 2 kg per person annually, with a growing demand in Europe. However, the annual global production of kishk was recorded in the thousands of tons, with the majority being produced in the regions mentioned. Many studies have highlighted the nutritional composition, microbiological quality, biophysical, and rheological properties of kishk and led to a tremendous development in the kishk industry (8-12). Even though kishk is a complete and balanced fermented food with excellent quality and extended durability, the safety of this product can be a very critical subject. Several studies have indicated that the artisanal nature of kishk, combined with the extensive handling during processing and inconsistent hygienic practices across countries, may enable the survival of milk or grain-generated pathogens or those from contaminated raw materials. *E. coli* O157:H7, *Salmonella*, *Listeria*, *Clostridium*, *Staphylococcus aureus*, and *Brucella* spp. along with coliforms, yeasts, and/or *Aspergillus* molds and AFM1, are common examples that can pose a potential health risk contributing to foodborne illnesses. (13-17).

Foodborne illness, particularly that resulting from the consumption of food contaminated with Shiga toxin-producing *Escherichia coli* O157:H7 and *Salmonella enterica* subsp. *enterica* remains a significant public health concern worldwide. According to the recent report by the World Health Organization (WHO), approximately 600 million people fall ill each year due to foodborne diseases linked to contaminated foods. These illnesses lead to 420 000 adult deaths and 125 thousand deaths among children under the age of 5, especially the vulnerable, resulting in a loss of 33 million healthy life years (HLYs) globally. In addition, WHO declared that nearly 70% of these foodborne illnesses in the Middle East and North Africa are linked to pathogens such as *E. coli* O157:H7, *Campylobacter*, Non-Typhoidal Salmonellosis (NTS), and Norovirus, emphasizing the considerable public health risk posed by these microorganisms (17-20).

This study aims to investigate the survival of *E. coli* O157:H7 and *Salmonella* during the production and storage of kishk.

# 3. GENERAL INFORMATION

## 3.1. The History of Fermentation

Fermentation is one of the oldest methods known in human history for preserving foods and was passed down orally from one generation to the next (1). This term originates from the Latin word *fervere*, which means "to boil" or "to cook," while its field of study is referred to as Zymology (4). As a significant milestone in the culinary cultures, fermentation is the process in which complex sugars and other macronutrients are broken down and converted into alcohol, carbon dioxide, and/or acids through the action of microorganisms (i.e., bacteria, yeast, and molds) in the absence of oxygen. Besides providing a unique savor to each food, fermentation is of great importance as it also enhances the nutritional value by increasing the digestibility and the synthesis of beneficial catabolites such as essential amino acids, Omega-3 fatty acids, and vitamins (e.g., B vitamins) (2,21).

Fermentation was known even before the invention of pasteurization and sterilization. The first fermentation was likely discovered by accident when salt was added to some foods, leading to the growth of harmless microorganisms that could ferment the raw ingredients, resulting in nutritious and palatable food. Since then, fermentation has been used to enhance both the preservation and sensory qualities of a wide range of food and beverages such as bread, beer, cheese, idli, tempeh, miso, sauerkraut, and fermented sausages (2,4,8). The earliest records of fermentation date back to antiquity, when Cagniard-Latour in 1837 proposed a direct link between fermentation and yeast cells. The history of yeast's association with human society is closely tied to the development of bread, beer, and wine as global food and beverage staples, with origins dating back approximately 5000 – 6000 years. Archaeological evidence suggests the production of a fermented beverage in China around 7000 BCE, the time of the appearance of an alcoholic drink produced from rice, fruit, and honey in the Neolithic Chinese settlement of Jiahu around 8000 BC. Referring to other reports, fermented products are believed to have been found in the Fertile Crescent region, specifically in the present-day city of Hillah in Iraq, encompassing Mesopotamia and the Eastern Mediterranean, dating back to around 6000 BCE. As well as the production of wine in Iran and Egypt around 6000 BCE and 3000 BCE,

respectively. The cultivation of grapevines and wine production spread throughout the Mediterranean, reaching Greece and Roman regions around 2000 BCE, Italy by 1000 BCE, Northern Europe by 100 CE, and the Americas by 1500 CE. Artisanal fermented food products were an important part of the human diet and were mostly produced locally or in small-scale industries (e.g., household and craft industries in rural regions in Africa). Today, many of these fermented foods are produced on a larger scale using innovative techniques following various industrial advancements in numerous developing countries, and that's the reason why only 27 years ago, reports focusing on Indigenous fermented products from various regions of the world began to emerge rapidly (22-26).

### 3.2. Types of Fermentation

Fermented foods are produced globally using various industrial methods, raw materials, and microorganisms. There are several classifications of fermentation depending on the product's physical status, the starter culture, the microorganisms used, and the byproducts obtained (Figure 1). The three types of fermentation based on the state of the fermented product are: solid-state (solid product like tempeh), liquid-state (liquid product like natto and soy sauce), and solid-liquid-state (semi-solid fermentation like the moist kinema). On the other hand, depending on the use of microorganisms, fermentation is divided into two main categories: natural fermentation (which can occur spontaneously in a natural environment without the addition of any starter culture) and controlled fermentation using specific starter cultures. Controlled fermentation with starter cultures is further divided into monoculture fermentation (e.g., natto fermentation) and multicultural fermentation (e.g., in cheese and air-dried sausage) using two or more cultures. Most traditional fermented products are made through solid-state fermentation, either spontaneously or using starter cultures, such as using the backsloping method at which a previously fermented product is inoculated into a new batch. During the fermentation process, molds in the form of threads or mycelium are commonly used in East and Southeast Asian countries, while bacteria and yeast-bacteria mixtures are prevalent in Africa, Europe, Australia, and America. Among the traditional foods of the Himalayas, there are fermented products that include all three groups (filamentous fungi, yeasts, and

bacteria). However, the bacteria that play a key role in the majority of fermented products are lactic acid bacteria (21,26,27). Nevertheless, fermentation is classified regarding the byproducts into: lactic acid fermentation, alkali fermentation, alcoholic fermentation, and acetic acid fermentation. Lactic acid fermentation, primarily driven by LAB, is commonly seen in the fermentation of milk and cereal grains. This process enhances the preservation, nutritional value, shelf life, and overall quality of a wide range of cereal-based foods. Alkali fermentation typically occurs in the fermentation of products such as fish and seeds, where protein hydrolysis results in the production of amino acids and ammonia. In alcoholic fermentation, the main byproducts are ethanol, with yeasts being the primary microorganisms involved. In acetic acid fermentation, certain microorganisms, such as *Acetobacter* species, convert alcohol into acetic acid in the presence of excess oxygen, playing a crucial role in food fermentation. One or more animal or plant raw food materials, such as cereals, meat, or fish products, can be fermented to obtain processed foods (28,29).

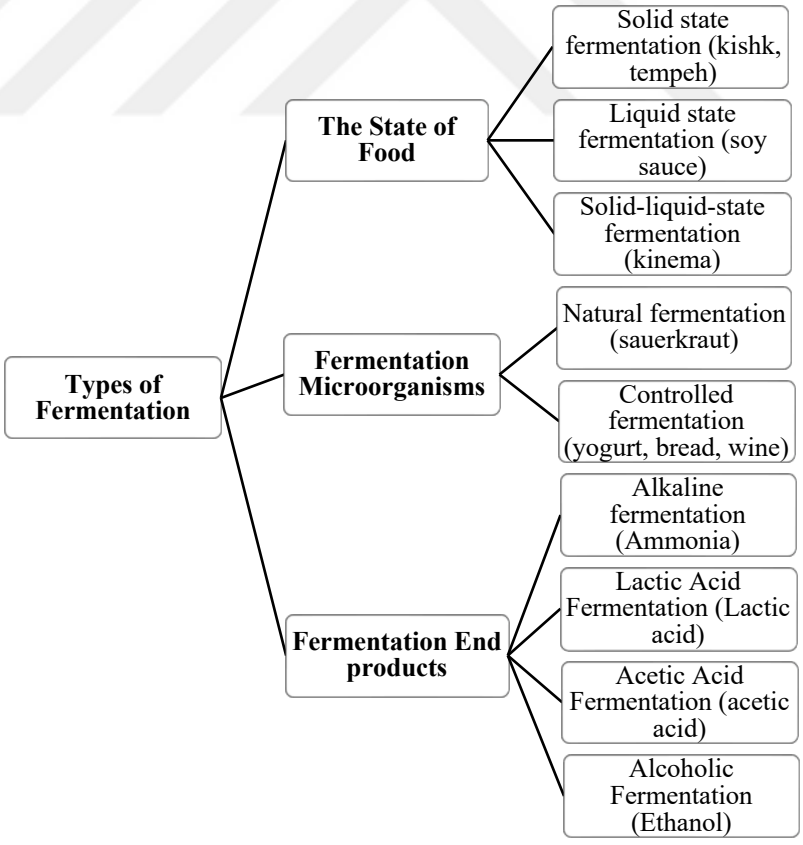


**Figure 1.** Classifications of the fermentation process upon different parameters.

### 3.3. Benefits of Fermentation

Preserving fresh and raw food materials for a long time throughout fermentation has always been a big advantage, as fermented food is characterized by a low pH of around 4,5 or below, at which the growth of most pathogenic microorganisms is restricted (1). Furthermore, fermented foods are superior to conventionally cooked foods in terms of nutritional value, digestibility (e.g., enhancing the quality and digestibility of plant-based proteins), availability of some amino acids, including lysine, and cooking or preparing time, providing microbiologically safer products with different desired tastes and textures. In addition, fermentation helps in the detoxification of toxic compounds in raw foods (e.g., pachyrrhizine, rotenone, tannins, and phytates), such as reducing 95% of lectins and other interfering substances in mineral absorption in tempe (30-33). Fermented products have antimicrobial properties due to their content of lactic acid bacteria's metabolites such as (lactic acid, acetic acid, hydrogen peroxide, ethanol, immunoglobulins, lysozymes, and lactoferrin compounds) along with their hostile characteristics to other microorganisms, which increases food safety by preventing the growth of harmful and spoilage microorganisms. This includes the mycotoxins and fungal growth, which are also suppressed due to the antifungal metabolites such as (lactic acid, phenyllactic acid, hydroxyphenyllactic acid, indole, and bioactive peptides) produced by LAB of *Lactobacillus*, *Bifidobacterium*, and *Lactococcus* genera. Research has demonstrated that some LAB, which dominate in various fermented foods such as Nigerian Ogi, Yakult, and Kefir, may facilitate the colonization of these microbes in the vagina and the gastrointestinal tract, thereby helping to reduce the risk of their infections (e.g., diarrheal and sexually transmitted diseases. These potential health benefits also include stimulating the immune system by inducing the presence of their associated microbiota, which also, in turn, suppresses the growth of disease-causing agents (34-37).

The global popularity of fermented products is increasing due to their health benefits. The antihypertensive effects of dairy products fermented by lactic acid bacteria have garnered significant attention over the past two decades. Studies have shown that fermented milk containing *Saccharomyces cerevisiae* can effectively lower both diastolic and systolic blood pressure, particularly in elderly individuals with

hypertension, attributed to the bioactive peptides, amino acids, and other bacterial metabolites produced during fermentation, which contribute positively to cardiovascular health. (38,39). Another example is soy, and others that have various bioactivities, including antioxidative, due to the phenolic compounds and folates, antiproliferative, and estrogenic effects. Soy sauce, natto, and kinema, which are soy-based fermented products, have antithrombotic and anticancer effects (40). The consumption of fermented products is linked to low blood cholesterol. Such as kefir, produced with *Lactobacillus acidophilus* and *Lactobacillus casei,* reduces serum cholesterol levels in the body and increases fecal *Lactobacilli* counts. Another example of this is Natto, which contains isoflavones, saponins, fibrinolytic enzymes, and vitamin K2 (38,41). The fermentation process increases vitamin K2 in Natto by 124 times, which is effective in bone formation and osteoporosis prevention in women. In Japan, fermented products were known to reduce osteoporosis symptoms when consumed post-menopause (41). They contain functional bacteria like *Lactobacillus*, *Lacticaseibacillus*, *Levilactobacillus*, and *Bifidobacterium* offer several health benefits, being GRAS (Generally Recognized As Safe). These include improving the gut microbiome, detoxifying the colon, treating chronic hepatitis, and alleviating intestinal and gut diseases such as bloating, flatulence, severe bacterial infections (e.g., *Clostridioides difficile* infection), and irritable bowel syndrome. In addition, there is an inverse relationship between the consumption of fermented dairy products (containing *Lactobacillus or Bifidobacterium* spp.) and the incidence of anxiety, depression, obesity, lactose intolerance, osteoporosis, cardiovascular diseases, and colon, lung, liver, and breast cancers. Consuming fermented products can also boost the oral microbiota and minimize tooth decay, gum disease, oral inflammation, and bad breath. In addition to their countless benefits, their management of lactose intolerance symptoms makes fermented foods anti-diabetic and FODMAP-reducing agents (41-43).

### 3.4. Microorganisms in Fermented Products

The microorganisms responsible for fermentation may either be naturally present in the food material or introduced as starter cultures, which are specifically selected to kick-start the fermentation. Fermentation involves the collaborative action

of bacteria (e.g., *Bacillus* and *Lactobacillus*), yeasts, and/or mycelial molds, which may function simultaneously or sequentially, with the microbial community evolving throughout fermentation. Among these microorganisms, yeasts (i.e., *Saccharomyces*) play a pivotal role in many fermented foods and alcoholic beverage production, specifically those rich in carbohydrates. In traditional fermentation, naturally occurring microorganisms are utilized for the preparation and preservation of diverse food products (44-46). Starter cultures are widely used around the world. The concept was well understood during the development of microbiology science in the twentieth century, leading to more uniformity in fermented food production. Starter cultures are specific, unadulterated, and well-defined microbial strains deliberately introduced into food under regulated conditions to achieve fermented products with desired sensory, nutritional, and preserving properties. Starter cultures used today are sold in liquid (fresh), lyophilized, frozen, or freeze-dried forms. They are microorganisms responsible for starting the fermentation process within food rapidly using their unique enzymes (47). The primary microorganisms of cultures used on a vast scale in the food industry are bacteria, particularly LAB (e.g., in cheese, yogurt, vinegar, fermented meats, and vegetables like sauerkraut and pickles), yeasts (in beer, bread, milk, and wine industry for ethanol production), and molds (in soy products, cheeses, and sausages. Starter cultures can consist of single-strain cultures (single microorganism type), multi-strain cultures, or mixed-strain cultures (including a mix of different strains of bacteria, yeast, or molds). In regions where fermented products are still produced in ranches and household areas, specifically in underdeveloped nations, the starter culture is often naturally soured milk with a pleasant flavor. In contrast, in more industrialized countries, specifically in large-scale fermented products industries, modern and controlled fermentation using selective pure cultures is preferred to impart the desired safety and quality to the final product (1,29,48).

### 3.5. Starter Culture Through Time

The first theory that describes the fermenters added to food substances as enzymes with an organic structure that break down carbohydrates through a catalytic effect was first proposed in 1836 by both Liebig and Berzelius. In 1856, French physicist Cagnar-Latour's discovery that the yeast in beer consists of cells, which

divide and multiply, brought new significance to the previous theory. In the late 19th century, researchers Storch, Weigman, and Conn recognized the role of flavor-producing and fermenting pure bacteria (such as citrate-fermenting diacetyl producers). By 1878, Christian Hansen launched a culture business that remains a prominent supplier of starter cultures for the dairy, meat, brewing, baking, and wine sectors to this day. In 1890, starter culture was produced and marketed commercially by Emil Christian Hansen and Christian D.E. Hansen in Hansen Laboratories. In 1906, the first commercial starter culture production took place at Marshall Laboratories (USA), marking the time when these cultures started to be known and produced around the world (46-49).

### 3.6. Lactic Acid Bacteria (LAB)

Bacteria that are used in the fermented products industry are divided into a) Lactic acid bacteria, b) Propionic acid bacteria, and c) Other bacteria. The most significant group utilized in the fermented food industry (e.g., yogurt, yakult, sour milk cream, cheese, kefir, kurut, kımız, fermented meat and fish) as starter cultures is the lactic acid bacteria. In the dairy industry, pasteurization and heat treatments are first applied to milk. After that, when milk reaches an optimum temperature (e.g., 110°F/43°C for yogurt), lactic acid bacteria are added, and fermentation takes place under anaerobic and controlled conditions. The decreasing pH and increasing acidity during fermentation, along with the production of antimicrobial substances (e.g., lactic acid, formic acid, $H_2O_2$, and bacteriocins), eliminate pathogens and enhance the texture, taste, and aroma of the final product. This process also extends the product's shelf life. Additionally, the low pH increases the antimicrobial potency of organic acids, making them more effective against harmful microorganisms. These bacteria ferment sugar to lactic acid as the end product. The fatty acids, amino acids, aromatic compounds (e.g., amines, ketones, esters, and acetaldehydes), postbiotics, and exopolysaccharides (EPS) produced from the lipolysis, proteolysis, and the breaking down of carbohydrates (e.g., lactose) by LAB contribute to the characteristic properties (e.g., casein hydrolysis in cheese) and safety standards of the product (45,50,51). Lactic acid is the oldest organic acid used in the dairy industry and can be utilized as an additive in preserving and improving the taste and texture by altering

food's acidity (e.g., grape juice, vinegar, and dough). Regarding safety, lactic acid and LAB are generally recognized as safe (GRAS) according to the U.S. Food and Drug Administration (FDA). LAB include a variety of Catalase (-), non-spore-forming, Gram (+), cocci (e.g., *Streptococcus* and *Leuconostoc*), and rods (e.g., *Lactobacillus*), nonmotile species across 11 genera, though only a few are commonly utilized as starter cultures. This group contains species of *Streptococcus*, *Leuconostoc*, and *Pediococcus* from the *Streptococcaceae* family, and species of *Lactobacillus* from the *Lactobacillaceae* family. Among them, *Lactococcus lactis* subsp. *lactis, L. bulgaricus, L. plantarum, S. lactis, S. cremoris, S. lactis* subsp. *diacetylactis, S. thermophilus, Leuconostoc, and Propionibacterium* are the most commonly used. *S. thermophilus* and *L. bulgaricus* are widely used in yogurt and Mozzarella cheese production (Table 1). LAB undergoes two types of fermentation: homofermentative (99% LA) and heterofermentative fermentation (50% LA, 30% other acids such as acetic acid). Producing propionic acid, formic acid, acetone, acetaldehyde, and diacetyl at the end of fermentation (Figure 2) (28,45,50,52).

**Table 1**. Some important lactic acid bacteria and their application as starter cultures

| **Microorganism** | **Fermented Product** |
|---|---|
| *Lactococcus lactis* subsp. *lactis* | Cheddar, Gouda, and Mozzarella cheeses and dairy products |
| *Lactococcus lactis* subsp. *cremoris* | Brie, Cheddar, Mozzarella and Cottage cheese and other dairy products |
| *Lactobacillus helveticus* | Swiss cheese, hard cheese and Italian cheeses such as Mozzarella and Parmesan. |
| *Lactobacillus bulgaricus* & *Streptococcus thermophilus* | Yogurt and cheese |
| *Lactobacillus sanfranciscensis* | Sourdough bread |
| *Leuconsotoc lactis* | Cheese & fermented dairy products |
| *Leuconsotoc mesenteroided* subsp. *cremoris* | Cheese, fermented dairy products, and fermented vegetables |
| *Lactobacillus sakei* | Sausages |
| *Oenococcus oeni* | Alcoholic beverages |
| *Pediococcus halophilus* | Soy paste |

*Data compiled from Wood & Holzapfel (1995), Leroy & De Vuyst (2004), and Kavitake et al. (2018) (28,45,52).

