THE NUTRIENT PROFILING OF COW AND GOAT MILK KEFIR AND THEIR CYTOTOXICITY EVALUATION OF CRUDE PROTEINS EVOLVED WITH BIOGENIC SILVER NANOPARTICLES

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DİYARBAKIR

GRADUATE SCHOOL
OF NATURAL AND APPLIED SCIENCES
OF DİCLE UNIVERSITY

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BY
RINAS VALİ

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Dedicated to My Family and Myself …

I hereby declare that all information in this document has been obtained and presented following the Thesis Manual (prepared by the Dicle University Graduate School of Natural and Applied Sciences), academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work. I shall be solely responsible if any evidence is found against my declaration.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Definition
°C	Degree Celsius
°SH	Soxhlet-Henkel degrees
µL	Microliter
g	Gram/s
h	Hour/Hours
kDa	Kilodalton
Mg	Milli gram/s
mL	Millilitre
nm	Nanometre
ppm	Part Per Million

Abbreviation	Definition
AcOH	Acetic acid
APS	Ammonium persulfate
ATCC	American type culture collection
B-AgNPs	Biogenic silver nanoparticles
BJ	Normal foreskin fibroblast
BSA	Bovine serum albumin
CK	Cow milk kefir
EDX	Energy dispersive X-ray analyser
GK	Goat milk kefir
LAB	Lactic acid bacteria
MeOH	Methanol
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
ROS	Reactive oxygen species

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
WSPE	Water-soluble protein extract

ABSTRACT

THE NUTRIENT PROFILING OF COW AND GOAT MILK KEFIR AND THEIR CYTOTOXICITY EVALUATION OF CRUDE PROTEINS EVOLVED WITH BIOGENIC SILVER NANOPARTICLES

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Fermented beverages and foods are essential components of the human diet, providing crucial nutrients and contributing to disease prevention. Lactic acid bacteria and yeasts play critical roles in fermentation, with specific strains, termed probiotics, offering distinct health benefits. This study focuses on the production of kefir from goat and cow milk, offering a comprehensive analysis of their nutritional profiles.

Our objective is to provide valuable insights into the unique characteristics of goat milk kefir (GK) and cow milk kefir (CK) by examining their pH values, titratable acidity, total free amino acid content, and total dry matter over an eight-day period. Additionally, this research focused on protein profiling using SDS-PAGE electrophoresis to elucidate the detailed water-soluble protein extracts (WSPE) composition of cow and goat milk kefir. Beyond these analytical endeavours, our emphasis on protein profiling lays the groundwork for exploring the cytotoxicity of proteins treated with biogenic silver nanoparticles (B-AgNPs). During the eight days of storage at 8 °C, the pH values and titratable acidity of both cow and goat milk kefir decreased. There was no significant difference between cow and goat milk kefir in terms of these parameters. However, the amino acid content increased, ranging from 1.35 to 2.45 for cow milk kefir and from 2.15 to 3.98 for goat milk kefir. The titratable acidity decreased from 0.92 to 0.74 and 0.95 to 0.81 for cow milk kefir and goat milk kefir, respectively. In terms of protein content, the Bradford method revealed a total protein range of 1.35 to 2.45 for cow milk kefir and 0.70 to 1.30 for goat milk kefir. When analysing protein profiles using SDS-PAGE, no significant difference was observed between cow and goat milk kefir, despite the initial variation indicated by the Bradford method.

When assessing the antimicrobial activity of proteins treated with B-AgNPs, only Gram-positive bacteria showed an effect, whereas Gram-negative bacteria did not exhibit any response. CK-WSPE and GK-WSPE showed no toxic effects on normal foreskin fibroblast (BJ, CRL-2522) cells, with proliferative concentrations determined as 62.5 and 500 µg/mL, respectively. Remarkably, while B-AgNPs exhibited high toxicity individually, this toxicity was mitigated when combined with CK-WSPE and GK-WSPE in the 1:2 mixtures. Only CK-WSPE demonstrated proliferation at a concentration of 15.625 µg/mL after being mixed with B-AgNPs, which is lower than the concentration required when CK-WSPE is applied independently to treat the cells.