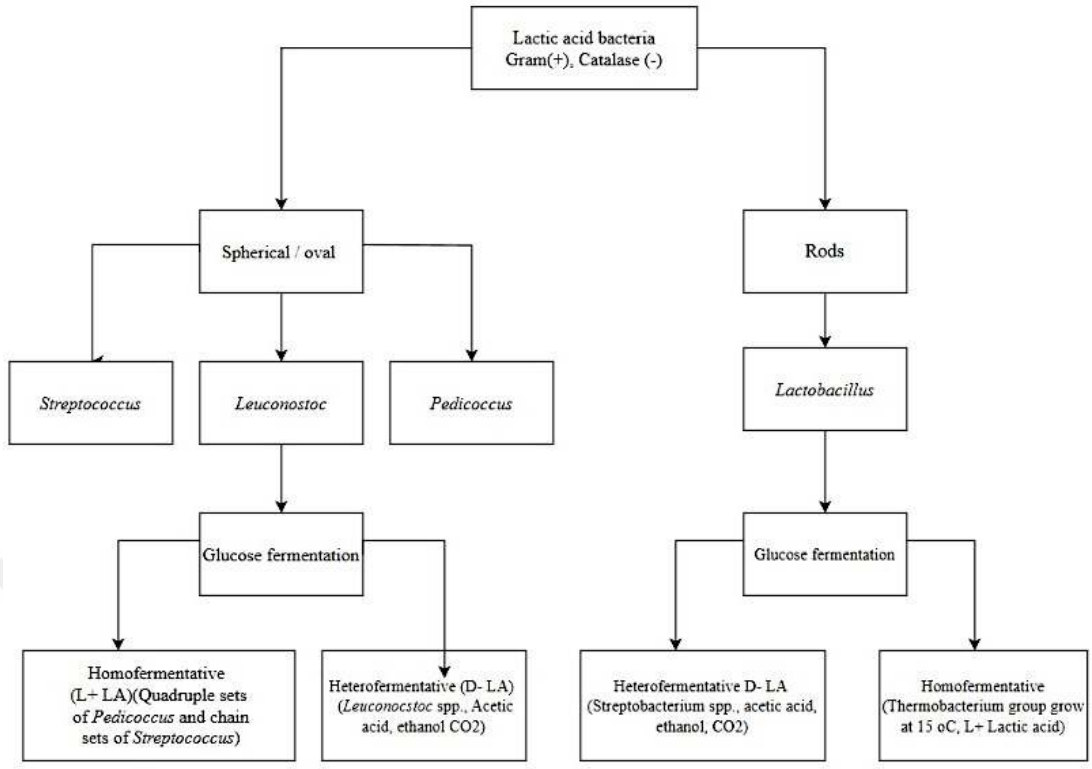


**Figure 2.** General distinction of lactic acid bacteria (LAB).

### 3.6.1. *Lactobacillus bulgaricus*

The species under the *Lactobacillus* genus are thin, long, or short rod-shaped, or more commonly, coccobacilli microorganisms. They require complex nutrients such as amino acids, peptides, nucleic acid derivatives, vitamins, salts, fatty acids or esters, and fermentable carbohydrates. The bacteria in this lineage break down glucose through a homofermentative pathway while only one species is heterofermentative (i.e., *L. brevis*). *Lactobacilli* are used as starter cultures primarily in dairy and sausage applications. The two main species that are used mainly for cheese and yogurt are *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus*. Other common dairy-related species include *L. casei* and *L. acidophilus,* which are used frequently in probiotic supplements, kefir, yogurt, and cheese production. *Lactobacilli* are acid-tolerant microorganisms. Their optimal temperature is 40-45°C, and their ideal pH is 4,8, but they can survive at lower pH levels (between 3,6 & 4), becoming more stressed specifically at levels of 2,5 – 3,5, and die below that margin (50,53).

According to their fermentation types, metabolic and genetic properties, *Lactobacilli* are divided into three main groups: Obligate homo-fermenting, facultative hetero-fermenting, and obligate hetero-fermenting species. The obligate homo-fermenters are bacteria that exclusively produce lactic acid as the major end product of sugar fermentation through the Embden-Meyerhof-Parnas pathway (glycolysis), such as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus helveticus.* They are used in the production of yogurt, cheese, and other dairy products (51,53). *Lactobacillus bulgaricus*, formerly *Lactobacillus delbrueckii* subsp. *bulgaricus, a* third species of the strain *L. delbrueckii,* is a thermophilic, dioxygen-sensitive, homo-fermenter, and rod-shaped bacterium that can ferment in both anaerobic and aerobic environments. It was Grigoroff, a Bulgarian graduate student, who first isolated and identified the *lactobacilli* bacteria from the starter culture used to produce Kiselo Mlyako (Bulgarian Yogurt). Grigoroff initially named the bacterium "Bacillus A," which is now recognized as *L. bulgaricus* according to Bergey's bacterial classification. It plays a significant role in industrial fermentation processes globally, especially in the manufacturing of yogurt, whereas it's commonly utilized with *Streptococcus thermophilus* to ferment pasteurized milk (54).

### 3.6.2. *Streptococcus thermophilus*

The genus *Streptococcus* contains many diverse species with a wide array of habitats. It includes human and animal pathogens, oral and intestinal commensals (e.g., cows, sheep, and goats), and one species, *Streptococcus thermophilus*, which is considered the second most important industrial starter after *lactococci* in the manufacture of fermented foods. In general, *Streptococci* are facultative anaerobes with a non-motile, obligate homofermentative metabolism. *S. thermophilus* or *Streptococcus salivarius* subsp. *thermophilus*, in novel nomenclature, is a member of the thermophilic LAB and has been traditionally used in combination with *L. bulgaricus* or *Lactobacillus helveticus* at high process temperatures (45$^{o}$C) for the manufacture of yogurt and so-called hard 'cooked' cheeses (e.g., Emmental, Gruyere, Grana). This bacterium is also used alone or in combination with *Lactobacilli* for the production of yogurt, Mozzarella, and Cheddar cheeses. Interestingly, some strains of *S. thermophilus* (e.g., *S. thermophilus* S85) also have a reputation as probiotics when

consumed in yogurt. Generally, *S. thermophilus* is more commonly associated with fermented dairy products (e.g., yogurt, kefir, sour cream, and cheeses such as cottage, mozzarella, gouda, and Swiss cheese) (55-57).

### 3.6.4. Lactic acid bacteria as probiotics

Probiotics are "live microorganisms that, when consumed in adequate amounts, provide health benefits to the host or consumer." The most common benefits of probiotic consumption include the modulation of the host's immune system and the enhancement of defense mechanisms. To comply with the National Yogurt Association's criteria for "live and active culture yogurt," the fermented product must contain live lactica acid bacteria at a concentration of $\geq 10^{6\text{-}8}$ CFU (Colony Forming Unit)/g, with the cultures remaining viable throughout the declared shelf life, that's when these cultures are considered probiotics. Some fermented products, such as kefir and kumis, have been associated with additional therapeutic effects, primarily due to the presence of probiotic lactic acid bacteria they may contain, including *Lactobacillus acidophilus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Propionibacterium*, *Bacillus*, and *Enterococcus* species (58-60). *Lactobacillus* and *Bifidobacterium* are widely recognized bacterial species commonly utilized as probiotics in the dairy products production. For instance, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* are widely used in the production of probiotic yogurts and dietary supplements due to their viability in fermented dairy matrices. In contrast, *Bifidobacterium bifidum* and *Bifidobacterium longum* are more frequently incorporated into infant formula products, owing to their prevalence in the intestinal microbiota of infants. The genus *Leuconostoc* commonly includes *Leuconostoc lactis*, which is found in certain cheeses, and *Leuconostoc mesenteroides*, which is typically associated with the fermentation of vegetables, including traditional foods like kimchi.

Regular inclusion of fermented products with probiotics in the diet is crucial for maintaining their health benefits. These foods provide a combination of probiotics, prebiotics, and postbiotics, making them highly nutritious. (60,61). Factors like fermentation conditions, pH, oxygen, storage temperature, and exposure to bile salts influence their survival. Recent studies confirm that *Lactobacillus bulgaricus* qualifies as a probiotic bacterium, with Nobel laureate Metchnikoff's research linking the health

and longevity of the Bulgarian population to their high yogurt consumption. Specific strains of *Streptococcus thermophilus* also exhibit probiotic properties by adhering to intestinal cells using their anti-inflammatory traits. Encapsulation is the current key strategy used to enhance the survivability of these probiotics in products like Greek-style yogurt, yogurt ice cream, and probiotic yogurts. This advancement has led to the use of these bacteria also in medical supplements (53,61,62).

### 3.7. Food-Related Illnesses and Some Important Foodborne Pathogens

The connection between human diseases and food was identified as early as 460 BC by Hippocrates. Food hazards can be present in food at any stage, from production to consumption. These hazards are either biological (e.g., bacteria, viruses, parasites), chemical (e.g., toxins), or physical. Food-related illnesses commonly occur when contaminated food or beverages are consumed and are usually caused by biological and chemical hazards. These illnesses are classified into six categories: bacterial infections, viral infections, parasitic infections, fungal infections, toxic chemicals, and foodborne toxins. Hence, foodborne diseases can be divided into (a) foodborne infection and (b) foodborne poisoning (63).

Foodborne diseases continue to pose a global public health threat. According to CDC estimates, forty-eight million people in the United States suffer from foodborne illnesses annually (https://www.cdc.gov/foodborneburden/index.html, Access date: 09 May 2025). Among these, 128 000 hospitalizations and around 2999 deaths were recorded. The recent report (1996-2023) documented by the Foodborne Diseases Active Surveillance Network (FoodNet) indicated that species of the *Enterobacteriaceae* (e.g., *Salmonella* spp., *Shigella* spp., *Yersinia,* and *E. coli* O157:H7), *Campylobacteraceae* (*Campylobacter* spp.), and *Listeriaceae* (*Listeria monocytogenes*) families, along with Norovirus, are the primary pathogens responsible for the highest number and incidence of laboratory-confirmed foodborne infections in the United States (https://www.cdc.gov/foodnet/reports/preliminary-data.html, Access date: 09 May 2025). In 2023, 27 European Union Member States, along with the United Kingdom (Northern Ireland), reported a total of 5961 food-related outbreaks, 52 127 cases of human illness, 2894 hospitalizations, and 65 deaths, marking the highest number of fatalities reported in the past decade

https://www.efsa.europa.eu/en/topics/topic/monitoring-foodborne-diseases, Access date: 09 May 2025). According to the European Union One Health Zoonoses report conducted in 2023 in 27 Member States (MSs), Northern Ireland (United Kingdom), and 10 non-Member States (non-MSs), campylobacteriosis and salmonellosis were the most commonly reported zoonoses in humans, ranking first and second, respectively (64). More than 246 000 human cases of campylobacteriosis are reported annually. According to EFSA, the annual economic burden of campylobacteriosis on public health systems and lost productivity in the EU is approximately €2,4 billion, with a belief that the true number of cases exceeds 9 000 000 each year.

In the same vein, *Salmonella* was the most frequently identified pathogen, responsible for 17,9% of all reported outbreaks, with *Salmonella* Enteritidis being the most common serovar detected by the European Food Safety Authority and European Centre for Disease Prevention and Control. 77 486 human cases (18 cases per 100 000 people) of Salmonellosis were reported in 2023, keeping it the second most reported foodborne disease since 2005. The top 2 of the *Salmonella enterica*'s non-typhoidal serovars are responsible for the vast majority of the cases acquired in the European Union were *S.* Enteritidis (responsible for 70,8% of non-typhoidal salmonellosis in Europe) and *S.* Typhimurium (8,9%), are considerable public health issues (64). On the other hand, STEC infections were the third most commonly reported gastrointestinal foodborne diseases in humans in the European Union, causing 3,1 cases per 100 000 people (64). While the CDC estimated that the O157:H7 strain of *E. coli* causes approximately 73 000 illnesses, 2200 hospitalizations, and 60 deaths annually in the United States of America, accompanied by about $405 million economic loss due to medical and livestock expenses and premature mortality upon last year's reports. (65). As Ota et al. (2019) reported, the most significant recorded Shiga toxin-producing *Escherichia coli* outbreak was caused by the O157 strain in 1996 in Sakai, Japan, impacting over 8000 people (66,67). The most recent *E. coli* O157:H7 outbreak in the US was likely linked to fresh onions served at McDonald's, affecting 104 people across 14 states in 2024. According to the CDC, FDA, and the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), 34 were hospitalized, 4 developed HUS, and one death was reported

(https://www.cdc.gov/ecoli/outbreaks/investigation-update-e-coli-o157-2024.html, Access date: 09 May 2025).

*Listeria monocytogenes* is often linked to the most severe outbreaks, characterized by high rates of hospitalizations and fatalities. Listeriosis was the most prevalent zoonotic infection reported following the previously mentioned diseases in the same year. Around three thousand confirmed invasive human cases of *L. monocytogenes* were reported (0,66 cases per 100 000 people) within the EU. This represents the highest number of cases reported since 2007, emphasizing a statistically significant increase over the 2019–2023 period (https://storymaps.arcgis.com/stories/629e6627e6c64111bfd5b9257473c74a, Access date: 09 May 2025). Recent outbreaks linked to fermented foods have highlighted the importance of proper food safety practices. For example, a large outbreak occurred across multiple regions in the Castellón province of Spain in 2011. This outbreak resulted in 83 confirmed *Salmonella* Typhimurium and *Salmonella* Derby cases that were directly linked to the consumption of dried pork fermented sausage. 80% of the patients were hospitalized and experienced symptoms from mild to severe gastroenteritis (68).

### 3.7.1. *Campylobacter* spp.

*Campylobacteraceae* is a family of microaerophilic, Gram-negative, non-spore-forming bacteria that comprises 27 species, including *Campylobacter jejuni*, *C. coli*, and *C. fetus*, among others. These bacteria are typically found in animals' intestines, including cattle, sheep, poultry, pets, and wild animals, with poultry and turkeys being the primary reservoirs. Often asymptomatic in animals, *Campylobacter* species thrive at temperatures around 41°C, making their survival outside hosts and during food processing and storage limited. Bacterial growth is inhibited by many factors such as thermal treatments (e.g., pasteurization) and pH levels below 4,9. Their optimal pH to develop ranges from 6,5 to 7,5. In humans, *C. jejuni*, *C. coli*, and *C. lari* are the main causes of campylobacteriosis, which is characterized by watery diarrhea, abdominal cramps, and fever, and it is reported by the CDC as the first most domestically acquired foodborne illness (https://www.cdc.gov/food-safety/php/data-research/foodborne-illness-

burden/?CDC_AAref_Val=https://www.cdc.gov/foodborneburden/burden/index.htm, Access date: 09 May 2025). According to the European Food Safety Authority (EFSA), campylobacteriosis is the leading zoonotic infection in Europe, with over 229 000 cases reported in 2014 and a rising incidence since 2008. The majority of infections are common in summer and associated with broiler meat, although the fatality rate remains low (around 0,03%). Notable outbreaks included one in East England in 2011, followed by another one that occurred in Australia in 2013 due to undercooked chicken liver pâté and others linked to cross-contamination in colleges and schools' restaurants, highlighting the importance of food-handling practices in preventing such infections (69).

### 3.7.2. *Salmonella* spp.

*Salmonellosis* outbreak (SO) is one of the top reasons for gastroenteritis worldwide, impacting 10% of the global population each year. *Salmonella* is a Gram-negative, non-spore-forming, rod-shaped, and facultatively anaerobic bacterium with over 2600 serovars. Serotypes are classified into two groups: typhoidal serotypes (responsible for typhoid and paratyphoid fevers) and nontyphoidal serotypes (such as *S.* Typhimurium, which can produce exotoxins and cause acute gastroenteritis in humans). All *Salmonella* species produce endotoxins since they are part of the structure of the bacterial cell wall, specifically lipopolysaccharides (LPS). Some like *Salmonella enterica* and *S. bongori* are commonly found in the intestines of mammals, birds, and humans, as well as in water and sediments. *Salmonella* can be eliminated through thermal processing with varying D-values depending on the food. The Non-typhoidal *S. enterica* subsp. *enterica*'s serovars are the most associated with foodborne illnesses and worldwide outbreaks, causing enteric fever, diarrhea, bacteremia, and chronic carriage (70). Non-typhoidal *Salmonella* (NTS) causes an estimated 150 million infections and 60,000 deaths globally each year (CDC Yellow Book: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/salmonellosis-nontyphoidal, Access date: 01 May 2025). In 2015, NTS infections (i.e., NT Salmonellosis) were the second most prevalent zoonotic disease in Europe, with *S.* Enteritidis and *S.* Typhimurium accounting for the majority of cases, typically linked to poultry, turkey, pig, and bovine meat. Salmonellosis peaks in

summer, due to inadequate sanitation, poor handling, and improper cooking practices (70). Notable outbreak was the 2009 incident in a London prison traced to egg cress rolls (71). In the same year, a 346-case outbreak in Diyarbakır in Türkiye, specifically in hotels and schools, was linked to contaminated chicken and potato main dish served with salad (72). Another large-scale multinational outbreak of *S. Typhimurium* in 2022 spread across 10 countries of the European Union (EU)/European Economic Area (EEA) and the United Kingdom (UK), affecting 150 individuals, mostly young children. It was traced to chocolate products made by a major multinational company, leading to widespread recalls of various product lines (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON369, Access date: 09 May 2025).

### 3.7.3. *Escherichia coli*

The *Escherichia* genus, classified within the *Enterobacteriaceae* family, part of the *Gammaproteobacteria* class, consists of Gram-negative, non-spore-forming, oxidase-negative, indole and catalase-positive, non-spore-forming, facultatively anaerobic, rod-shaped coliform bacteria. These bacteria are classified into six species: *E. coli*, *E. blattae*, *E. hermannii*, *E. vulneris*, *E. fergusonii*, and *E. albertii*. Among these, *E. coli*, first identified in 1885 by the German Microbiologist Theodor Escherich, is the most significant due to its role in causing gastrointestinal diseases. *E. coli* grows most effectively at 37°C (98°F), though some laboratory subtypes are capable of thriving at temperatures as high as 49°C (120,2°F). Due to its minimal nutritional demands, *E. coli* can be readily cultivated on standard media, including Nutrient agar, MacConkey agar, Eosin Methylene Blue (EMB) agar, and some strains on blood agar (73). Renamed in 1919 to honor its discoverer, *E. coli* classification has evolved to include unique features that distinguish it from other enteric species. Certain strains are recognized as beneficial probiotics and are utilized in pharmaceutical products, while most strains are harmless; some can cause illnesses like diarrhea, urinary tract infections (UTIs), and kidney or respiratory infections when ingested through contaminated food or water. Pathogenic *E. coli* strains acquire virulence factors through mechanisms like plasmids, toxins, bacteriophages, and pathogenicity islands and are classified based on serogroups and clinical symptoms.