Keywords: Biogenic silver nanoparticles, Biological activity, Cow milk kefir, Goat milk kefir, Kefir.

ÖZET

İNEK VE KEÇİ SÜTÜ KEFİRİNİN BESİN PROFİLİ VE BİYOJENİK GÜMÜŞ NANOPARTİKÜLLERLE GELİŞTİRİLEN HAM PROTEİNLERİNİN SİTOTOKSİSİTELERİNİN DEĞERLENDİRİLMESİ

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Fermente içecekler ve gıdalar, insan diyetinin vazgeçilmez bir parçasıdır ve sağlıklı beslenmenin önemli bir unsuru olarak hastalıkların önlenmesine katkı sağlarlar. Laktik asit bakterileri ve mayalar, fermantasyonda kritik roller oynarlar ve özellikle belirli suşları probiyotik olarak adlandırılarak sağlık üzerinde belirgin faydalar sunarlar. Bu çalışma, kefir üretimi için keçi ve inek sütüne odaklanarak, onların besinsel profillerinin kapsamlı bir analizini sunmaktadır.

Amacımız, keçi sütü kefiri (GK) ve inek sütü kefirinin (CK) benzersiz özellikleri hakkında değerli öngörüler sağlamak için, sekiz günlük bir süre boyunca pH değerleri, titrasyon asitliği, toplam serbest amino asit içeriği ve toplam kuru madde miktarı üzerinde analizler gerçekleştirmektir. Ayrıca, bu araştırma, inek ve keçi sütü kefirinin suda çözünen protein ekstrelerinin (WSPE) bileşimini aydınlatmak için SDS-PAGE elektroforezi kullanarak protein profillemesine odaklanmaktadır. Bu analitik çabalardan öte, protein profillemesine verdiğimiz önem, biyojenik gümüş nanopartiküllerle (B-AgNPs) muamele edilen proteinlerin sitotoksisitesini araştırmak için bir temel oluşturmaktadır. 8 °C'deki sekiz günlük depolama esnasında hem inek hem de keçi sütü kefirinin pH değerleri ve titre edilebilir asitliği azaldığı gözlemlendi. Bu parametreler yönünden inek ve keçi sütü kefiri arasında anlamlı bir fark görülmedi. Ancak amino asit içeriği bakımından inek sütü kefiri 1,35'ten 2,45'e keçi sütü kefirinin ise 2,15'ten 3,98'e yükseldiği belirlendi. Titre edilebilir asitlik inek sütü kefirinde 0,92'den 0,74'e, keçi sütü kefirinde ise 0,95'ten 0,81'e düşmüştür. Bradford yöntemine göre yapılan protein içerik analizlerinde inek kefirinin 1,35 ile 2,45, keçi sütü kefirinin ise 0,70 ile 1,30 arasında protein içerdiği belirlendi. SDS-PAGE kullanılarak protein profilleri analiz edilirken, Bradford yöntemiyle belirlenen başlangıç varyasyonuna rağmen inek ve keçi sütü kefirleri arasında anlamlı bir fark gözlenmedi.

B-AgNPs ile muamele edilen kefir proteinlerinin antimikrobiyal aktivitesi incelendiğinde, mikrobiyal büyümeyi inhibe etme yeteneğinin Gram-pozitif bakterilere karşı olduğu ve Gram-negatif bakterilere karşı aktivitesinin olmadığı görülmüştür. CK-WSPE ve GK-WSPE'nin, normal sünnet derisi fibroblast (BJ, CRL-2522) hücreleri üzerine toksik etki göstermediği ve sırasıyla 62,5 ve 500 µg/mL konsantrasyonlarda proliferatif etki gösterdiği belirlendi. B-AgNP'ler tek başına dikkat çekici bir biçimde yüksek toksisite sergilerken, 1:2'lik karışımlarda CK-WSPE ve GK-WSPE ile birleştirildiğinde bu toksisitenin azaldığı görülmüştür. Sadece CK-WSPE, B-AgNPs karışımı 15,625 µg/mL konsantrasyonda proliferasyon göstermiş olup, bu konsantrasyon, CK-WSPE'nin hücreleri tedavi etmek için tek başına uygulandığında gereken konsantrasyondan daha düşüktür.

Anahtar Kelimeler: Biyojenik gümüş nanopartiküller, Biyolojik aktivite, İnek sütü kefiri, Keçi sütü kefiri, Kefir.

1. INTRODUCTION

Food fermentation stands as one of the oldest and most cost-effective methods for processing and preserving food, a practice deeply ingrained in human history for centuries (Steinkraus et al., 2004). This age-old technique involves the collaboration of bacteria, yeasts, or molds, either independently or in tandem, to transform a diverse range of raw foods into fermented products (Medina, 2019). The microbial and enzymatic processes inherent in fermentation play a pivotal role in altering the sensory characteristics (including appearance, texture, aroma, and flavour) and nutritional composition of raw materials, resulting in the creation of desirable food product properties and nutrients (Sharma et al., 2020). Moreover, fermentation contributes to heightened digestibility of foods and bolsters food safety by eliminating undesirable substances present in raw foods, such as tannins and phytates (Soetan and Oyewole, 2009). The intricate dance of microorganisms during fermentation not only transforms raw ingredients into delectable products but also serves as a mechanism for improving both the palatability and safety of our food supply. In recent times, there has been a heightened interest in crafting fermented dairy beverages infused with probiotics. This enthusiasm is fuelled by numerous health assertions associated with the regular intake of such products (Magalhães et al., 2011).

Fermented products now contribute significantly, accounting for 20-40% of the global food supply (Adesulu and Awojobi, 2014). A diverse array of raw ingredients, ranging from milk to fruits, tea, and vegetables, serves as substrates in the fermentation process, ultimately transforming into an array of popular fermented foods and beverages. This encompasses a wide spectrum, including wine, beers, sauerkraut, sausages, kombucha, tempeh, cheese, yogurt, and the most important products is kefir (Tamang et al., 2020). Kefir originating centuries ago in the Caucasus Mountains of Russia, is a traditional fermented dairy drink. Today, kefir's popularity has spread worldwide, and it is well-known for being a nutritious and healthful product thanks to its probiotic content (Plessas et al., 2016). The term "kefir" has its roots in the Turkish word "keyif" signifying a delightful taste or a sense of well-being (Nielsen et al., 2014). Most of the previous studies dealt with cow's milk kefir, and there are not many studies on goat's milk kefir, and in this research, the study was on both.

This study aims to conduct a comprehensive nutritional analysis (nutrient profiling) of cow and goat milk kefir, followed by the evaluation of cytotoxicity of the crude proteins produced in the presence of biogenic silver nanoparticles (B-AgNPs).

a) Fermentation and Nutritional Profiling: Over an 8-day period, cow and goat milk undergo fermentation, resulting in the creation of kefir. Nutritional parameters, including pH values, titratable acidity, total free amino acids, and total dry matter were meticulously analysed, providing a holistic understanding of kefir's composition.

b) Protein Profiling with SDS-PAGE Electrophoresis: Protein composition analysis of cow and goat milk kefir was performed using SDS-PAGE electrophoresis, revealing distinct protein bands and their abundance.

c) Assessment of Cytotoxicity and Antimicrobial Activity: In addition to cytotoxicity evaluation, the antimicrobial activity of kefir proteins in the presence of biogenic silver nanoparticles was assessed. Biogenically synthesized silver nanoparticles play a pivotal role in enhancing both cytotoxicity and antimicrobial activity. This innovative intervention presents promising avenues for maximizing the therapeutic potential of kefir in biomedical applications.