*E. coli* is categorized into 150 to 200 serotypes determined by three key antigens: the somatic (O) antigen, the capsule's (K) antigen, and the flagella (H) antigen (73). In humans, it can be categorized into three main groups: commensal, intestinal pathogenic *E. coli* (IPEC), and extra-intestinal pathogenic *E. coli*. Pathotypes of IPEC include enteroaggregative (EAEC), Enteroinvasive (EIEC), diffusely adherent (DAEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), and Shiga toxin-producing (STEC, VTEC) or enterohemorrhagic (EHEC) strains. They are the natural inhabitants of the gastrointestinal tract of farm animals, pets, wild animals, and humans. *E. coli* contamination from cattle, especially STEC, is particularly concerning due to its risk in meat, milk, and related products. Thermal treatments, such as pasteurization, can effectively reduce *E. coli* levels, varying D-values depending on food type (73,74). However, when thermal processing fails, or contamination occurs, ingestion can lead to severe health risks, ranging from mild diarrhea, fever, abdominal pain, and nausea to more serious conditions affecting the urinary tract, bloodstream, and central nervous system such as meningitis in newborns and dehydration in children caused by ETEC and EPEC (73,74).

#### 3.7.3.1. Enterohemorrhagic *Escherichia coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC)

Recent research has identified the seven main phylogenetic groups (A, B1, B2, C, D, E, F) using various methods such as PCR, MLST, and 16S rRNA sequencing. The most common pathogenic phenotypes related to foodborne illnesses fall into group E, including EHEC, such as *E. coli* O157:H7 (73,74). EHEC is the major pathogenic group linked to human diarrhea, with strains producing verotoxins (VTs), which are toxic to Vero cells. Due to their similarity to Shiga toxins (stx1-stx2) from *Shigella dysenteriae* type 1, these strains are also called STEC, and both are considered EHEC. In other words, EHEC is a subset of STEC, referring to strains that cause bloody diarrhea and more severe outcomes such as hemolytic uremic syndrome (HUS) and often death. EHEC/STEC infections range from mild diarrhea to severe conditions such as hemorrhagic colitis, thrombotic thrombocytopenic purpura (TTP), and HUS, a condition marked by kidney failure and bloody or non-bloody diarrhea caused by Stx1 and Stx2. Around 19% of cases develop serious complications, with acute kidney

failure from HUS being the most severe. Cattle serve as the primary reservoir for EHEC, which is commonly found in animal-based foods like beef, as well as raw milk, dairy products, and leafy greens. The pathogen is highly infectious, with even a small dose, as few as 10 CFU, capable of causing illness (73,74).

#### 3.7.3.1.1. *E. coli* O157:H7

The most well-known and the first recognized EHEC/STEC serotype as a human pathogen associated with bloody diarrheal outbreaks and kidney failure in the U.S., specifically in children, is *E. coli* O157:H7. However, it is a major cause of food and waterborne outbreaks in several countries, including the United States, Europe, Japan, the Middle East, and the United Kingdom. The "O157" refers to the somatic antigen, and "H7" represents the flagella antigen of this strain. Cattle are the main carriers of *E. coli* O157:H7, often without showing symptoms, though it can also be present in animals like pigs, sheep, and turkeys. The bacteria can spread through contaminated food, water, leafy greens, direct human contact, or exposure to infected animals and feces. The production of Shiga toxins by *E. coli* O157:H7 within the intestinal epithelium (intoxication) and bloodstream plays a crucial role in causing HUS, which leads to severe gastrointestinal diseases from diarrhea to hemorrhagic colitis. HUS can progress to systemic complications, including hemolytic anemia, thrombocytopenia, acute kidney failure, and long-term health issues. The STEC outbreaks show seasonal peaks in summer. The initial recorded incident of *E. coli* O157:H7 occurred in 1982, originating from tainted hamburgers served at a U.S. fast-food chain, subsequently earning it the moniker "burger bug" (73-77). To minimize infections from contaminated meat products and other vehicles (e.g., irrigation water, leafy greens, and dairy products), proper handling, heat treatments, and industry practices must be aimed at reducing cross-contamination at all stages of production (75-78). In August 2012, a massive outbreak of enterohaemorrhagic *Escherichia coli* O157 infection occurred in Hokkaido, Japan, affecting 94 nursing home residents. The investigation was conducted by the City of Sapporo and the Hokkaido Prefectural Government and reported 107 total cases, including five deaths, of which 95% of the cases had consumed pickles from a single production facility. The outbreak was caused by improper vegetable sanitation at the production site (79). Similarly, the first Canadian

*E. coli* O157 outbreak related to kimchi consumption was investigated in January 2021-2022. Across two provinces in the western part of Canada, 91% of 14 cases were confirmed to be sick due to their consumption of fermented vegetables (80). Illness spikes during the current century include the large STEC outbreak that happened in 2015 in the EU and resulted in 5901 infected people with eight fatalities sourced from contaminated meat and dairy products (https://www.ecdc.europa.eu/sites/default/files/documents/STEC-infection-AER-2020-JD-FINAL.pdf, Access date: 09 May 2025).

### 3.7.4. *Listeria monocytogenes*

The *Listeria* genus comprises 17 recognized species of Gram-positive, rod-shaped, catalase-positive, non-spore-forming, and non-capsulated bacteria. Of these, *L. monocytogenes* and *L. ivanovii* are pathogenic to humans. *Listeria* is commonly found in soil and water, plants, animals, and humans. Food contamination with *Listeria* can be reduced through pasteurization (D-values for *L. monocytogenes* in minced beef ranging from 0,15 to 36,1 minutes at 50 - 60°C). Their growth is inhibited at pH values below 4,3. Infection with *L. monocytogenes* causes abdominal pain, diarrhea, fever, myalgia, confusion, and neck stiffness, with severe symptoms like facial paralysis and pleural effusion in pregnant women. Listeriosis outbreaks have been linked to contaminated foods such as ice cream, leafy greens, mushrooms, deli meats, dairy products, cooked chicken, hard-boiled eggs, frozen vegetables, bean sprouts, and soy products, resulting in significant fatalities. A 2023 outbreak in the U.S. involved illnesses from leafy greens, particularly iceberg and romaine lettuce, and packaged salads across 16 states, which were contaminated with *L. monocytogenes*, leading to 19 cases of listeriosis. According to the CDC, a similar outbreak linked to vanilla ice cream in Florida in 2022 resulted in 28 hospitalizations and one death. Over the past decade, about 30 *Listeria* outbreaks led to 524 reported cases of listeriosis and 80 fatalities, with a case fatality rate of 15,26% (81, https://www.cdc.gov/listeria/outbreaks/index.html, Access date: 09 May 2025). In the EU, the listeriosis cases peaked at their highest level in 2023 since 2007. To date, 2200 listeriosis cases were reported in 2015, with a mortality rate of 270, primarily affecting elderly people. Over 400 cases and 60 deaths (50% of the reported cases) caused by

dairy product-related listeriosis have also been recorded, being one of the common causes of illness (https://www.efsa.europa.eu/en/news/zoonotic-diseases-rise-eu-listeriosis-cases-hit-highest-levels-2007, Access date: 09 May 2025). Listeriosis outbreaks, like the one in Switzerland occurred between 1983 and 1987 linked to unpasteurized soft cheese, the one in Austria, caused by unpasteurized milk in 1986, and the other one in France in 1995 that involved a Brie-style cheese also made from raw milk, highlight the risks associated with consuming raw milk or fermented cheeses made from unpasteurized milk (81,82).

### 3.7.5. *Staphylococcus aureus*

*Staphylococcus aureus* (*S. aureus*) is a Gram(+) bacterium and a leading cause of food poisoning worldwide due to its widespread presence on human skin and nasal passages, its ability to spread through the air, and its strong resilience in various environments. It can outcompete other microorganisms, surviving high temperatures, osmotic pressure, and low humidity. *S. aureus* can harbor various Staphylococcal Enterotoxins (SEs) genes and generate a range of heat-stable toxins (i.e., SEs) along with SE-like toxins, which are the key contributors to food poisoning outbreaks. Staphylococcal food poisoning (SFP) occurs when *S. aureus* produces the SEs in contaminated dairy, meat, and fish after poor handling practices when introduced by an infected or colonized person. These infections are distinguished by rapid-onset symptoms such as vomiting, diarrhea, nausea, and abdominal cramps within one to seven hours of consumption (83). In August 2019, an SFP outbreak was caused by a contaminated chicken salad prepared at a nursing home in Piedmont, Italy. Among 33 individuals who ate the chicken salad, 11 (9 guests and two healthcare workers) developed gastrointestinal symptoms that resolved within 10 hours (83,84). About 20 types of SEs have been identified, with the five main types responsible for most food poisoning cases (toxin-mediated illnesses). However, *S. aureus* can cause fatal health problems that affect the skin, internal organs, and wound sites of surgeries. Examples include skin and soft tissue infection (SSSI), Staphylococcal Scalded Skin Syndrome (SSSS), and Toxic shock syndrome (TSS). Osteoarticular infection, Ocular infections, Infective Endocarditis, and Pneumonia are the common forms of *S. aureus* infection

symptoms in children and infants. Beyond foodborne illnesses, *S. aureus* contributes to ruminant mastitis, a significant economic concern in agriculture. (83,84).

### 3.7.6. Others

Other bacterial pathogens, such as *Clostridium perfringens, Bacillus cereus, Shigella*, *Vibrio,* and *Yersinia,* along with parasitic and viral pathogens such as *Toxoplasma gondii, Cyclospora*, *Cryptosporidium,* and Norovirus, also cause severe gastrointestinal infections worldwide. About 9,9 million foodborne diseases in the US are related to such pathogens, estimated in 2019 by the CDC. Immunocompromised people, infants, children younger than 5 years old, the elderly, hospitalized individuals, and pregnant women are the most affected groups. (https://www.cdc.gov/foodborneburden/burden/index.html, Access date: 09 May 2025).

## 3.8. Fermented Foods

Fermented foods and beverages are fundamental components of the dietary culture of every society, embodying the cultural heritage of ethnic communities. Although the role of microorganisms in fermented foods was not understood, these products were skillfully produced on small scales a long time ago. With the emergence of microbiology as a scientific discipline in the mid-19th century, biological knowledge has facilitated a better understanding of fermented products and the health benefits attributed to their cultures, resulting in a global interest in them. Broadly produced and consumed fermented products are lately defined by a specialist committee from the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "foods produced through controlled microbial growth and enzymatic transformation of food components." This classification encompasses all fermented products from different food origins, regardless of whether live microbes are present, as long as they are part of the food matrix at the time of consumption (1,2,8,25). Globally and locally, there is a diverse range of fermented products produced from various ingredients {including dairy (e.g., kefir, kumis, kurut, and dried yogurt), vegetables (e.g., East Asian Kombucha, Korean Kimchi, Sauerkraut, Himalayan Gundruk, and Japanese Sunki), legumes and beans (e.g., Chinese *Douchi* and Soy Sauce, Nigerian Iru and Origi, Indonesian Tempeh, Japanese Natto and Miso, and

Korean Meju, Doenjang, and kochujang), honey and shrubs (e.g., the Ethiopian T'ej or Mies), grains (e.g., the Turkish drink *Boza*, Zimbabwean drink *Mahewu*, and Southeast Asian *Idli*, *Ogi,* and *Dosa*), combinations of grains and milk (e.g., tarhana, and kishk), fish products (e.g., Fish Sauce and Norwegian *Rakfisk*), meat products (e.g., Salami and dry fermented sausages) and alcoholic beverages (Mexican *Pulque*, and the East African *Pombe* and *Tari*)}. All are generally regarded as safe (GRAS) considering their acidic environment and microbial content, and favored for their taste. They offer benefits such as preserving perishable foods, enhancing nutritional value, providing antioxidants, therapeutic properties, and positive immunological effects. Nowadays, 5-40% of the total daily intake (about 50-400 grams per person) is made up of fermented foods and beverages worldwide (3,4-7,85-87).

### 3.8.1. Cereal-milk-based fermented products

Cereal-milk-based fermented products are those that combine cereals with curdled milk. A well-known example is the fermented blend of milk and wheat, 'Kishk'. Nations of the Southern Mediterranean share similar food habits that have mostly relied on such fermented products for centuries. This is because milk has always been considered a superfood representing existence, acceptability, nutrition, and productivity. Usually, cow's milk is preferred due to its availability and economic advantages, while some Asian and African areas prefer camel and buffalo milk due to their nutritional value. On the other hand, Cereals play a significant role in human nutrition, filling stomachs, and protecting individuals from hunger in many developing countries. Being composed of key nutrients like starch granules and essential nutrients (e.g., amino acids, sugars, and lipids), the use of cereals in fermented products serves as a source of plant-based protein, carbohydrates, minerals, and fiber in the daily diet. Although the protein content of cereals is low, with a deficiency in essential amino acids such as lysine and threonine, fermentation is the easiest and most economical method to enhance the nutritional value of cereals by increasing plant-protein digestibility, and helps break down proteins into amino acids. When cereals are combined with a fermented milk base, the insufficient nutrients in milk are supported by cereal nutrients, and vice versa. The highest demand for milk and cereal-based

fermented products worldwide is recorded in Europe and North America. One of the most prevalent examples of such fermented products is Kishk (3,88-91).

### 3.8.2. Some pathogens in cereal-milk-based fermented products

With their significant role in diets of numerous cultures, such as African and Middle Eastern societies, cereal-based dairy fermented products such as Akpan (West African fermented beverage made of corn/millet and milk (89,91), tarhana, and kishk can sometimes be vehicles for not only their nutrients but also for foodborne pathogens. These pathogens are usually *Salmonella*, *Yersinia enterocolitica, Campylobacter* spp., *E. coli* O157, *L. monocytogenes*, *B. cereus*, *C. botulinum*, aflatoxins, and parasites. Considering the main ingredients, these products can contain both milk-originated and/or cereal-originated pathogens. For example, fungi pose significant challenges to cereal crops worldwide, producing heat-stable mycotoxins, which can cause gastroenteritis, kidney failure, or cancer in humans and animals. Approximately 5-10% of global food production is lost to fungal spoilage, particularly affecting the cereal industries. Sometimes fermentation and heat treatment reduce fungal contamination, but may not eliminate it; that's why following safety standards is important (92). For example, 21 out of 40 samples of tarhana chips (52.5%) tested positive for AFM1 according to Özçam et al.'s study (93). and those conducted also by Colak et al. in 2011, in which out of the 138 tarhana powder samples, 29 samples contained Aflatoxin B1, ranging from 0,2 to 13,2 μg/kg (94). The presence of other pathogens, such as *E. coli* O157:H7, *Staphylococcus*, *Salmonella*, and *Shigella*, is also possible in such products. Such as those detected in Nigerian Ogi samples studied by Ajayi and Ogunleye (2022) (95).

In turn, milk may also serve as a source of harmful microbes or toxins in fermented products and threaten health if it undergoes poorly controlled or non-sterile production, transportation, and storage conditions, especially in underdeveloped or developing countries that lack Good Manufacturing Practices (GMPs) (96). Microorganisms in raw milk are classified into three groups: lactic acid bacteria, coliforms, and pathogens. Thus, aflatoxins ($AFM_1$) might be contributed by the milk base in cereal-milk-based fermented products, as reported in the studies by Aydın et al. (2023), Ipek and Erkan (2024), and Yeşil et al. (2019) (97,98). Moreover,

*Salmonella, Staphylococcus,* and *E. coli* O157:H7 can also exist, such as those identified in all the yogurt samples collected from regional Egyptian markets in Giza by Motawee et al. (2016) (99). Yakubu et al. (2018) confirmed the presence of *E. coli* O157: H7 within both raw milk and 2% of the fermented milk samples collected from Sokoto City's markets in Nigeria (100). Such studies suggest possible fecal contamination within milk-based fermented products and underscore that proper production conditions are critical in preventing potential outbreaks and safeguarding public health (96-100).

### 3.10. Kishk

Kishk, also spelled keshk or kishik, is a highly functional dried fermented food that is commonly produced and consumed in many rural populations, especially in the Eastern Mediterranean, the Middle East (e.g., Egypt, Lebanon, Syria, Jordan, and Iraq), Africa, and the Indian subcontinent. During the traditional production of kishk, grains or cereals (usually the parboiled dry, hard, and cracked bulgur wheat made from *Triticum vulgare*, known as 'the common 'wheat') and fermented milk (plain yogurt, low-fat yogurt, buttermilk or fermented condensed buttermilk 'Laban Zeer') are the two main ingredients. 1-6% (w:w) of salt is added to the mixture while mixing yogurt and bulgur. The type of ingredients (e.g., cow, goat, sheep, buffalo, camel milk, white or brown bulgur, etc.) and the preparation methods, including fermentation and preservation conditions, vary among regions. This is what gives the kishk and kishk-like products their variety and identity. For example, kishk made with goat milk is much more acidic than cow milk kishk. Kishk, when made with some differences in production method, is called 'Thanu' in Hungary. In contrast, Kishk Sa'eedi (KS) in Egypt is traditionally made from Laban Zeer (sour condensed buttermilk) and whole wheat grains. 'Talkuna' in Finland and Russia is another kishk-like product made from roasted barley, rye, maize, oats, and sometimes pea flour. In Qatar, it is called 'Hogut,' and in Syria, it is known as 'Kushuk' (3,101-106). These different names are due to the different grain bases (e.g., wheat, rice, oat, or barley). Another example is 'Kashk,' a mixture of yogurt and herbs instead of bulgur in Iran. When wheat flour, spices, and garlic are used together, a kishk-like product called 'Zhum' is obtained, which is a well-known fermented food in Yemen (Table 2). Researchers have studied kishk

worldwide to collect sufficient information about its advantages and obstacles. Moreover, other similar products that are widely consumed around the world include 'Tarahana' or 'Tarhana' in Greece and Türkiye (9,101-104,106).

In this regard, researchers have begun to use different flours (e.g., soybean, purslane, malt, chickpea, rice, or corn) instead of the commonly used cereal (i.e., bulgur or wheat flour) to develop kishk products in Middle Eastern countries. Recently, due to their health benefits, natural products like kishk have become a popular area of research. Among these, agents with particularly high polyphenol content are being studied and targeted for use in kishk due to their health-enhancing effects. Today, various combinations of fermented milk and grains are present in the kishk's formula. For example, yogurt can be made from cow's milk, goat's milk, or a mixture of both. Hence, different researchers have studied and examined the chemical, nutritional, and sensory values of kishk and modified kishk in detail. As the prevalence of malnutrition, colon diseases, and foodborne infections increases, some researchers have integrated nutrient-rich animal milk (e.g., buffalo milk) for immunocompromised individuals or intestinal disorders. Other researchers have focused on using plant-based flours, like substituting bulgur and wheat flour with oat or carob flour in effective proportions, providing a good source of β-glucan, fibers, antioxidants, and unsaturated fatty acids. This helps to produce a functional, nutritious, and well-textured kishk mixture. The combination of buffalo milk and oat flour is believed to play a significant role in human nutrition, science, and public health (3,106-115).