1.1 Food Fermentation

For millennia, fermentation has stood as one of the most ancient food processing technologies employed by humans to craft foods and beverages possessing desirable properties (Ray and Joshi, 2014). The term 'fermentation' finds its roots in the Latin word 'fervere', signifying "to boil". This term describes the process in which yeast ferments malted grains or fruit extracts, producing carbon dioxide bubbles that create a boiling-like effect, a phenomenon particularly evident in the making of alcoholic beverages (Soto, 2019). The convergence of scientific advancements and the industrialization of fermented food production proved to be advantageous. It became clear that traditional methods, such as back slopping or natural fermentation, were insufficient for large-scale industrial operations. The advent of retailing and mass marketing required consistently high-quality and safe products. Particularly for fermented foods, especially those made from milk, the identification of fermentation

microorganisms in the late 19th century enabled the isolation and mass production of starter cultures. These cultures could then be supplied to factories for large-scale production, ensuring consistency and reliability in the fermentation process. This significant development greatly impacted manufacturing methods, guaranteeing uniformity and dependability in the final products (Tamang et al., 2016).

These advancements coincided with significant technological progress in milk processing and handling, making dairy fermentations some of the most sophisticated and thoroughly researched food fermentations to date. Research on lactic acid bacteria, crucial for producing many fermented foods, particularly those based on milk, has advanced rapidly throughout the 20th century. Our understanding of these bacteria, along with our ability to manipulate and control them, has achieved levels of sophistication that were unimaginable just two decades ago (Caplice and Fitzgerald, 1999). One of the key features of food fermentation is its ability to not only extend the shelf life of perishable foods but also to enhance their nutritional value and sensory characteristics. During fermentation, microorganisms break down complex carbohydrates, proteins, and fats into simpler compounds, unlocking additional nutrients and bioactive compounds in the process. Furthermore, fermentation imparts unique flavours, textures, and aromas to foods (Ghosh et al., 2022; Sharma et al., 2020)

1.2 Probiotic Definitions and Mechanisms

Intestinal probiotics are living microorganisms naturally found in the mammalian intestines that, when consumed in adequate amounts, exert beneficial effects on human health (Sánchez et al., 2017). They encompass substances produced by microorganisms that foster the growth of other microorganisms (Dhanker et al., 2023), as well as organisms and substances that aid in maintaining a balanced intestinal microbial environment (Leser and Mølbak, 2009). These probiotics are live microbial supplements that, when ingested, positively influence the host animal by enhancing its intestinal microbial balance (Ezema, 2013). They are viable mono- or mixed-cultures of microorganisms that confer benefits to animals or humans by improving the characteristics of the native microflora (Ayichew et al., 2017). The mechanism by which probiotics operate varies according to the particular strain, and it can be categorized into three main modes. To begin with, probiotics have the potential to

impact the host's immune system, specifically targeting gut-associated immune cells and gut epithelial cells. This interaction between probiotics and host immune cells leads to the modulation of the immune response (Lebeer et al., 2008). Secondly, probiotics can compete with pathogens, effectively thwarting their attachment to the intestinal lining. Thirdly, probiotics have the ability to modify specific host substances, such as bile salts, food constituents, and microbial toxins (Saarela et al., 2000). Various factors such as oxygen levels, pH, storage temperature, hydrogen peroxide, and others can impact the viability of probiotics (Karimi et al., 2011). Additionally, probiotics produce metabolites such as peptides, amino acids, and vitamins, which play a role in regulating the host immune system. This immune modulation can potentially mitigate the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections (Infusino et al., 2020).

1.3 Probiotics and Human Health

The history of probiotics dates back to the early 20th century, when Metchnikoff in 1907 first highlighted the benefits associated with consuming fermented dairy products (Martín and Langella, 2019). He proposed that aging could be attributed to the putrefaction processes in the large intestine. Around the same time, another scientist, Tissier, observed that the predominant component of gut flora in breast-fed infants was bifidobacteria (Rad et al., 2012). Probiotics are now available in various forms such as food, dietary supplements, and medications. Even earlier historical references, including biblical recommendations, have recognized yogurt as significant for treating certain ailments (Lourens-Hattingh and Viljoen, 2001).

Products containing strains of *Bifidobacterium* are not limited to yogurt, and they are also added to milk, some types of cottage cheese, and specialized products like rice milk. Kefir, a fermented milk product popular in Turkey, is among the most well-known sources of probiotics. Research suggests that probiotics may offer potential benefits in managing human disorders such as inflammatory bowel diseases like colitis, non-specific ileitis, and Crohn's disease. Studies have also explored probiotics' role in alleviating conditions such as irritable bowel syndrome and lactose intolerance (Silva et al., 2023), as well as in preventing peptic ulcers and colorectal cancer (Lanas, 2009).

1.4 Cow and Goat Milk

Fresh cow milk typically has an acidity ranging from 6.0 to 7.5 °SH (Soxhlet-Henkel degrees). Milk with acidity below 6.0 °SH may indicate mastitis in cows. Alternatively, milk acidity can also be expressed in terms of pH, typically falling between 6.4 and 6.8, with a lactic acid content of 0.14 to 0.16%. Cow's milk contains higher levels of total solids, and protein compared to goat's milk. However, cow's milk has lower fat content than goat's milk. These differences have significant implications: goat's milk, with its higher fat content and smaller fat globules, is easier to digest and more nutrient-dense. This makes it particularly effective for absorbing fat-soluble vitamins such as vitamin E, vitamin D, vitamin A, and vitamin K. Understanding these distinctions is crucial when making dietary choices based on nutritional requirements and health considerations (Ceballos et al., 2009).

1.5 What is Kefir?

Kefir is a fermented beverage produced through the action of lactic acid bacteria (LAB), acetic acid bacteria, and yeasts on milk. This combination of microorganisms creates a distinctively fermented milk product with unique characteristics. Historically, the origins of fermented foods likely arose serendipitously, with fermented milk becoming a popular preservation method before modern refrigeration or preservation techniques like canning and pasteurization were developed. According to oral tradition, fermenting milk in skin bags for preservation led to the discovery of the first kefir grains, marking the beginning of the longstanding tradition of kefir production.

Kefir is traditionally made from the milk of cows, sheep, goats, or buffalo and has been known by various names including kefir, kiaphur, kephir, kefyr, kéier, kepi, kippe, and knapon. In recent times, commercial production of kefir has complemented traditional methods in many countries, contributing to increased consumption and reinforcing its reputation as a health-promoting product. Recognized as a functional food and probiotic, kefir shows promising evidence for its potential to alleviate various health conditions. Early research on kefir originated primarily from the former Soviet Union. Despite its health benefits, kefir's distinct taste may pose challenges for consumer

acceptance. Further scientific exploration of this unique product's composition is necessary to enhance its appeal to consumers (Farnworth and Mainville, 2003).

1.5.1 Characteristics of kefir grains

Kefir grain, which is the main element of kefir. The kefir grains exhibit a cauliflower floret shape, characterized by their creamy colour, slimy texture, and varying sizes, elastic and irregular features. It has a structure similar to a burst and an elastic structure with a diameter varying from 1-2 mm to 3-6 mm. When kefir grains are added to milk, they swell and turn white (Yüksekdağ and Beyatlı, 2003).

These grains consist of a complex symbiotic microbial ecosystem, comprising LAB, acetic acid bacteria, and yeast. According to study by Tan et al. (2020), when kefiran is embedded in an exopolysaccharide matrix, this ecosystem is composed of approximately equal ratios of D-galactose and D-glucose (Figure 1.1). Kefiran, making up around 24 to 25% of the kefir grain's dry weight, serves to safeguard the microbiota within the grain from unfavourable environmental conditions (Izquierdo-González et al., 2019). Kefir grains can be reused indefinitely under proper conditions, making them a valuable and sustainable resource in kefir production. Their unique composition and fermentation capabilities contribute to the distinctive qualities and health benefits of kefir as a fermented beverage.