### 3.10.1. Kishk's history

The history of kishk dates back centuries. For a long time, traditional dried fermented milk products (e.g., kurut, kishk) have been made by fermenting milk spontaneously in various regions, including the Balkans, eastern Mediterranean, western Asia, and Turkestan. These products are typically prepared at home using whole milk (i.e., cow, sheep, goat milk, or a mix) or buttermilk. Nomadic people, desert inhabitants, and people in the countryside often preserve surplus summer produce by transforming it into various forms with a longer shelf life (9). Egyptian original fermented milks were the earliest recognized worldwide in food reserves holding therapeutic purposes in rural districts (116). One of these products was the

fascinating kishk, made using basic tools. Examples of such products are kishk seiami (vegetable mixture), laban Jamid, or kurut, preserved in olive oil, laban kerbah, laban kad, laban rayeb, laban zabadi, laban zeer, and labneh. That's where kishk (also KS) was first produced, specifically in Upper Egypt and some parts of North Africa, during the Pharaonic era (3100 – 332 BCE) (3,9,115-117). The time when fermented foods started to be considered in many African communities. As the introduction of weaning foods is linked to a rise in diarrhoeal diseases, often due to poor hygiene, contaminated water, and unsafe food handling practices, fermentation was a low-cost method to enhance food safety and nutrition during weaning. Cereals and milk production fluctuated differently over time due to various changes, including political shifts at the national and regional levels, agricultural policies, crop varieties, topography, local and international markets' economies, and climate and social upsets. However, this made kishk gain its unique identity. The low handling cost, great taste, long shelf life, and nutritional characteristics of kishk contributed to the culture being known in Lebanon during the period of 900 to 999 AD, then spread throughout the nearby Arabic countries such as Syria, Jordan, Sudan, Iraq, and the Middle East. After that, kishk became known in Persia, Romania, Bulgaria, South Türkiye (e.g., Mardin, Hatay), and worldwide. A few years ago, kishk was produced annually with above 6000 MT only in Lebanon and surrounding villages. To this day, kishk is still the most popular food-preserving method (Mouneh) in such areas (1-4,3,9,22-24).

**Table 2.** Synonyms for kishk and related products in different countries

| Traditional Name | Ingredient/additive[b] | Region |
|---|---|---|
| Kishk, Kushuk, Keshkeh, or Kichk | Bulgur, Burgul or Burghol[c] | Syria, Lebanon & Egypt |
| Sa'eedi Kishk | Bulgur, Laban Zeer | Egypt |
| Hogut | Bulgur | Qatar |
| Kishk Siyamy | Vegetables | Egypt |
| Zhum | Wheat flour, garlic and pepper | Yemen |
| Kushuk, Kushik | Wheat flour and herbs | Iraq |
| Kashk, Kaskg | Herbs | Iran |
| Curpi or Zurpi | None | Nepal |
| Chura | Wheat flour and tea | Nepal, Tibet, India |
| Tarhana, Kapestoes | Veggies, herbs, and crushed wheat or flour | Greece, Cyprus & Türkiye |
| Talkuna | Crushed rye, oats, and barley | Finland & Russia |
| Korot | Lemon, spices and sugar | Soviet Union |
| Kurut | None | Türkiye |
| Keshk | None | Tibet |
| Teschurra | None | Central Asia |
| Madeer, Oggt | None | Saudi Arabia |
| Tamar Oggt | Dates | Saudi Arabia |
| Klila | None | Algeria & Morocco |

*Data compiled from Tamime et al. (1995) (9).
b: major ingredients besides the salt added to a fermented milk base.
c: parboiled cracked wheat.

### 3.10.2. Kishk's production and storage

The preparation process of kishk and the ingredients used in its production may differ among different regions and ages, as do its chemical and microbial composition, physical, rheological, and sensory properties. However, the common goal among all kishk producers is the same: to create a fermented food product that has a long shelf life, good consistency, pleasing taste, and health benefits. Kishk is traditionally made by first washing and drying the partially boiled, dried, and roughly crushed cereal origin, known as 'Burghol/Bulgur', from dirt and then combining it with cow, sheep, buffalo, or goat yogurt, known locally as 'Laban', (almost 86% moisture) at a ratio of 1 part bulgur to 2 or 4 parts of yogurt, along with 1-8,9% of sodium chloride or salt (w: w) to form the kishk dough, known as the 'Hamma' paste. Sometimes, full-cream sour milk, low-fat yogurt, or buttermilk is preferred rather than the normal cow yogurt by various social classes. The quantity of plain yogurt is chosen based on Hamma's absorption rate, which is influenced by the fermentation speed, temperature, relative humidity (RH), bacterial culture, and moisture levels. Labneh or strained yogurt can be combined with yogurt or used alone with bulgur. The mixture is then hand-kneaded daily and frequently for 3 to 8 days in plastic or stainless-steel containers and then covered with a mesh at ambient temperatures (25-35°C). The fermented dough is then shaped by hand into balls or nuggets, and either preserved in glass jars soaked with olive oil or distributed on a mat or fabric, and sun-dried on roofs until reaching 4-12 % moisture. This is why kishk production is preferred during summer, when drying under sunlight and heat is the most effective, as it can be preserved and consumed on winter days. Kishk balls are kept as they are, or can be ground into a fine powder before being packed in air-tight glass jars or well-sealed plastic pouches. Kishk is non-hygroscopic and usually stored at room temperature or sometimes in a refrigerator for 1-3 years without spoiling. It is typically consumed as a thick soup after being rehydrated with boiling water, resembling tarhana and other fermented milk-cereal blends (3,7,9,101,104,105,114,115). Today, the modern methods utilized in large factories in urban regions are largely similar to those implemented in industrialized countries.

### 3.10.3. Kishk consumption

Kishk has many forms of consumption. Nevertheless, it's commonly consumed as a thick soup after its powder is reconstituted with boiled water, adding olive oil, some onion, herbs, and garnish, similar to other fermented milk-cereal and kishk-like blends. However, kishk can be enjoyed in different forms; for instance, a semi-solid puree, typically accompanied by vegetables or eggs; a thick resembling oatmeal with vegetables, spices, garlic, or dates; raw as a Hamma paste spread on biscuits or bread; as Meeykeh, a kishk salad made with wild mint, onion, olive oil, and tomatoes; as Kebbeh b kishk, a stuffed kibbeh with kishk filling; and as Shish Barak with kishk: kishk-filled dumplings (3,9,88).

### 3.10.4. Kishk's physical properties

The color, texture, and particle size of kishk, besides the other important physical properties, vary significantly depending on fermentation-storage conditions and the methods and ingredients used, regarding the geographical region and food sources. Physical analyses for kishk are usually performed on the color parameters that are evaluated by measuring the L* (brightness), a* (green-red balance), and b* (blue-yellow balance) values using the CIE Lab* system. Moreover, the microstructure and particle size distribution (using the electron microscope and laser granulometry (SEM)), along with the water absorptions, are also important properties to be considered. For instance, significant differences have been observed in kishk's color when different types of milk and grains were used by Salameh et al. (2016). Samples made with goat milk were brighter (had higher L* values) than those made using cow milk, explained by the absence of β-carotene pigment in goat milk. Similarly, kishk samples made with white bulgur exhibited greater L* readings compared with samples made with brown bulgur, which showed significantly lower L* values (6). Furthermore, the Pearson correlation coefficient revealed a strong negative correlation (-0,92) between the drying time in the sun and the brightness of the kishk, indicating that kishk samples became darker with longer exposure to sunlight. This is consistent with the expected browning after heat treatment and the evaporation of water during sun or air-oven drying. Moreover, regarding the type of milk, kishk has been observed under the scanning electron microscope as a complex network of macronutrients (i.e.,

milk proteins and wheat starch in a thick fatty layer). The more oily this network is, the more agglomerates it forms, and the more advanced microstructure kishk gains. Thus, kishk's physical quality varies depending on the surrounding conditions and the type of milk and grain used (13,101,103,129-131).

### 3.10.5. Kishk's chemical properties

The chemical composition of commercial kishk broadly varies from one country to another. The impact of these variations on the chemical, nutritional, and sensory properties of kishk has been thoroughly investigated. This is caused by many factors, such as the method and time of fermentation and drying step, the separation of fat from milk to get buttermilk before fermenting it with bulgur, the addition of certain dairy high-protein products, the type of the main ingredients used (i.e., bulgur, fermented milk (yogurt) and salt), the utilization of strained yogurt or labneh (26-50% dry matter), the ratio of bulgur to fermented milk (1:2, 1:3, or 1:4 w:w), and calculating protein content using different multiplication factors (6,25 or 6,38) (3,16,101,103). Dried Kishk is non-hygroscopic and can be stored in open containers for 2-3 years without degradation, remaining stable at room temperature. This is due to its low moisture content (3-13% w:w), the acidic nature pH which usually fluctuates between 3,80-4,80 with titratable acidity between 1,3-2,50%), and the high salt content (NaCl ranges from 1.0% to 8.9%) help protect its quality and safety against the proliferation and spoilage by harmful microorganisms. Along with its ash content (5-10% g / 100 g), which indirectly contributes to the final product by its role in balancing pH, indicating low processing and microbial stability. Kishk undergoes chemical transformations that occur during the processing of wheat and yogurt together have been investigated by many researchers a long time ago (1,3,6,16,118-120). For a better understanding of kishk's chemical quality, in 2016, Salameh and colleagues studied 20 samples of kishk collected from local markets in different Lebanese regions (Al-Bekaa, Mount Lebanon, the North, and the South). Ten samples were made from cow's milk, five from goat's milk, and five from a mixture of goat and cow's milk. Brown bulgur was used in two samples made from goat's milk and a goat-cow milk blend. The remaining samples were prepared with white bulgur (101). The chemical properties were analyzed, and the samples made from goat's milk were significantly

richer in fat and protein compared to the others. However, their pH was the lowest due to higher moisture content and hydrogen ions (Table 3). Similarly, strained yogurt made from goat's milk exhibited higher fat content. Other studies have also shown that kishk products based on goat's milk have higher fat content than those made from cow's milk (101). In general, the type of milk primarily influences the fat content in kishk, which usually ranges from 8 to 10%, while the moisture is affected by both the milk base and the drying method. The ash content, on the other hand, is dependent on the amount of salt added to the product. When it comes to protein content, it is mainly influenced by the type of milk and grain used, as well as the proportions of each. These findings resulted in positive changes in the physical properties of the product (3,101,118-120).

Numerous studies have incorporated many substitute ingredients and plant-based milk varieties into traditional kishk products to enhance their chemical composition (3,111-114). For example, in 2020, Salman et al. used purslane powder to improve the overall biological and nutritional value of kishk. Purslane powder was added in varying concentrations as 0%, 0,25%, 0,5%, 1,0%, 1,5%, and 2% to the kishk samples. The results indicated that kishk samples with higher concentrations of purslane exhibited increased levels of K, P, Ca, Na, protein, and ash content. However, these samples had lower fat and pH values than the control samples of kishk (112). The obtained results were consistent with the pH values reported by Tamime et al. (1999a), Gadallah and Hassan (2019), and Abd-Rabou et al. (2020) (3,9,13,101,108,112-114).

**Table 3**. Chemical composition of 20 kishk samples collected from different areas in Lebanon ($\bar{x}$±SD g/100 g kishk)

| Kishk [M&B] | N | MC% | Ash | Protein | Fat | pH |
|---|---|---|---|---|---|---|
| Cow kishk[w] | 10 | 6,08±0,34 | 6,27±0,65 | 21,83±1,10 | 16,90±1,54 | 4,27±0,16 |
| Goat kishk ($4^w$+$1^b$) | 5 | 6,65±0,30 | 7,33±0,24 | 21,03±0,71 | 20,36±1,04 | 4,18±0,12 |
| Mixed kishk ($4^w$+$1^b$) | 5 | 6,55±0,56 | 6,71±1,44 | 20,10±1,24 | 17,13±1,92 | 4,21±0,14 |

*Data compiled by Salameh et al. (2016), (101).
M&B: kishk products are made mainly from bulgur and milk base.
w: kishk samples made with white bulgur.
b: kishk samples with brown whole wheat bulgur.
MC%: moisture content percentage.
SD: standard deviations. $\bar{x}$: Mean values.

### 3.10.6. Kishk's nutritional properties

Kishk is a nutritionally complete protein-dense food with 8,9-54,5% protein, 8-25% higher digestibility, and 13-23,5% amino acids availability. It is a potential source of various vitamins, minerals, and growth factors resulting from microbial fermentation. It is a great nutritional food for all ages, as the nutrients lacking in milk are provided by cereals, and vice versa. Moreover, it offers significant dietary values, serving as a good source of carbohydrates (40-70 g / 100 g), fiber (0,5-3 g/ 100 g), vitamins (thiamine, riboflavin, niacin), minerals (such as iron, phosphorus, calcium, magnesium, selenium, and manganese), fats from bulgur (1,6-19,9% g / 100 g), essential amino acids (e.g., phenylalanine, threonine, isoleucine, leucine, arginine, valine, tyrosine, and lysine, with tiny portions of tryptophan and sulfur-containing amino acids). It's also worth mentioning that the niacin content is 3 times higher in kishk than in milk itself. Nevertheless, tryptophan content in kishk has been considered by FAO and WHO (1985) as a limiting and temporary factor as a result of the exposure to sunlight during the fermentation and drying process that triggers the thermal degradation along with the loss of the sulfur-containing amino acids. However, Kishk produced under controlled laboratory conditions has shown good levels of tryptophan and other sulfur-holding amino acids. Some authors like Damir and Mohamed (1992)

indicated the presence of 6 important organic acids in kishk (i.e., butyric, propionic, acetic, formic, lactic, and succinic acids), as the major is Lactic and the minor is formic acid. Lactic acid content is usually way higher in laboratory-made kishk samples than in commercial kishk products. The opposite is true for acetic acid, which is usually half lost in experimental kishk samples (3,9,101,120-124,127). Kishk has fluctuating amounts of L (+) and D (-) lactic acid isomers. D (-) lactic acid accumulates in the blood of individuals with short bowel syndrome and intestinal failure, potentially leading to the development of D (-) lactic acidosis and encephalopathy. This is due to the colonization of intestinal *Lactobacilli* that produce D (-) lactate. Additionally, newborn infants may be unable to fully metabolize the D (-) lactate produced by ingested food or intestinal microorganisms due to the immaturity of their liver function. As a result, D (-) lactic acid is not recommended for infants or young children (122,123).

In order to improve the nutritional quality and obtain a functional kishk, researchers started to replace bulgur with oats, cinnamon, purslane, Faba Bean (*Vicia Faba*, L.), whole wheat, chickpea, and barley flour (*Hordeum vulgare*), and other nutrient-dense cereals or milks, such as caprine milk or plant-based milks (e.g., soy or coconut milk). For example, kishk made with bulgur, oats, or barley was considered a beneficial source of β-glucan, dietary fibers, and unsaturated fatty acids (107-112,126). That’s how it contributes to lowering the glycemic index, reducing plasma cholesterol and blood glucose levels, mitigating the risk of colon cancer, cardiovascular diseases, and various intestinal disorders. (129). On the other hand, incorporating whole wheat flour in kishk production increased the bioavailability of calcium, iron, magnesium, and zinc without negatively impacting the product's acceptability (3). In another direction, a study conducted by El-Kenany et al. (2006) replaced buffalo milk with cultured soy milk to create a more nourishing alternative for vegetarians known as "vegi kishk." (3,121).

### 3.10.7. Kishk's microbiological profile

Kishk production offers an effective method for preserving yogurt/milk and cereals for later use, particularly in African and Middle Eastern regions, where temperatures are too high, by creating an environment where harmful microorganisms cannot thrive or spoil the product. This is significantly attributed to its microbial identity. The microbiological profile of kishk is influenced by several factors: the fermentation process, which involves a variety of microorganisms, the initial microbial load of grain and milk base, the drying method, and the hygienic conditions maintained throughout production (3,14,105,127). The predominant ones are the obligate heterofermentative lactic acid bacteria, specifically *Lactobacilli* (e.g., *L. bulgaricus*) and *Streptococci* (e.g., *S. thermophilus*), as such bacteria are commonly found in raw milk and fermented dairy products (e.g., yogurt). *L. bulgaricus* and *S. thermophilus* are commonly present in kishk in ranges of 6 to 8 log cfu/g and 7 to 9 log cfu/g, respectively. Elevated levels of lactic acid bacteria in kishk play a crucial role in ensuring the product's safety throughout manufacturing and storage (3,9,89,99,105). According to several studies, the microbiological profile of kishk is predominantly composed of lactic acid bacteria, particularly genera such as *Streptococcus* (notably *S. thermophilus*), *Leuconostoc*, *Lactobacillus* (especially *L. delbrueckii* subsp. *bulgaricus*), *Pediococcus*, and sometimes *Enterococcus*. In addition to these vegetative bacteria, the microbial community may include certain spore-forming organisms and a minor fraction of salt-tolerant microorganisms. Homofermentative and facultatively heterofermentative *Lactobacillus* and Streptococcus species typically proliferate during the initial stages of kishk production, particularly throughout fermentation, which represents a critical phase in determining the survival or inhibition of pathogenic bacteria. However, obligate heterofermentative LAB are always detected throughout all stages of kishk production, while *Lactococcus* occupies a minor portion. An increase in total bacterial populations is commonly observed during the conditioning (fermentation) period. (3,9,14,105,130). On the other hand, the presence of *Bifidobacterium*, *Leuconostoc*, and *Pediococcus* species in kishk may vary depending on the type of fermented milk utilized in its production.

The microbiological profile of kishk plays a crucial role in its flavor, texture, safety, chemical, and nutritional composition, giving kishk its long history. However,

kishk continues to be produced using traditional methods, thus it may contain other microbial contaminants due to the uncontrolled production, fluctuations in incubation temperature and relative humidity, and frequent intensive handling during fermentation or sun-drying that negatively affect the final product's quality. For example, fermented milk in kishk products can be contaminated with *Escherichia coli, Salmonella,* or some coliforms, which are generally considered hygiene red flags (14,15,115).

One of the current research priorities is the development of kishk using selected probiotic cultures, such as *Bifidobacterium animalis* subsp. *lactis*, to enhance its functional, nutritional, and microbial properties (130,118,131). One of their super beneficial effects is the inhibition of pathogenic organisms such as *Salmonella*, *Shigella*, *E. coli*, and *Helicobacter.* Defense mechanisms include the production of inhibitory substances against pathogens and competing for essential nutrients and adhesion sites. However, other studies showed that adding *L. acidophilus* or *L. casei* subsp. *casei* to yogurt starter culture did not result in any noticeable improvement in kishk's quality (3,130-132). In 2006, Petros et al. detected certain strains of *Lactobacillus* (i.e., *L. casei* ACA-DC 6002, *L. plantarum* ACA-DC 146, and *L. paracasei* subsp. *tolerance* ACA-DC 4037) that were able to prevent the adhesion of *Escherichia coli* and *Salmonella Typhimurium* to Caco-2 cells in the intestinal epithelium (133). Additionally, in 2010, Schillinger and Jéssica observed lactic acid bacteria's antifungal activity, at which several strains produced inhibition zones against *Penicillium nordicum* (BFE 487) (134). Other studies showed that *Lactobacillus* strains from *kishk* exhibited antifungal activity, particularly inhibiting *Penicillium* species with 48-63% inhibition zones. Others identified fourteen probiotic isolates from yogurt and *kishk* with varying antibacterial effects against *Staphylococcus aureus*, with LZb8, S4b1, and RC2b3 showing the highest activity. That demonstrated similar antibacterial activity against *Escherichia coli* (131-134).

### 3.10.8. Pathogens in kishk

Pathogenic bacteria can be detected in kishk, including *E. coli* O157:H7, *Shigella*, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium* spp., *Staphylococcus*

*aureus*, *Campylobacter* spp., *Brucella* spp., and *Yersinia* spp. Using raw milk and poor sanitary conditions during production often leads to contamination with these pathogens. They can be found in many fermented dairy products and are considered major foodborne threats (9,14,115).