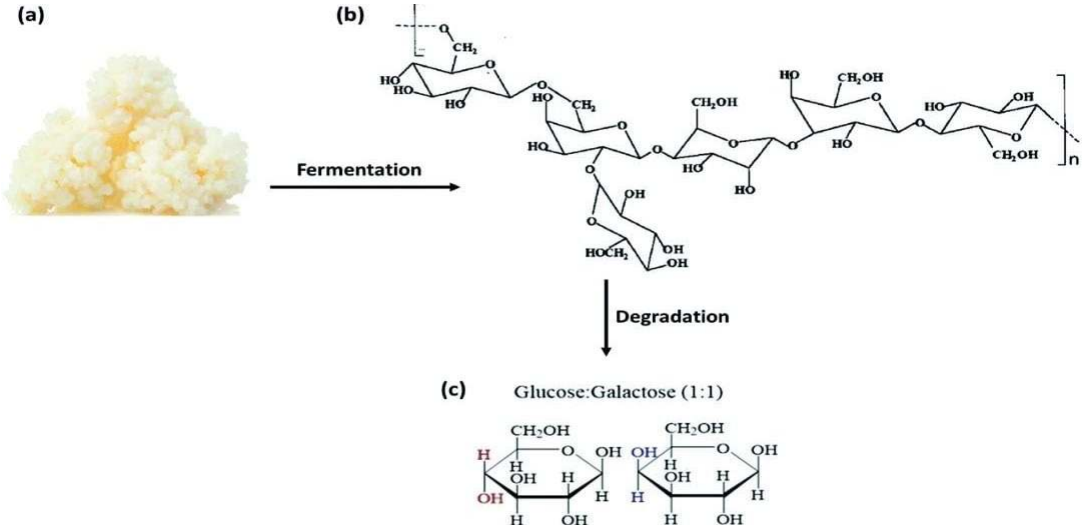

Figure 1.1 Structure of (**a**) laboratory-crafted milk kefir grains, (**b**) polysaccharides, and (**c**) monosaccharides (Tan et al., 2020).

1.5.2 Kefir production

Traditional and industrial production methods are used in kefir production. In making kefir at home using the traditional method, first the milk is boiled for about 5 min. It is cooled to room temperature (25 °C). After removing the cream layer upon cooling, 15-20 g of kefir grains is added to 1 L of milk, and left to ferment at 22-25 °C, approximately 18-24 h. Kefir grains are separated with the help of a strainer and kept in the refrigerator (Terzi, 2007). In the realm of industrial kefir production, three different methodologies are utilized. While certain enterprises still adhere to traditional techniques, employing kefir grains as a starter culture and leveraging their inherent microbial diversity, others utilize kefir grains but rely on the kefir produced as the primary culture. Nevertheless, the majority of businesses tend to favour commercial starter cultures as their primary choice for initiating fermentation processes (Yaman, 2011). The traditional production of kefir in businesses is slightly different from home production. Instead of boiling raw milk in businesses, boil it at 85-95 °C for 2-20 min. Pasteurized milk, up to 20-25 °C is cooled and 2-10% culture is inoculated into the milk (Terzi, 2007). As shown in the Figure 1.2, the kefir grains are separated from the kefir using a sterile strainer after incubation (Rosa et al., 2017). Kefir grains can be washed with sterile distilled water and used again. It is kept in the refrigerator. Separated from kefir grains after fermentation kefir is cooled and packaged (Altay et al., 2013; Schoevers, 1999). Utilizing kefir grains for kefir production is not conducive to large-scale operations due to the heterogeneous distribution of the grains when introduced into milk. While a portion of the grains sinks to the bottom, others remain buoyant on the surface of the milk (Richard, 2016). The diversity of the microflora present in kefir grains contributes to the variability observed in kefir produced from these grains. Furthermore, this method yields kefir with notable variations in taste, aroma, and flavour, alongside significant differences in texture (Garofalo et al., 2020) in the industrial production of kefir, the use of the kefir grain is difficult since they must be separated after fermentation (Farnworth and Mainville, 2003; Wang et al., 2012). More importantly, kefir is produced through the use of kefir grains during the preparation phase. Kefir grains are used many times and are microbial. The risk of contamination is quite high, as is the carbon dioxide gas production. The formation of yeasty taste and odour shortens the shelf life of kefir (Kim et al., 2018). Kefir, even when produced according to proper techniques, has a limited shelf life and is

susceptible to handling errors. It is noted that the shelf life of kefir may further decrease depending on certain factors (Dinç et al., 2008).

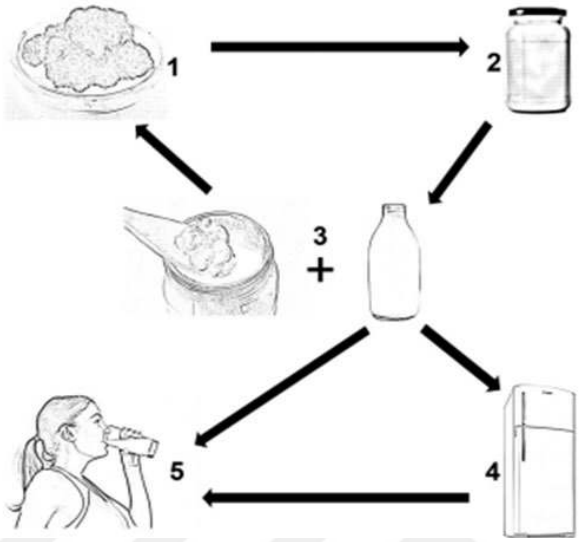

Figure 1.2 Local production of kefir: 1. Preparing kefir yeast .2. Add kefir grains to the milk in an open bowl for 24 h at 25 °C and then for another 24 h at 8 °C. 3. Grain separation and filtration.4. Cool the kefir at 8 °C. 5. Kefir is ready to drink (Rosa et al., 2017).

1.5.3 Health effects of kefir

The microbiota found in kefir has been linked to a diverse range of health advantages. These include properties such as antioxidative, immunomodulatory, anticarcinogenic, antihypertensive, hypocholesterolaemia, antidiabetic, anti-inflammatory, antiallergic, as well as antimicrobial and antiviral activities. These positive effects are ascribed to the array of bioactive compounds produced during fermentation, exopolysaccharides, bacteriocins, comprising organic acids and bioactive peptides (Ahmed et al., 2013). As shown in the Figure 1.3, the unique microbiota present in kefir plays a crucial role, potentially acting independently or in collaboration to contribute to these health-promoting properties (El-Rayes et al., 2015; Rosa et al., 2017).