In this context, such pathogenic microorganisms were present in kishk and all fermented dairy products collected randomly from various locations in Sohag Governorate, Egypt, by Abd Alla AA et al., 2020. The average counts of *E. coli*, *Staphylococcus aureus*, *Salmonella* & *Shigella*, in kishk samples were 3,89, 4,31, and 4,83 log cfu/g, respectively, which was considered a threat to public well-being (15). Similarly, many other studies, such as the study conducted by Saleh et al. in 2009, investigated the bacterial contamination in Lebanese kishk samples collected from the Beqaa Valley, where *E. coli* O157:H7 was confirmed in all kishk samples (139). El-Shreef MTA and El-Gendi (2014) also detected the presence of *Enterococci* species in 61,6% and 77,8% of the kishk samples collected from Assiut shops in Egypt, with average counts of $6{,}95x10^4$ and $3{,}9x10^4$, respectively (130). Apart from this, fungal contamination and mycotoxins (Aflatoxin M1; AFM1) can also pose a serious challenge to the fermented food and kishk industry due to their demonstrated carcinogenic, mutagenic, nephrogenic, hepatotoxic, neurotoxic, and other effects on health (138,140). Milk containing mycotoxins from animals fed contaminated feed may include AFM1 and transfer it to kishk. AFM1 is the primary metabolite produced by storage molds from the *Aspergillus* genus. AFM1 in milk and dairy products is classified as a Group 2A human carcinogen by the International Agency for Research on Cancer (IARC) in 1993 (134-138). Although kishk presents an inhospitable environment for pathogens, certain kishk products may still pose public health risks as vectors for various pathogens. This underscores the need for improved safety protocols throughout production and storage stages. Accordingly, the present study aimed to examine the extent to which proper production practices influence the survival of existing pathogenic microorganisms in kishk. Given the ongoing traditional methods of production and the widespread consumption of kishk, ensuring its microbiological safety today remains a critical public health priority. (3,9,14,115,141-143).

# 4. MATERIALS AND METHODS

## 4.1. Materials

### 4.1.1. Materials and study design

Bulgur (coarse bulgur), yogurt, strained yogurt, and coarse salt used in kishk production were purchased from a supermarket in the Southeastern Anatolian Region within the borders of Diyarbakır province, Turkiye. The purchased materials were transported to the laboratories of the Department of Food Hygiene and Technology at Dicle University within 35-40 minutes in their original packaging under aseptic conditions, with the cold chain intact (refrigerator temperature maintained at 4°C).

## 4.2. Methods

### 4.2.1. Preparation of kishk

Kishk was produced following the method described by Toufeili et al. (1999), with slight modifications (144). 500 g of bulgur, 1 kg of yogurt, 30 g of coarse salt, and 800 g of strained yogurt were used to produce 800 g of kishk. Bulgur was heated at 100°C / 20 minutes and cooled to room temperature. Later on, 1 kg of yogurt and 30 g of coarse salt were weighed separately using sterile aluminum containers under aseptic conditions and placed into a sterile stainless bowl, in which they were mixed with bulgur manually for 10 minutes to form the ‘Hamma’ paste. The bowl was covered with a sterile aluminum foil and incubated at 35°C to be initially fermented for 24 h. After that, the strained yogurt was entirely added to the fermented mixture and kneaded thoroughly before being incubated for another 24 hours. At the end of fermentation, kishk was shaped by hand aseptically into small balls approximately 2-4 cm in diameter, then placed separately into sterile aluminum trays and dried at 53°C in a drying oven for 96 hours (4 days) (Figures 3 and 4).

### 4.2.2. Determination of chemical and microbiological quality of ingredients and the uninoculated kishk mixture

Prior to inoculation, the microbiological quality of yogurt, strained yogurt, and kishk mixture was determined. Ten grams of each sample was placed in a sterile filter sample bag (Bag Filter, France), and homogenized with 90 ml of sterile 0,1% Peptone water (PW) (104, LABM, UK), using a stomacher device (AES, Easy Mix-G560E, France). Then decimal dilutions were prepared in tubes containing 9 ml of sterile 0,1% PW. The enumeration of the lactic acid bacteria (i.e., *Lactobacillus* and *Streptococcus* spp.), total mesophilic aerobic bacteria (TMAB), total psychrophilic aerobic bacteria (TPAB), coliforms, *Staphylococcus* & *Micrococcus* spp., yeasts, and molds in yogurt, strained yogurt, and the uninoculated kishk mixture was carried out (Table 4). The pH and dry matter content (%) of yogurt, strained yogurt, bulgur, and raw kishk mixture were determined using a digital pH meter with a glass electrode (EcoMet P25, Korea) and a dry matter analyzer (UniBloc, Shimadzu MOC63u, Japan), respectively. Titratable acidity (lactic acid%) was also obtained for yogurts and the kishk mixture by acid-base titration using 0,1 N NaOH and 3-4 drops of phenolphthalein indicator until complete neutralization. For pH and titratable acidity, 10 grams were taken from yogurt, strained yogurt, and the raw mixture were taken separately into a filter sample bag and homogenized for 2 minutes with 90 ml of distilled water in a stomacher, while only 5 g of samples were taken for the dry matter analysis (145).

### 4.2.3. Contamination of kishk with pathogens

#### 4.2.3.1. Bacterial strains

In the present study, one *Salmonella* Enteritidis (RSKK 91), one *Salmonella* Typhimurium (NCTC 14028), and two *E. coli* O157:H7 strains (ATCC 43894 and ATCC 43895) were used. All strains were obtained from the culture collection of the Department of Food Hygiene and Technology at Dicle University Faculty of Veterinary Medicine (Diyarbakır, Türkiye). Pure cultures were maintained as frozen stocks at -20°C. The strains used in all experiments were freshly prepared using the same procedure as given below.

#### 4.2.3.2. Preparation of the inoculum and pathogens inoculation

Cultures were prepared by inoculating 0,1 mL of thawed frozen stock into 10 mL of fresh tryptic soy broth (TSB) (LABM, Lancashire, UK) and incubated at 37°C for 24 hours. For *E. coli* O157:H7, individual strains were streaked onto Sorbitol MacConkey agar (SMAC) and incubated at 37°C for 24 hours. *Salmonella* strains were streaked onto Xylose Lysine Deoxycholate (XLD) agar under the same conditions. A working culture for each strain was then prepared by transferring colonies into TSB and incubating at 37°C for 24 hours. Cultures were centrifuged (Nüve NF 800 R, Ankara, Türkiye) at 4192 × g for 10 minutes at 4°C, and the resulting pellets were washed with 0,1% PW (LABM, UK) to remove organic residues. After a second centrifugation under the same conditions, the supernatant was discarded, and each pellet was resuspended in 0,1% sterile PW. The suspensions were then combined in a single tube and adjusted to a final volume of 50 mL with 0,1% sterile peptone water. This mixed *E. coli* O157:H7 and *Salmonella* inoculum was used immediately as the pathogenic inoculum. The inoculation was carried out immediately after the Hamma's mixture preparation, as shown in Figure 3.

#### 4.2.3.3. Present/absent analysis

The presence/absence of the target pathogens was confirmed throughout the production and storage of kishk. Briefly, for *E. coli* O157:H7, 25 g of sample was placed into a sterile stomacher bag containing 225 mL of modified Tryptone-Soy Broth (mTSB) with Novobiocin (LABM, UK), then homogenized using a stomacher (Easy Mix-G560E, France). The homogenates were incubated at 37°C for 24 hours and subsequently streaked onto Sorbitol MacConkey agar (SMAC) (LABM, UK) for selective isolation and absence confirmation. For *Salmonella* spp., 25 g of sample was homogenized with 225 mL of buffered peptone water (BPW) and incubated at 37°C for 24 hours. Following pre-enrichment, 100 µL aliquots were transferred into 10 mL of Rappaport–Vassiliadis broth (LABM, UK), respectively, and incubated at 37°C and 42°C for 24 hours. A loopful from each enrichment was streaked onto Xylose Lysine Deoxycholate agar (XLD; LABM, UK) and incubated at 37°C for 24 hours to confirm the absence of *Salmonella* spp.

### 4.2.4. Storage

After the inoculation, fermentation, and drying, dried kishk balls were placed into sterile polyethylene bags and stored at 4°C and 24°C for 36 days (Figure 5). Kishk samples from preparation till the end of the storage period (at both temperatures) were subjected to chemical and microbiological analyses as declared in Table 4. The experiment was repeated three times. In each experiment, two samples were analyzed at each sampling time.

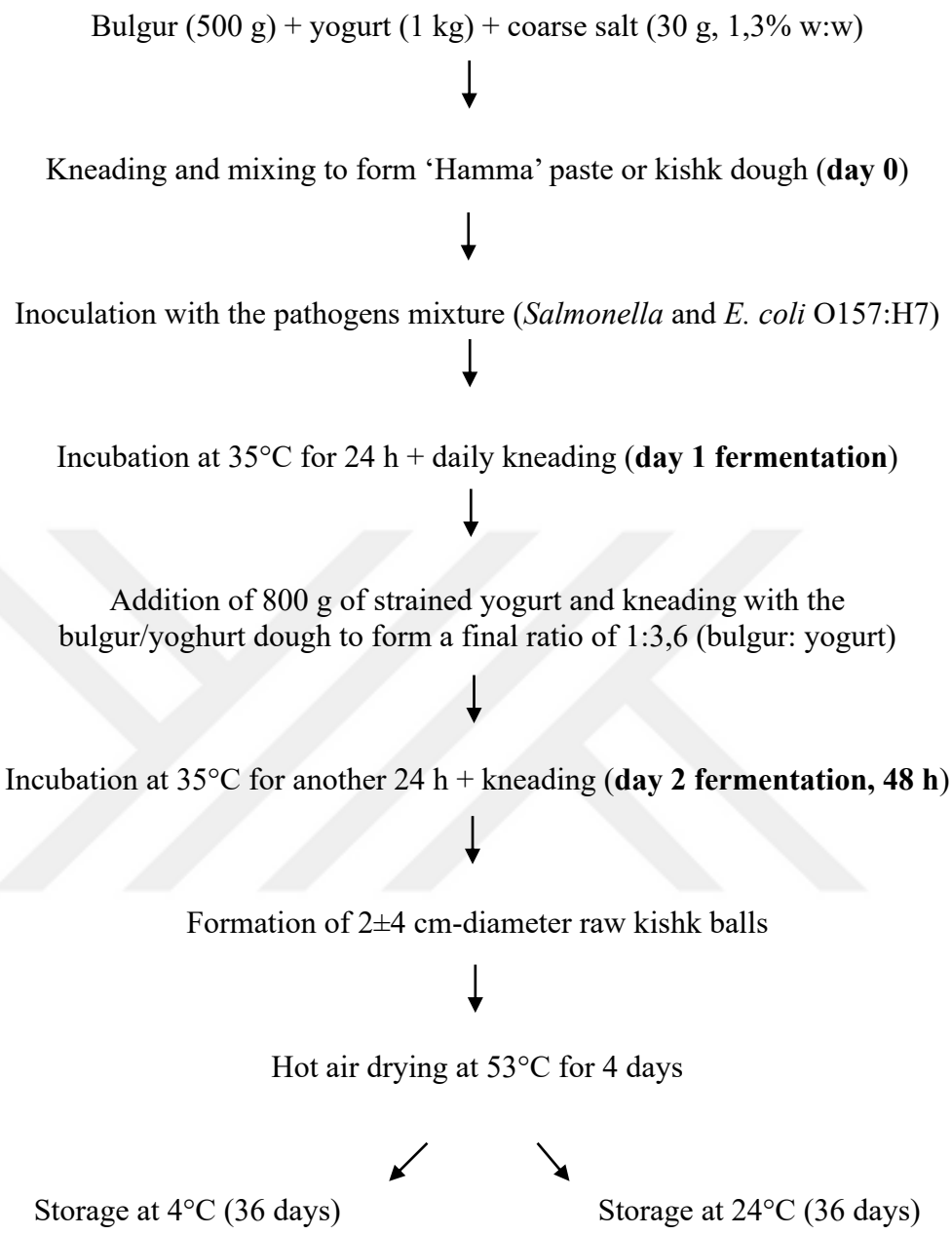


**Figure 3.** Kishk production and storage procedure


**Figure 4.** Kishk production

**Figure 5.** Kishk packaging

**Table 4.** Sampling days, chemical and microbiological analyses carried out during kishk production and storage

| Time (Days/h) | Chemical analyses | | | Microbiological analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | TA (%) | DM (%) | *E. coli* O157:H7 | *Salmonella* | TMAB | TPAB | Coliform | Yeast and molds | *Streptococcus* & *Lactobacillus* spp. | *Staphylococcus* & *Micrococcus* spp. |
| Yogurt | + | + | + | + | + | + | + | + | + | + | + |
| Strained yogurt | + | + | + | + | + | + | + | + | + | + | + |
| $KM_0$ | + | + | + | + | + | + | + | + | + | + | + |
| KMp | | | | + | + | + | + | + | + | + | + |
| $KM_{24f}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{48f}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{24d}$ | + | + | + | | | | | | | | |
| $KM_{48d}$ | + | + | + | | | | | | | | |
| $KM_{72d}$ | + | + | + | | | | | | | | |
| $KM_{96d}$ | + | + | + | + | + | + | + | + | + | + | + |
| $KM_{1s}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{3s}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{9s}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{18s}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |
| $KM_{36s}$ | + | + | + | +[a] | +[a] | + | + | + | + | + | + |

[a]: Enrichment was carried out.
$KM_0$: kishk mixture (after mixing the main ingredients at day 0).
KMp: kishk mixture after inoculation with the pathogenic mixture.
$KM_{24f}$: kishk mixture after 24 h fermentation.
$KM_{48f}$: kishk mixture after 48 h fermentation.
$KM_{24d}$: kishk mixture after 24 h drying.
$KM_{48d}$: kishk mixture after 48 h drying.
$KM_{72d}$: kishk mixture after 72 h drying.
$KM_{96d}$: kishk mixture after 96 h drying.
$KM_{1s}$: kishk day 1 storage, at 4°C and 24°C.
$KM_{3s}$: kishk day 3 storage, at 4°C and 24°C.
$KM_{9s}$: kishk day 9 storage, at 4°C and 24°C.
$KM_{18s}$: kishk day 18 storage, at 4°C and 24°C.
$KM_{36s}$: kishk day 36 storage, at 4°C and 24°C.
TA (%): titratable acidity as lactic acid (%).
DM (%): dry matter content percentage.

### 4.2.5. Chemical analyses of kishk

For the chemical analyses, pH and titratable acidity (lactic acid%) were measured by taking a 10 g sample of kishk on each sampling day (Table 4). Samples were placed into a filter sample bag, in which 90 ml of distilled water was added to each sample bag containing the 10 grams of sample. Sampling mixtures were homogenized for 2 minutes in a stomacher (Easy Mix-G560E, France) to be analyzed as previously described. For dry matter analysis, only 5 g of kishk was taken per sample, following the established procedure mentioned previously (145).

### 4.2.6. Microbiological analyses of kishk

The samples of kishk (10 g) were taken as specified in Table 4, and placed aseptically in a stomacher bag (Bag filter, France) containing 90 ml of sterile 0,1% PW and then homogenized for 2 min (Easy Mix-G560E, France). The samples were then subjected to serial 10-fold dilutions using 9 mL of sterile 0,1% PW and microbiologically analysed for the following microorganisms as declared in Table 4. When pathogen numbers decrease below the detection limit, an enrichment method was applied. The average of the results was reported as CFU per gram (145).

#### 4.2.6.1. Total mesophilic aerobic bacteria count (TMAB)

The total mesophilic aerobic bacteria in kishk were enumerated by pour plating on sterile Plate Count Agar (PCA) (149, LABM, Lancashire, UK). The PCA plates were incubated at 37°C for 24 h, and colonies were counted (145).

#### 4.2.6.2. Total psychrophilic aerobic bacteria count (TPAB)

The total Psychrophilic Aerobic Bacteria were enumerated by pour plating on sterile Plate Count Agar (PCA) (LABM, UK). The plates were then incubated at 4°C for 7 days, and typical colonies were counted (145).

#### 4.2.6.3. Yeasts and molds count

Yeasts and molds were enumerated by pour plating on sterile Potato Dextrose Agar (PDA) (098, LABM, UK) containing 10% tartaric acid. The plates were incubated at 25°C for 5 days (145).

#### 4.2.6.4. Coliform bacteria count

Coliform bacteria were enumerated by pour plating on sterile Violet Red Bile Lactose Agar (VRBA) (Merck, Darmstadt, Germany). The Petri plates were incubated at 37°C for 24 h, and typical colonies were counted (145).

#### 4.2.6.5. *Lactobacillus* spp. count

The *Lactobacilli* were enumerated by pour plating on sterile de Man, Rogosa, and Sharpe agar (MRS) (Biokar, Diagnostics, France). The plates were incubated at 30°C for 48-72 hours (145).

#### 4.2.6.6. *Streptococcus* spp. count

*Streptococcus* spp. were enumerated by pour plating on sterile M17 agar (Biokar Diagnostics, France). The plates were incubated at 30°C for 48-72 hours. (145).

#### 4.2.6.9. *Staphylococcus* & *Micrococcus* Count

*Staphylococcus* and *Micrococcus* spp. were enumerated by spread plating on Baird-Parker agar (BPA) containing 5,263% egg yolk emulsion (Merck, Darmstadt, Germany). The plates were incubated at 37°C for 24 h. Typical colonies were counted (145).

#### 4.2.6.7. *E. coli* O157:H7 Count

*E. coli* O157:H7 was enumerated by spread plating on Sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC) (Oxoid, UK). The plates were incubated at 37°C for 24 h, and typical colonies were counted (64). In cases where the number of *E. coli* O157:H7 was below the detection limits, a presence/absence test was performed on the samples. For this purpose, 90 ml of Modified Trypticase Soy Broth with Novobiocin (mTSB) (LABM, UK) was added to another sample (10 g) in sterile sampling bags on each sampling day, followed by mixing properly for 2 minutes in a

stomacher and incubating at 37°C for 18-24 hours. After incubation, an aliquot of each enriched sample was streaked onto Sorbitol MacConkey agar with Cefixime Tellurite (CT-SMAC) (Oxoid, UK), and incubated at 37°C for an additional 24 h. The results were evaluated as present/absent (146).

#### 4.2.6.8. *Salmonella* count

*Salmonella* was enumerated by spread plating on Xylose Lysine Deoxycholate Agar (XLD) (Chemsolute, Germany). The plates were incubated at 37°C for 24 hours. In case the *Salmonella* count was below the detectable limits, a presence/absence test was performed on the samples. For this purpose, 90 ml of buffered peptone water (BPW) (LABM, UK) was added to another sample (10 g) in sterile sampling bags on each sampling day and kept at 37°C for 18-24 hours (pre-enrichment). After that, 0,1 ml of the pre-enriched liquid was transferred to polypropylene conical tubes containing 10 ml Rappaport Vassiliadis Soy Peptone broths (RVS/RVSEB) (LABM, UK). After incubation at 42°C for 18-24 hours, an aliquot of each enriched sample was streaked onto XLD agar and incubated at 37°C for 24 h. Any detected growth was considered "injured bacteria" (146).

### 4.2.7. Statistical Analyses

Analyses of pH, titratable acidity, and dry matter content were carried out using SPSS 22 software (IBM, SPSS Statistics, Version 22). Microorganism numbers were log-transformed ($log_{10}$ cfu/g) and analyzed using the variance analysis (ANOVA) test, based on a 3x2x5 (repetition x storage temperature x sampling days) factorial experimental design, and interactions between variables were assessed. Subsequently, Mean values were separated using Fisher's Least Significant Difference (LSD) test to assess both within-group and between-group differences, based on the General Linear Models (GLM) procedure, with a significance level set at 5%. All statistical analyses were performed using the Statistical Analysis System (SAS) software (Version 8, 1999, SAS Institute Inc., Cary, NC, USA). Statistically significant levels were expressed as $P<0,05$.

# 5. RESULTS

## 5.1. Chemical Properties of Yogurt, Strained Yogurt, Bulgur, and the Uninoculated Kishk Mixture

The chemical properties of the main ingredients (i.e., yogurt, bulgur, and strained yogurt) used in kishk production are presented in Table 5. Yogurt's pH, titratable acidity (SH/TA), and dry matter content (DM%) were 4,25±0,06, 0,75±0,03, and 17,44±0,88, respectively. These values were observed in strained yogurt as 3,91±0,07, 1,35±0,03, and 28,92±0,99 %, respectively. Bulgur, as the cereal base, showed the relatively highest pH value and dry matter content, recorded as 6,59±0,81 and 91,82±0,80, respectively. On the other hand, the pH, titratable acidity, and dry matter of the uninoculated raw kishk mixture (Hamma) were found to be 4,22±0,08, 1,32±0,16, and 47,59±0,08, respectively.

**Table 5.** Some chemical results of the main ingredients and the uninoculated kishk mixture

| **Product** | **Parameter** | **$\bar{x}$ ± SD** |
|---|---|---|
| Yogurt | pH | 4,25±0,06 |
| | TA (%) | 0,75±0,03 |
| | DM (%) | 17,44±0,88 |
| Strained yogurt | pH | 3,91±0,07 |
| | TA (%) | 1,35±0,03 |
| | DM (%) | 28,92±0,99 |
| Bulgur | pH | 6,59±0,81 |
| | DM (%) | 91,82±0,80 |
| KM | pH | 4,22±0,08 |
| | TA (%) | 1,32±0,16 |
| | DM (%) | 47,59±1,71 |

KM: kishk mixture (after mixing yogurt, bulgur, and salt).
DM (%): dry matter content percentage.
TA: titratable acidity as lactic acid (%).
SD: standard deviation, $\bar{x}$: mean value.

### 5.2. Microbiological Properties of Ingredients and Uninoculated Kishk Mixture

The microbiological quality of yogurt and strained yogurt used in kishk production is presented in Table 6. Data indicate that both yogurt and strained yogurt exhibit good microbiological characteristics, particularly in terms of total mesophilic and psychrophilic aerobic bacterial counts, as well as lactic acid bacteria, including *Streptococcus* & *Lactobacillus* species. A noticeable difference of about 2 $\log_{10}$ units was observed in the TMAB counts between yogurt and strained yogurt. In the uninoculated kishk mixture, following the initial blending of yogurt, bulgur, and salt, the respective microbial counts were 4,46±0,43 for TMAB, 5,70±0,54 for TPAB, 4,61±0,40 for *Lactobacillus*, and 6,56±0,73 for *Streptococcus*. Remarking higher counts of *Streptococcus* spp. and TPAB and lower counts of *Lactobacillus* spp. and TMAB compared to those found in yogurt. Neither *Escherichia coli* O157:H7, *Salmonella*, nor molds were detected in any of the yogurt, strained yogurt, or the uninoculated kishk mixture. However, the average counts of yeasts and coliforms in yogurt, strained yogurt, and the uninoculated kishk mixture were relatively comparable. Counts of Staphylococcus and Micrococcus were below detectable limits in all main ingredients and the uninoculated mixture (i.e., <2 log cfu/g).

**Table 6.** The microbiological quality of yogurt, strained yogurt, and the uninoculated kishk mixture used in kishk production ($\bar{x}$±SD $\log_{10}$ cfu/g)

| | **Microorganisms** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TMAB | TPAB | Yeasts & molds | Coliform | *Lactobacillus* spp. | *Streptococcus* spp. | *Staphylococcus & Micrococcus* | *E. coli* O157:H7 | *Salmonella* |
| **Yogurt** | 8,43±0,35 | 4,95±0,67 | 5,01±0,15 | 2,14±0,38 | 5,06±0,62 | 5,08±0,56 | <2 | ND | ND |
| **Strained yogurt** | 5,98±1,31 | 6,17±1,10 | 4,82±0,50 | 1,96±0,32 | 6,71±0,88 | 5,56±0,54 | <2 | ND | ND |
| **KM** | 4,46±0,43 | 5,70±0,54 | 4,16±0,64 | 1,97±0,58 | 4,61±0,40 | 6,56±0,73 | <2 | ND | ND |

TMAB: Total mesophilic aerobic bacteria, TPAB: Total psychrophilic aerobic bacteria, KM: Kishk mixture.
SD: standard deviation, $\bar{x}$: mean value.
ND: not detected after present/absent analysis.
* <2 $\log_{10}$ cfu/g.

## 5.3. Microbiological Changes Determined During the Production and Storage of Kishk

The microbiological changes observed in kishk during the fermentation, drying, and storage (at 4ºC and 24ºC) are shown in Tables 7 and 8.

### 5.3.1. Total mesophilic aerobic bacteria (TMAB) count

The microbiological analyses showed that kishk production had a significant impact on total mesophilic aerobic bacterial counts at all stages. Following pathogen inoculation, TMAB levels increased markedly, reaching 8,65±0,71 $\log_{10}$ cfu/g. After fermentation, a slight reduction (~ 1 log cfu/g) in TMAB counts was observed, with levels decreasing to 7,79±0,20 $\log_{10}$ cfu/g. Counts measured before and after drying were found to be relatively comparable, since the difference didn't exceed 0,11 $\log_{10}$ cfu/g (Table 7). As shown in Table 8, total mesophilic bacterial counts exhibited an initial slight increase within the first 24 hours of storage, reaching 8,18±0,18 and 8,30±0,18 $\log_{10}$ cfu/g at 4°C and 24°C, respectively. After that, a gradual decline in bacterial counts was observed throughout the storage period. By day 36, TMAB levels had decreased to 6,70±0,18 $\log_{10}$ cfu/g at 4°C, while remaining higher and averaged 7,26±0,18 $\log_{10}$ cfu/g at 24°C ($p < 0,05$). The statistically significant difference in mesophilic bacterial counts at the end of storage suggests that, while bacterial levels declined over time at 4°C, the reduction was relatively less pronounced at 24°C (Figure 6).

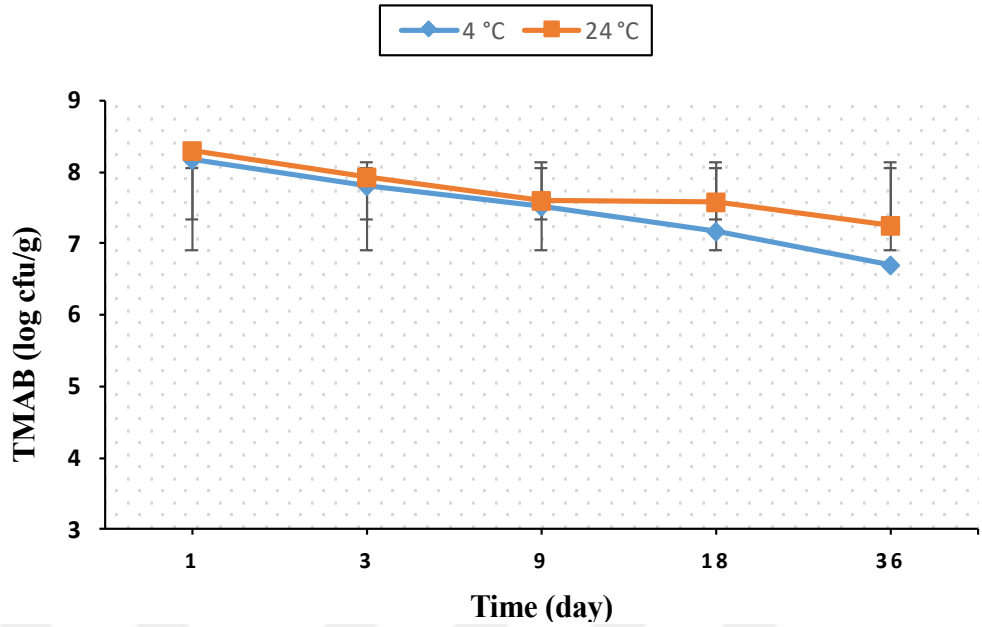


**Figure 6**. Changes in TMAB counts of kishk during storage

### 5.3.2. Total psychrophilic aerobic bacteria (TPAB) count

The microbiological analyses demonstrated that the making of kishk had an important effect on the TPAB counts during all steps of production (Tables 7 and 8). The psychrophilic aerobic bacteria exhibited a significant decrease during the first 24 hours of fermentation, with a minor drop observed on the second day. However, the drying impact on psycrophilic aerobic bacterial counts after 96 hours was observed when their levels dropped about 0,55 $\log_{10}$ cfu/g. Tables 7 and 8 revealed that the TPAB count, which was 5,86±0,07 $\log_{10}$ cfu/g in dried kishk, significantly fluctuated during the 36 days of storage at both temperatures. During the first 24 hours of storage, the psychrophilic aerobic bacterial counts in both temperature groups were found to be similar, recording a slight increase (4°C: 7,33±0,07, 24°C: 7,18±0,09). These counts increased slightly after 72 hours, followed by a gradual decline throughout the entire period at 4°C, while the decline at 24°C was more pronounced. By the end of the storage period, the average counts of TPAB were 6,19±0,09 and 4,53±0,11 for 4°C and 24°C, respectively. Significant differences in psychrophilic aerobic bacterial counts were observed as a steady decline at 4°C and a rapid decrease at 24°C, compared to the initial counts ($p<0,05$) (Figure 7).

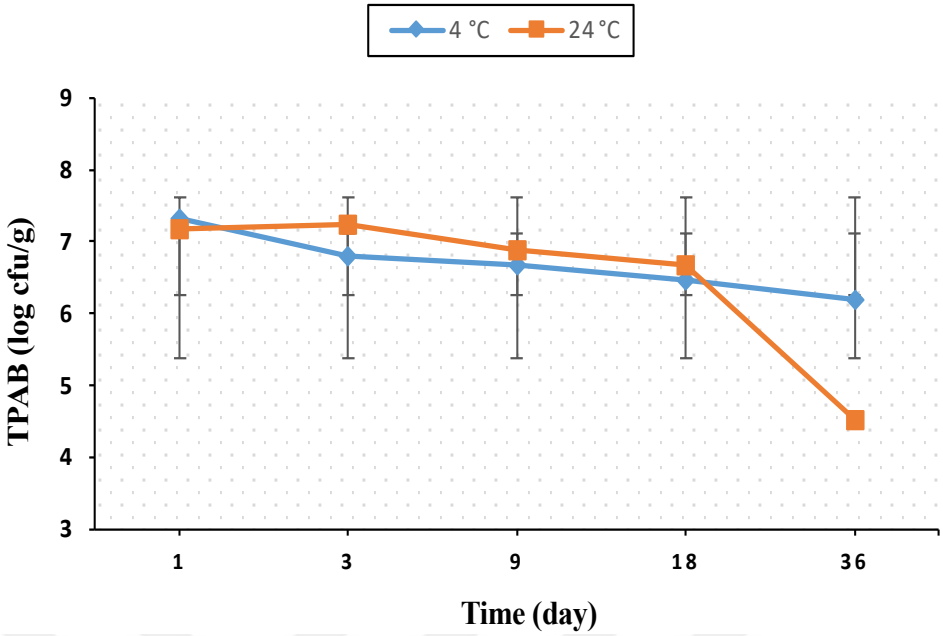


**Figure 7.** Changes in TPAB counts of kishk during storage

### 5.3.3. Yeasts and molds count

Fermentation and drying of kishk had a negative effect on the growth patterns of yeasts and molds in it. As shown in Tables 7 and 8, and as no molds were detected in the main ingredients, yeast counts detected in the raw mixture decreased significantly after undergoing 48 hours of fermentation at 35°C, to a level of 3,80±0,27 $\log_{10}$ cfu/g. Additionally, this decline continued, reaching undetectable levels by the end of the drying stage ($p<0,05$). No yeast or mold growth was detected in kishk balls at either 4°C or 24°C throughout the entire storage period.

### 5.3.4. Coliform count

Microbiological analyses indicated that the production and drying durations of kishk had a significant impact on the coliform count. A closer look at Tables 6 and 7 revealed that the initial coliform count in the kishk mixture was 1,97±0,58 $\log_{10}$ cfu/g. The count showed a significant increase following the addition of the pathogenic inoculum ($p<0,05$). However, coliforms experienced a statistically significant drop during fermentation from 6,51±0,34 to 1,50±1,40 $\log_{10}$ cfu/g. By the 96th hour of drying, these counts fell under the detectable limits of enumeration ($p<0,05$). As shown

in Tables 7 and 8, coliform counts were under the detectable limits after the drying process and during the entire storage period at both temperatures.

### 5.3.5. *Lactobacillus* spp. count

The microbiological analysis revealed that kishk production and storage periods significantly impact the *Lactobacillus* spp. counts. Data in Tables 7 and 8 show that *Lactobacillus* counts increased slightly from 4,61±0,40 to 4,80±0,66 $\log_{10}$ cfu/g following the pathogenic inoculation. During fermentation, the highest levels were recorded on the first day. Over the next 24 hours, the count of *Lactobacillus* spp. decreased slightly, followed by a modest increase observed at the end of the drying process. These counts were close and comparable to those of *Streptococcus* species isolated from the dried kishk product (7,51±0,25 $\log_{10}$ and 7,59±0,31$\log_{10}$ cfu/g, respectively). Table 8 demonstrated that *Lactobacillus* counts showed no significant changes over time during storage at 4°C, accompanied by a gradual decline at 24°C. At the end of the storage period, the counts of *Lactobacillus* spp. were 7,13±0,08 and 6,11±0,13 $\log_{10}$ cfu/g at 4 and 24°C, respectively. A more rapid decrease in *Lactobacillus* counts was observed at room temperature compared to refrigeration temperature, with an approximate difference of 1 log unit, indicating a tenfold greater reduction in microbial load at room storage temperatures (Figure 8).

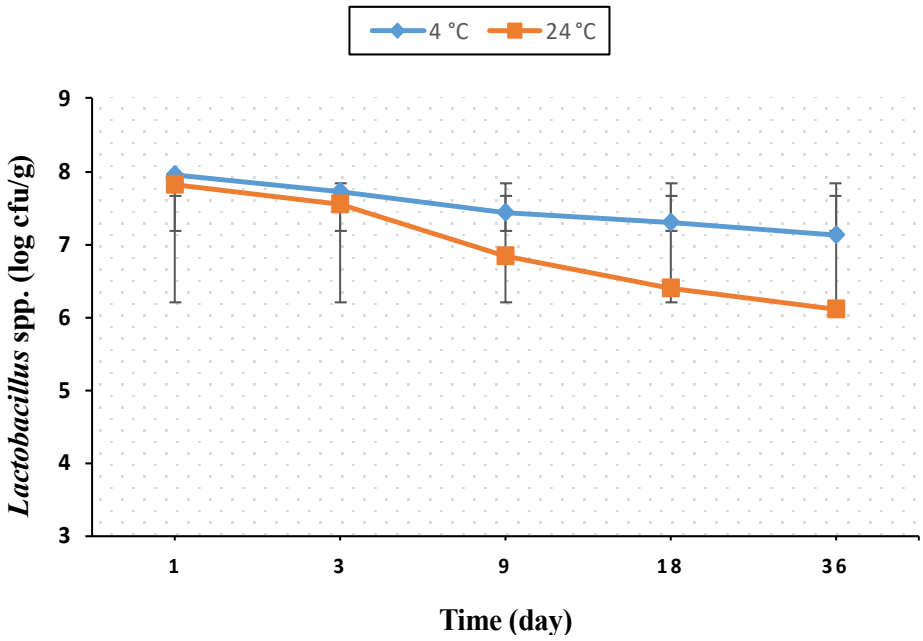


**Figure 8.** Changes in *Lactobacillus* spp. counts of kishk during storage

### 5.3.6. *Streptococcus* spp. count

As shown in Tables 7 and 8, *Streptococcus* counts had a slight decline after the pathogenic inoculum was added to the kishk mixture, contrasting with the increase noted in *Lactobacillus* spp. counts. This was followed by a considerable increase reaching the highest levels observed on the first day of fermentation, recorded as 7,99±0,57 $\log_{10}$ cfu/g. This increase was comparable to the rise observed in *Lactobacillus* species. Although *Streptococcus* spp. were noted to decrease after drying, this reduction was not statistically significant ($p>0,05$). Table 8 demonstrates that storing kishk at 4°C supported this bacterium's persistence. *Streptococcus* count in the dried kishk (7,59±0,31 $\log_{10}$ cfu/g) started to increase significantly during the first day of storage at both temperatures, recording higher numbers at 24°C, averaged as 7,92±0,08, while it was 7,74±0,24 at 4°C. Following the second day of storage, these counts steadily decreased throughout the entire period, reaching means of 6,35±0,12 and 6,89±0,30 at the end of the storage at 24°C and 4°C, respectively. The decline in bacterial counts at 24°C was faster than that observed at 4°C (Figure 9).

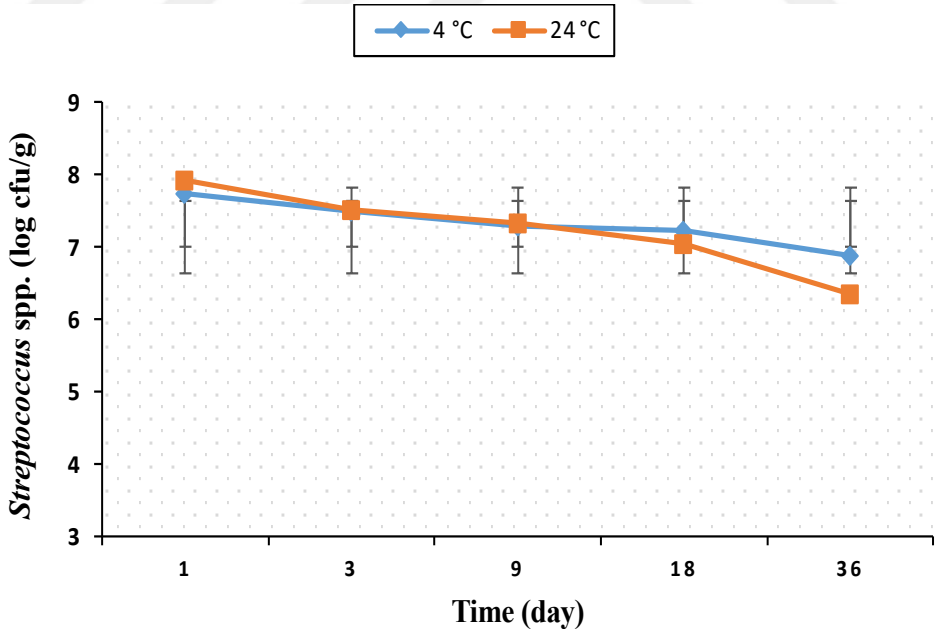


**Figure 9**. Changes in *Streptococcus* spp. counts of kishk during storage

### 5.3.7. *Staphylococcus* and *Micrococcus* spp. count

This study revealed that kishk production did not significantly affect *Staphylococcus* and *Micrococcus* spp. in it ($p>0,05$). The initial counts of *Staphylococcus* and *Micrococcus* spp. in both yogurt and strained yogurt were already below the detectable limit (<100 cfu/mL) and remained consistently undetectable throughout kishk production and storage.

### 5.3.8. *E. coli* O157:H7 count

Analyses revealed that the production duration of kishk significantly influenced the levels of *E. coli* O157:H7. As shown in Table 7, the pathogen count after inoculation (inoculum load: 9,02±0,77 $\log_{10}$ cfu/g) was found to be about 3 logs lower (i.e., 6,28±0,39 $\log_{10}$ cfu/g) in the kishk mixture. After the first 24 hours of fermentation, *E. coli* O157:H7 levels exhibited a significant decrease below the detection limit (< 1 $\log_{10}$ cfu/g) ($p<0,05$). Based on the applied enrichment procedures and present/abscent analyses results, no growth of *E. coli* O157:H7 was detected in any of the tested kishk samples from the end of fermentation through day 36 of storage, indicating the absence of both viable and injured pathogenic cells under the tested conditions and marking the death of the pathogenic cells and the microbiological safety of the product. (Tables 7 and 8).

### 5.3.9. *Salmonella* count

Results indicated that kishk production showed a significant inhibition impact on *Salmonella* counts ($p<0,05$). Considering the inoculum load of *Salmonella* was 8,82±0,99 $\log_{10}$ cfu/ml, the initial inoculation count in the raw kishk mixture on day 0 of production was 6,68±0,90 $\log_{10}$ cfu/g. However, after only 24 hours of fermentation, this count significantly decreased below the detectable limits in the kishk mixture ‘Hamma’. On day 2, marking the end of the fermentation process (48 h), the *Salmonella* count was also recorded as not detected. In all three trial repetitions, kishk samples were all negative for *Salmonella,* which was recorded as ‘absent’ in all enrichment tests during fermentation, drying, and storage (Tables 7 and 8).

**Table 7.** Changes in microorganisms' counts of the pathogen-inoculated kishk during fermentation and drying ($\bar{x}$±SD $\log_{10}$ cfu/g)

| | **Microorganism** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TMAB | TPAB | Yeasts & molds | Coliform | *Lactobacillus* spp. | *Streptococcus* spp. | *Staphylococcus & Micrococcus* | *E. coli* O157:H7 | *Salmonella* |
| **KMp** | 8,65±0,71 | 4,71±0,23 | 4,91±0,11 | 6,51±0,34 | 4,80±0,66 | 6,32±1,06 | < 2 | 6,28±0,39 | 6,68±0,90 |
| **$KMp_{24f}$** | 8,47±0,25 | 6,60±0,38 | 4,99±0,88 | 2,53±1,41 | 7,73±0,10 | 7,99±0,57 | < 2 | < 1 | ND |
| **$KMp_{48f}$** | 7,79±0,20 | 6,41±0,06 | 3,80±0,27 | 1,50±1,40 | 7,47±0,16 | 7,66±0,47 | < 2 | ND | ND |
| **$KMp_{96d}$** | 7,90±0,54 | 5,86±0,07 | < 1 | < 1 | 7,51±0,25 | 7,59±0,31 | < 2 | ND | ND |

SD: standard deviation, $\bar{x}$: mean value.
TMAB: Total mesophilic aerobic bacteria, TPAB: Total psychrophilic aerobic bacteria, KMp: Kishk mixture contaminated with pathogens, $KMp_{24f}$: the contaminated kishk mixture after 24 h fermentation, $KMp_{48f}$: the contaminated kishk mixture after 48 h fermentation, $KMp_{96d}$: the contaminated kishk mixture after 96 h drying at 53°C.
ND: not detected after present/absent analysis.
*<2 $\log_{10}$ cfu/g.

**Table 8.** Changes in microorganisms' counts of kishk during storage ($\bar{x}$±SD $\log_{10}$ cfu/g)

| Microorganism | Storage temperature | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 9 | 18 | 36 |
| TMAB | 4°C | 8,18±0,12$^{Ax}$ | 7,81±0,13$^{Ay}$ | 7,53±0,10$^{Ayz}$ | 7,18±0,09$^{Az}$ | 6,70±0,10$^{Az}$ |
| | 24°C | 8,30±0,09$^{Ax}$ | 7,94±0,07$^{Axy}$ | 7,61±0,13$^{Ayz}$ | 7,59±0,12$^{Bz}$ | 7,26±0,11$^{Bz}$ |
| TPAB | 4°C | 7,33±0,07$^{Ax}$ | 6,79±0,10$^{Ax}$ | 6,68±0,12$^{Ay}$ | 6,46±0,14$^{Ayz}$ | 6,19±0,09$^{Az}$ |
| | 24°C | 7,18±0,09$^{Ax}$ | 7,23±0,11$^{Axy}$ | 6,88±0,09$^{Ay}$ | 6,68±0,08$^{Ay}$ | 4,53±0,11$^{Bz}$ |
| *Streptococcus* spp. | 4°C | 7,74±0,24$^{Ax}$ | 7,49±0,14$^{Axy}$ | 7,30±0,11$^{Ayz}$ | 7,23±0,06$^{Ax}$ | 6,89±0,30$^{Az}$ |
| | 24°C | 7,92±0,08$^{Ax}$ | 7,52±0,08$^{Axy}$ | 7,33±0,06$^{Ay}$ | 7,04±0,05$^{Ay}$ | 6,35±0,12$^{Bz}$ |
| *Lactobacillus* spp. | 4°C | 7,96±0,09$^{Ax}$ | 7,72±0,10$^{Axy}$ | 7,44±0,09$^{Ayz}$ | 7,30±0,17$^{Az}$ | 7,13±0,08$^{Az}$ |
| | 24°C | 7,81±0,13$^{Ax}$ | 7,54±0,08$^{Ax}$ | 6,83±0,11$^{By}$ | 6,40±0,12$^{Bz}$ | 6,11±0,13$^{Bz}$ |
| Yeasts & molds | 4°C | < 1 | < 1 | < 1 | < 1 | < 1 |
| | 24°C | < 1 | < 1 | < 1 | < 1 | < 1 |
| Coliform | 4°C | < 1 | < 1 | < 1 | < 1 | < 1 |
| | 24°C | < 1 | < 1 | < 1 | < 1 | < 1 |

$^{A, B}$: results expressed with different letters in the same column are statistically significant (P<0,05).
$^{x, y, z}$: results expressed with different letters in the same row are statistically significant (P<0,05).
TMAB: Total mesophilic aerobic bacteria, TPAB: Total psychrophilic aerobic bacteria.
*None of the analyzed samples was found to contain *E. coli* O157:H7 and *Salmonella* (ND: not detected after present/absent analysis).
** *Staphylococcus-Micrococcus* found to be under the detectable limits throught the storage period.
SD: standard deviation, $\bar{x}$: mean value.

### 5.4. Chemical Changes Determined During the Production and Storage of kishk

The chemical changes of kishk during the fermentation, drying, and storage at different temperatures (4 and 24°C) are presented in Tables 9 and 10.

#### 5.4.1. pH value

The chemical analyses revealed a significant effect of the production and storage of kishk on pH values across almost all stages ($p<0,05$). As shown in Tables 9 and 10. By the second day of fermentation, a significant decrease in pH, accompanied by a corresponding increase in titratable acidity, was observed in the fermented kishk ($p<0,05$), decreasing from 4,08±0,08 to 3,87±0,07. Following that, no significant decrease in pH was observed during the first hours of drying. Nevertheless, the last two days of dehydrating changed the pH values again, recording averages of 4,02±0,09 and 4,18±0,14, respectively. Throughout the storage period (Table 10), significant pH differences were observed in kishk stored at 24°C. In contrast, the pH values of kishk balls stored at 4°C remained approximately stable over 36 days, with no significant difference from the initial values. The pH value in kishk balls was following a gradual decrease at both temperatures, with a more pronounced manner at 24°C (reaching an average of 3,81±0,08). Figure 10 illustrates the pH changes during the storage of kishk.

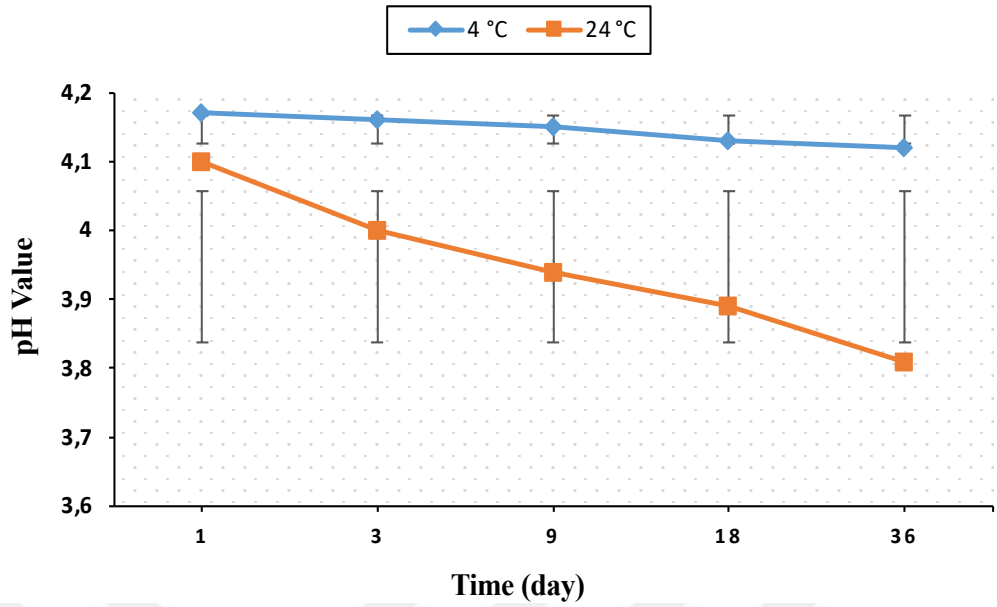


**Figure 10.** Changes in pH of kishk during storage

### 5.4.2. Titratable acidity (as lactic acid%)

Upon chemical analyses, the impact of the kishk production and storage on the total acidity (lactic acid%) is demonstrated in Tables 9 and 10. It was observed that the initial total acidity in the raw kishk mixture (1,32±0,16) had significantly increased during fermentation ($p<0,05$) to reach an average of 1,66±0,03. During drying, no significant increase in acidity was observed ($p>0,05$). However, the acidity in dried kishk balls (1,35±0,19) increased significantly day by day from the first day of storage at 24°C, reaching a peak of 1,72±0,14 on day 36 ($p<0,05$). In contrast, no significant increase in titratable acidity was observed during the same storage period at 4°C, with a final value of 1,40 ± 0,20, closely aligning with the initial level conducted under refrigeration temperature (1,36±0,19). Following the 36-day storage period, the total acidity values for both temperature groups over the last storage days were found to be incomparable, as shown in Figure 11.

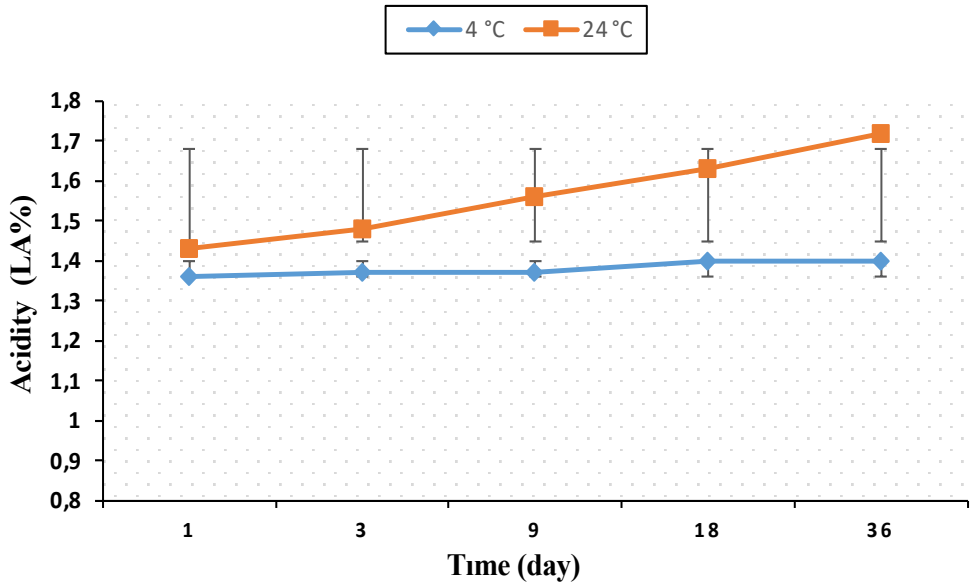


**Figure 11.** Changes in the titratable acidity of kishk during storage

### 5.4.3. Dry matter content (DM %)

The statistical analyses revealed that the production and storage duration of kishk significantly influenced its moisture and dry content (%). Table 9 shows that the dry matter content of kishk at the end of fermentation was 45,40±1,74, which didn't differ significantly from that of the raw kishk mixture observed before fermentation (Table 5). However, a significant increase in dry matter content was observed on the first day of drying ($p < 0,05$), continuing progressively to reach its peak at the 96th hour, rising from 55,41±1,01% to 95,20±0,40%. Data in Table 10 present that throughout the subsequent storage period, the moisture loss was more pronounced and the dry matter was greater in kishk balls stored at 24°C compared to that in kishk stored at 4°C (95,94±0,11% and 95,31±0,19% on day 36, respectively). Although these differences were not statistically significant, the data indicated a consistent and more substantial increase in the dry matter content at 24°C. Figure 12 illustrates the changes in the dry content (%) over the storage period regarding the varying temperatures.

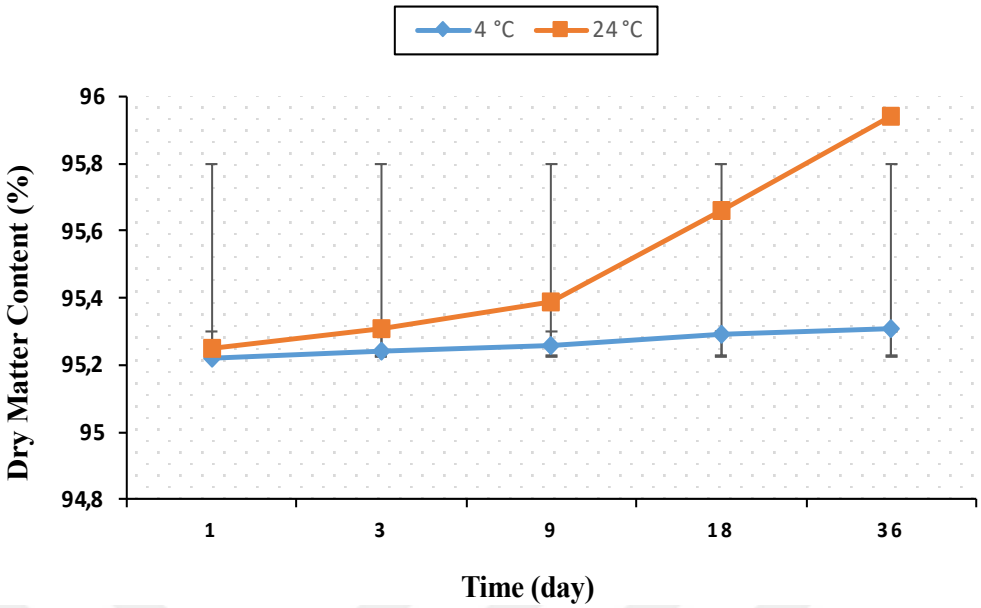


**Figure 12.** Changes in the dry matter content (%) of kishk during storage

**Table 9.** Chemical changes in kishk during production ($\bar{x}$±SD)

| Groups | pH | Acidity (LA%) | Dry matter (%) |
|---|---|---|---|
| $KM_{24f}$ | 4,08±0,08 | 1,51±0,07 | 49,35±1,82 |
| $KM_{48f}$ | 3,87±0,07 | 1,66±0,03 | 45,40±1,74 |
| $KM_{24d}$ | 3,91±0,05 | 1,63±0,03 | 55,41±1,01 |
| $KM_{48d}$ | 3,95±0,04 | 1,61±0,03 | 72,20±1,16 |
| $KM_{72d}$ | 4,02±0,09 | 1,55±0,04 | 83,23±1,35 |
| $KM_{96d}$ | 4,18±0,14 | 1,35±0,19 | 95,20±0,40 |

$KM_{24f}$: kishk mixture after 24 h fermentation.
$KM_{48f}$: kishk mixture after 48 h fermentation.
$KM_{24d}$: kishk mixture after 24 h drying.
$KM_{48d}$: kishk mixture after 48 h drying.
$KM_{72d}$: kishk mixture after 72 h drying.
$KM_{96d}$: kishk mixture after 96 h drying.
SD: standard deviation, $\bar{x}$: mean value.

**Table 10**. Chemical analyses results of kishk during storage (x̄±SD)

| Parameter | Storage temperature | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | **1** | **3** | **9** | **18** | **36** |
| pH | 4°C | 4,17±0,14 | 4,16±0,14 | 4,15±0,14 | 4,13±0,14 | 4,12±0,15 |
| | 24°C | 4,10±0,10 | 4,00±0,11 | 3,94±0,16 | 3,89±0,08 | 3,81±0,08 |
| Titratable acidity (LA%) | 4°C | 1,36±0,19 | 1,37±0,19 | 1,37±0,26 | 1,40±0,19 | 1,40±0,20 |
| | 24°C | 1,43±0,13 | 1,48±0,14 | 1,56±0,23 | 1,63±0,12 | 1,72±0,14 |
| Dry matter (%) | 4°C | 95,22±0,38 | 95,24±0,37 | 95,26±0,32 | 95,29±0,27 | 95,31±0,19 |
| | 24°C | 95,25±0,31 | 95,31±0,30 | 95,39±0,29 | 95,66±0,16 | 95,94±0,11 |

SD: standard deviation, x̄: mean value.

# 6. DISCUSSION

In this study, the survival of specific foodborne pathogens, namely *E. coli* O157:H7 and *Salmonella strains*, recognized for their potential risks to human health, was investigated during the production and storage of kishk. Kishk was made from yogurt, strained yogurt, salt, and bulgur and was experimentally contaminated with *E. coli* O157:H7 and *Salmonella* strains. The observed chemical parameters of the main ingredient (i.e., pH, acidity, and dry matter content) were consistent with the standards set by the Turkish Food Codex Communique, validating the appropriateness of the ingredients for kishk production (147,148).

## 6.1. Total Mesophilic Aerobic Bacteria (TMAB) Count

Table 7 presents the initial total mesophilic aerobic bacteria (TMAB) counts, which significantly increased in the Hamma mixture following pathogen inoculation. This rise can be attributed to the mesophilic and facultative aerobic nature of *Salmonella* and *E. coli* O157:H7, which affected the total mesophilic aerobic bacterial counts. Moreover, both can survive alongside other microbial populations in a nutrient-rich matrix such as kishk, before being decreased by the inhibitory effects of fermentation-associated acidification (141). Similar observations were reported in the TMAB count by Dalioglu et al. (2002) after adding *E. coli* O157:H7 and *S. aureus* strains to tarhana. Similarly, Kivanc & Funda (2017), Erbaş et al. (2005), Ibanoğlu et al. (1999), and Karagozlu et al. (2008) detected a comparable manner of TMAB during tarhana's fermentation and drying (107,149-151). The reduction in TMAB counts may be due to multiple inhibitory factors, including moisture loss, increased acidity, and the production of antimicrobial compounds such as carbon dioxide, hydrogen peroxide, acetaldehyde, and ethanol, as well as intensified microbial competition — all of which create unfavorable conditions for the survival of TMAB. However, storage exhibited a more pronounced effect on TMAB in kishk. After drying, the initial total viable counts in dried kishk balls were comparable with El-Gendy (1983) and Atia and Khattab (1986)'s findings (9,12,115). Data in Table 8 declare that from day 1 to the 36th day of storage at 4°C (Table 8), total aerobic mesophilic bacteria showed a more significant decrease than those in kishk balls stored at 24°C. The values

observed at room temperature were consistent with those reported in previous studies by Erbaş et al. (2005) in tarhana, Abd El-Razik et al. (2016), who applied the HACCP system in kishk production, and Ismail and Rayan (2022) (110,150,152). TMAB counts exhibit optimal metabolic activity within a temperature range of 20–45°C, as widely supported in the literature, leading to a gradual, but slight decline in bacterial counts during storage at room temperature, due to cellular stress and gradual loss of viability. In contrast, TMAB became metabolically inactive at 4°C, supporting the view that lower storage temperatures prevent mesophilic bacterial activity, resulting in more stress-induced injured cells and an increased product's shelf life (115,143,153,154). The final values of TMAB observed on day 36 of room temperature storage were similar to those conducted by Ismail & Rayan (2022) (152).

### 6.2. Total Psychrophilic Aerobic Bacteria (TPAB) Count

The significant increase in TPAB counts during the first 24 hours of fermentation suggests that the psychrotolerant species were outcompeting mesophilic aerobic bacteria under such conditions (Table 7). Similar findings were reported by Dikici (2008), who reported an initial increase in total psychrotrophic aerobic bacterial counts at the beginning of the fermentation period of Şavak cheese, followed by an expected decline within the subsequent 24 hours (155). The decrease in TPAB after 96 hours of drying was likely due to the exposure to high temperatures well above their optimal range, leading to denaturation and enzymatic inactivation, thus ending up with cell injury or death. TPAB continued to decline gradually throughout the storage period, with a more pronounced reduction observed at 24°C, particularly during the final 18 days (Table 8, Figure 7). This sharp drop can be attributed to the differing metabolic characteristics of these bacteria, as their optimal growth and sustained activity occur at low (refrigeration) temperatures, whereas exposure to higher or warmer temperatures induces stress and ultimately leads to cell death or injury (156,157).

### 6.3. Yeasts and Molds Count

Based on Table 7, yeast counts may have increased following the pathogenic inoculation and during the initial hours of fermentation, likely due to the nourishing composition of the initial Hamma's mixture, before being affected by fermentation conditions. However, a significant decrease in yeast counts was observed during the last hours of fermentation, reaching a level of 3,80±0,27 $\log_{10}$ cfu/g, then continued to be under the detectable limits at the end of the drying stage. The reason could be the increasing acidification during fermentation and the moisture reduction through drying, which led to the non-hygroscopic status of kishk. Although the microbiological quality of both yogurt and strained yogurt did not meet the expected recommendations for yeasts and molds (upper limits: 2-3 $\log_{10}$ cfu/g) as outlined in the Turkish Food Codex Communiqué for fermented dairy products, the controlled production process of kishk was effective to eliminate the presence of yeasts and molds throughout the whole storage period (158). These results align with the findings of Nassar et al. (2023), Ismail and Rayan (2022), and Abd-Rabou (2020), and those reported by Abd El-Razik et al. (2016) and Tamime et al. (2000), in which no presence of yeast or mold was detected in any of the kishk samples (105,110,114,117,152). Similar patterns of yeasts and molds have also been conducted in tarhana by Daglioglu et al. (2002), Erbaş et al. (2005), Sağdıç et al. (2005), and Turantaş and Kemahlıoğlu (2012) (141,148,159,160). A recent study, by Chedid et al. (2025), further supported these findings (161). However, this was not the status in other kishk products studied by Elshreef et al. (2014), Elewa and Metry (2006), Tamime & McNulty (1999), and Elewa NAH (2006), at which yeasts and molds counts reached maximum averages of 5,76, 5,34, 5,93, 6,73 log cfu/g, respectively (14,109,123,130). The decreasing pH and moisture content in kishk balls stored at 24°C, along with the unfavorable refrigeration temperature, played an important role in limiting the potential growth of yeasts and molds during storage (162).

### 6.4. Coliform Count

Coliforms are a broad group of bacteria commonly used as indicators of hygiene and potential contamination in food and water. Their presence in dairy products, like yogurt or kishk, often points to insufficient heat treatment, post-processing contamination, or poor sanitation during production. While not all coliforms are harmful, their detection raises food safety concerns. Therefore, microbiological standards, such as TS 1330, set strict limits for their acceptable levels to protect public health (163). The significant increase in coliform counts in the kishk mixture immediately after the pathogenic inoculation can be attributed to the fact that *E. coli* O157:H7 is a coliform bacterium. Meaning that their inclusion in the kishk mixture directly influences the final coliform counts in it. Although the inoculated kishk mixture initially contained coliform levels exceeding the upper limits established by the Turkish Food Codex Microbiological Criteria Communiqué (Communiqué No: 2010/4), a significant reduction in coliform counts was observed during the fermentation and drying processes involved in kishk production (158). By 96 hours of drying, coliforms had declined to below detectable levels, and this status was maintained throughout the subsequent storage period. Our results were in alignment with Tamime et al. (2000), Ismail & Rayan (2022), Abd El-Razik et al. (2016), Abd-Rabou (2020), Daglioglu et al. (2002), Erbaş et al. (2005), Sağdıç et al. (2005), Chedid et al. (2025), and Turantaş & Kemahlıoğlu (2012) (105,110,114,141,150,152, 159-161). The findings underscore the critical importance of stringent control over coliform contamination throughout the production process, including the handling of raw materials, processing stages, and storage conditions.

### 6.5. Lactic Acid Bacteria (LAB) Count

Lactic acid bacteria played a key role in kishk fermentation, lowering the pH and increasing acidity. *Lactobacillus* and *Streptococcus* spp. showed a sharp growth during the early stages of fermentation, reaching their highest levels after the first 24 hours (7,73±0,10 and 7,99±0,57 cfu/g, respectively) (9). The gradual, slight decrease during the last hours of fermentation and drying was similar to patterns of LAB observed by Gaddallah and Hassan (2019), Elewa (2006), and Salama et al. (1992) (13,123,124). The decline is attributed to the high acidity, limited substrate availability, and the

subsequent effect of temperature on moisture loss. Tamime et al. (2000), Ismail and Rayan (2025), Abd El-Razik et al. (2016), Hajj et al. (2019), and Salama et al. (1992) in kishk, and Erbaş et al. (2005) in tarhana had also observed a similar manner of LAB during production (105,110,124,125,150,152). Other studies that employ the traditional fermentation and drying method under sunlight, such as that by Damir et al., may conduct similar LAB counts, but at the same time, lead to a poor microbiological quality of the product. The gradual decline in LAB observed during storage (Table 8) is consistent with the LAB dynamics reported in kishk and tarhana by Ismail and Rayan (2022), Erbaş et al. (2005), and Turantaş and Kemahlıoğlu (2012). These numbers followed the same gradual decrease in a month or a half at room temperature, from 7,13 to 6,88±0,02, 5,44±0,10 to 4,17±0,50, and from 7,6 to 5,10 log cfu/g, respectively (150,152,160). However, both species' counts were well-maintained throughout storage at 4°C, due to the slowdown of bacterial respiration, similar to Erbaş et al. (2005) findings, as LAB counts didn't change in the F type of refrigerated tarhana (150). Cooler storage temperatures are generally more favorable for sustaining functional bacterial populations in the final product. Thus, the high metabolic activity leads to faster nutrient depletion, bacterial competition, and by-product accumulation at room temperature, a much decreased pH, and a faster decline in LAB. This contributes to a long shelf life, as the acidic environment inhibits the growth of many spoilage microorganisms and pathogens (10,105,161).

### 6.6. *Staphylococcus* and *Micrococcus* spp.

In this study, *Staphylococcus* and *Micrococcus* spp. counts remained undetectable throughout the production and storage. Consistent with our findings, previous studies by Gaddallah & Hassan (2019), Tamime et. al. (2000), Abd El-Ghani et al. (2014), Ismail & Rayan (2022), Abd El-Razik et al. (2016), Hajj et al. (2019), and Uçar & Çakiroğlu (2011) reported neither the presence of pathogenic microorganisms nor contamination with *Staphylococcus* or *Micrococcus* spp. in Kishk and Tarhana (13,16,105,110,113,152,164).

### 6.7. *E. coli* O157:H7 and *Salmonella* Count

Tables 7 and 8 show that the initial inoculation levels of both *E. coli* and *Salmonella* have immediately dropped in the raw kishk mixture (of bulgur, yogurt, and salt) from 9,02±0,77 to 6,28±0,39 $\log_{10}$ cfu/ml and from 8,82±0,99 to 6,68±0,90 $\log_{10}$ cfu/ml, respectively. This can be attributed to the antimicrobial properties of the mixture components; the combined effects of low pH from fermentation, osmotic stress induced by the salt level (2 %), and competition from naturally occurring LAB likely contributed to the rapid decline in the pathogens' viability following inoculation into the 'Hamma' mixture. These counts experienced a significant reduction at pH 4,08 ($p<0,05$) during the early stages of production, particularly after 24 hours of fermentation. However, *E. coli* O157:H7 strains demonstrated greater resistance compared to *Salmonella* during the experimental kishk production process, as *Salmonella* was not detected in either the kishk samples or on selective media after just 24 hours of fermentation. In contrast, *E. coli* required the same 24 hours of fermentation to fall below detectable limits, and approximately 48 hours to be eliminated from the kishk, at a pH of 3,87 ($p < 0,05$) (Table 7). In the present/absent tests performed at all steps, no significant changes were observed after 48 hours of fermentation, drying, and storage regarding both pathogens' counts. STEC has an exceptionally low infectious dose, with as few as 10 bacterial cells potentially causing infection (165-167). In contrast, non-typhoidal *Salmonella* strains typically require an infectious dose ranging from $10^3$ to $10^6$ cfu to cause illness (70-72). Notably, neither pathogen was detectable in the final product, the 'dried kishk'. This is thought to be associated with several stress factors (cumulative hurdles): increased competitive flora, accumulation of metabolic and antimicrobial by-products, a drop in pH and moisture content, increased acidity, and the addition of NaCl, leading to metabolic stress (70,165-167).

Similar findings were reported by Sagdıc et al. (2005) and Erbaş et al. (2005), in tarhana in which *E. coli* O157:H7 became undetectable by the end of the second day of fermentation, which was attributed to the reduction in pH (3,75-3,84) (150,159). Turantaş and Kemahlıoğlu (2012) demonstrated that *S. Typhimurium* counts significantly declined during the early stages of tarhana's fermentation, while *E. coli* O157:H7 decreased gradually over time, and significantly after day 4 of

fermentation at a pH of approximately 4 (160). However, Daglioglu et al. (2002) detected *E. coli* O157:H7 survival under similar fermentation, as it took more than 96 hours to inhibit this pathogen at a pH of 4,34 (141). Aytac, S.A. (1996), which followed a traditional production method, indicated that *E. coli* O157:H7 remained viable during the entire fermentation period, while it decreased to below $10^2$ cfu/g at 4 pH after drying, and was inhibited after the 8th day at a pH of 3,10 (168). Such variations likely stem from differences in pathogenic strains, initial microbial loads, fermentation or drying methods, ingredients, and production conditions. Additionally, the lack of standardized processing protocols across studies may contribute to inconsistencies, including elevated pH levels or potential cross-contamination. However, Gadaga, T.H. et al. (2007) in Africa, Saleh et al. (2009) in Lebanon, Abd-Alla et al. (2020) in Egypt (Sohag), Anyogu, A. et al. (2021) in Africa, Salameh and Hosri (2019), and Chedid et al. (2025) in Lebanon, had all detected one or more of these pathogens in kishk and kishk-like products and thus failed to comply with the national hygiene and safety standards (15,17,139,161,169,170). Nonetheless, literature presents conflicting findings concerning the persistence of *E. coli* O157:H7 and *Salmonella,* commonly encountered in fermented foods. Research has predominantly concentrated on the roles and benefits of lactic acid bacteria within fermented products (9,14,106,171). Results highlight the significance of the fermentation and drying processes in kishk production, which enhance acidity and reduce moisture content, thereby playing a crucial role in improving the microbiological safety of the final fermented product.

### 6.8. pH Value and Titratable Acidity (LA%) of Kishk

*Salmonella* and *E. coli* O157:H7 had been highly affected by decreasing pH levels (18,34). As presented in Table 9, the significant decline in pH (from 4,22±0,08 to 3,87±0,07) in line with the increasing acidity (from 1,32±0,16 to 1,66±0,03) found at the end of fermentation, was similar to that conducted in acidophilus milk-based kishk by Elewa & Metry (2006) (i.e., 3,90), and in Rayeb Kishk by Damir & Salama (1992) (4,00±0,07) during the fifth and third day of fermentation at reduced temperatures (109,124,127). The decrease in pH during fermentation is due to the lactic acid and organic acids (e.g., acetic acid) produced by the LAB, which exhibit an inhibitory effect on such pathogenic microorganisms, leading them to fall under the

detectable limits (3,109,127). This underscores the critical role of temperature control during production in maintaining both the safety and quality of the final product. On the other hand, the gradual increase in pH during drying can be attributed to the moisture loss (acid-base balance and concentration effect), evaporation of some water-bound lactic acid, decreased microbial activity of LAB that further limits the lactic acid production, and the proteolysis of milk proteins (e.g., casein) releasing basic nitrogenous compounds (28,45).

As illustrated in Table 10, the chemical composition of kishk was monitored over a 36-day storage period at two different temperatures (4°C and 24°C), and slight variations were observed in pH, titratable acidity (TA), and dry matter content. A gradual decline in pH was recorded across all time points, indicating increased acidity as storage progressed. The reduction was more pronounced at 24°C, with pH dropping from 4,10±0,10 on day 1 to 3,81±0,09 on day 36, compared to a smaller decline at 4°C (4,17±0,14 to 4,12±0,15). This trend correlates with a progressive increase in titratable acidity, especially at 24°C, where values reached 1,72±0,14 on day 36, suggesting enhanced lactic acid accumulation due to the continued microbial activity of LAB at ambient temperatures. This was in contrast to the suppression of their metabolic activity and lactic acid production under refrigeration conditions (4±1°C) (28,45,172). Our findings are consistent with the chemical analyses reported by Nassar et al. (2016), Ismail and Rayan (2022), Elewa and Metry (2006), El-Attar et al. (2015), Gadallah and Hassan (2019), Elewa NAH (2006), and El-Den and Kamaly (2017) (13,109,117-119,123). El-Nawawy and El-Kenany (2005) also reported that lactic acid bacterial counts in kishk products were significantly higher when stored at 4°C compared to those stored at 24°C over storage periods of 30, 60, and 90 days (121). Similar findings were introduced by Attia and Khattab (1985), Elham & Kamaly (2017), Hussein et al. (2013), and Tamime et al.(1997) (105,116,118,173).

### 6.9. Dry Matter Content (%) in Kishk

Table 9 shows that the dry matter content of kishk was increased during production, to reach 95,2±0,40 % after 96 hours of drying at 53°C. The small diameter of kishk balls gives a larger surface area, which facilitates more rapid moisture evaporation (101). This process is influenced by several factors such as adequate air

circulation, low humidity, and a controlled drying temperature. Additionally, maintaining a consistent air velocity at the outset of the drying process has been shown to enhance moisture loss from kishk. In contrast, sun drying of kishk, while traditional, demands significant manual labor to ensure uniform drying and poses considerable risks of microbial and physical contamination (103,104). Despite the similarity in dry matter content between the dried experimental kishk and the values reported by Abd-Alla et al. (2020), which averaged 95,13±0,39 %, none of the Egyptian kishk samples were free from pathogenic contaminants (15). This is likely attributable to improper drying practices, as mentioned before (15). Another study by Salameh et al. (2019) applied different drying techniques under hot air drying at 53°C for 4 days, which positively impacted the chemical and microbiological properties of kishk, despite the traditional method (170). Our findings in Table 10 indicate that only slight fluctuations were observed in the dry matter content (%) throughout the storage period. While values remained relatively stable at 4°C, a slight increase was recorded at 24°C, peaking at 95,94±0,11% on day 36. This might be attributed to mild dehydration or concentration effects at higher storage temperatures, which is more preferable for kishk's microbiological safety (24°C). Based on the chemical profile observed at day 36 of storage at room temperature, kishk is expected to maintain characteristics comparable to those of Grade A premium kishk, as defined by Dimassi et al. (2024), throughout subsequent storage periods (120).

# 7. CONCLUSION AND RECOMMENDATIONS

This study highlights the inhibitory impact of kishk production and storage on the viability and survival of *E. coli* O157:H7 and *Salmonella* spp. Key findings confirm that the adherence to controlled production and storage conditions plays an important role in reducing moisture content and pH, increasing acidity, minimizing contamination, suppressing the pathogenic survival, and thus ensuring the product's safety and quality. Fermentation proved to be the most effective phase, resulting in the complete elimination of *E. coli* and *Salmonella* strains. However, the subsequent drying and storage stages are equally critical in sustaining this microbial suppression over time. Findings also indicate that, from a health perspective, storage at 4°C helps maintain lactic acid bacteria viability, while storing kishk at room temperature (24°C) might be effective for prolonging shelf life and enhancing its chemical stability. The results provide a comprehensive understanding of the chemical and microbiological changes in kishk during production and storage. This also supports kishk's potential as a long-lasting and culturally significant fermented food when produced under hygienic and safety standards.

This research addresses potential public health concerns associated with kishk produced traditionally and emphasizes the need for developing effective strategies and standardized production that aim at minimizing microbial risks and foodborne illnesses, while preserving desirable characteristics. By bridging cultural heritage with food safety standards, the study offers practical recommendations to mitigate specific pathogenic threats and presents novel insights into the antimicrobial effect that a standardized kishk production process can offer. In this context, kishk produced properly under controlled conditions may be considered a microbiologically safe product against *E. coli* O157:H7 and *Salmonella*, holding its long-lasting, cultural, and nutritional attributes. To validate this assumption, further studies investigating the survival of wild-type strains are necessary. The fermented dairy sector remains competitive, and the need for further studies for safer traditional fermented products, like those properly produced, is always essential.

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| **Foreign Language Exam Score** | | | | | | | | |
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# ORIGINALITY REPORT

DETERMINATION OF CHANGES IN ESCHERICHIA COLI O157:H7 AND SALMONELLA NUMBERS DURING THE PRODUCTION AND STORAGE OF KISHK

ORİJİNALLİK RAPORU

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