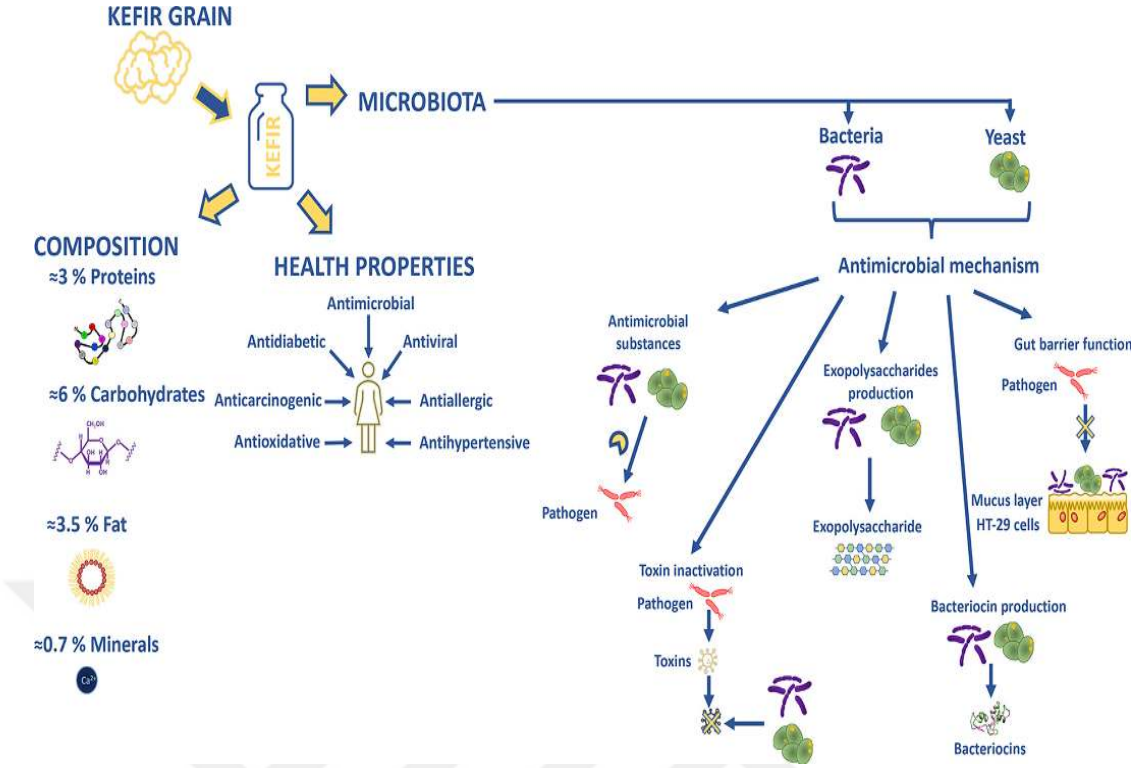

Figure 1.3 Graphical depiction of the kefir product composition and kefir grain, microbiota, health benefits, and their antimicrobial properties (González-Orozco et al., 2022).

1.5.4 The nutritional composition of kefir

The nutritional composition of kefir varies significantly, influenced by factors such as the specific characteristics of the grains used, fermentation conditions, storage methods, and the composition of the milk (Farag et al., 2020). Despite this variability, research offers insights into its chemical composition. Kefir is predominantly composed of moisture (about 90%), followed by protein (approximately 3%), sugars (around 6%), ash (about 0.7%), and fat (about 3.5%) (Coultate, 2016). During the fermentation process, proteins undergo changes, becoming more easily digestible due to proteolysis and acid coagulation. Kefir demonstrates a similar amino acid profile to the milk obtained from different animals used as the fermentation substrate. Compared to unfermented milk, kefir exhibits higher levels of essential amino acids such as serine, threonine, tryptophan, alanine, isoleucine, lysine, phenylalanine, methionine, and valine. These amino acids are listed in descending order in kefir: 376mg lysine, 262mg isoleucine, 231mg phenylalanine, 220mg valine, 183mg threonine, 137mg methionine, and 70mg tryptophan per 100g of kefir. These findings highlight the

nutritional richness of kefir, with enhanced levels of essential amino acids compared to unfermented milk (Rosa et al., 2017).

1.5.5 Protein in kefir

Protein concentration in kefir can be determined using various methods that involve the binding of different dyes to the protein. This can be achieved either by staining the protein bands after their electrophoretic separation on sodium dodecyl sulfate (SDS) gels, or by staining the protein in suspension and then measuring the absorption spectrophotometrically (Bradford, 1976).

1.6 Nanoparticles

Nanoparticles are extremely small particles, generally ranging from 1 to 100 nanometres in size. They exhibit unique properties owing to their diminutive size and substantial surface area, making them valuable for diverse applications in medicine, agriculture, food, cosmetic, electronics, and environmental science (Araujo et al., 2017; Buzea et al., 2007; Luque and Clark, 2010; Savage and Diallo, 2005). Nanoparticles can be divided into different categories according to their size, morphology, and chemical and physical properties. These include metal nanoparticles, carbon-based nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, lipid-based nanoparticles, and polymeric nanoparticles (Joudeh and Linke, 2022).

Metal nanoparticles are nanoscale colloids of metals, synthesized through chemical or biological reduction, microemulsion, or thermal decomposition of metal salts, representing zero-dimensional nanomaterials (Yasin et al., 2013). The size of these nanoparticles is influenced by several key factors, such as the physical or chemical conditions during synthesis, the type of reducing agent employed, the choice of stabilizing agent to prevent aggregation, and the reaction temperature (Pasinszki and Krebsz, 2020). Nanoparticle synthesis methods are broadly categorized into two main groups: (1) top-down synthesis and (2) bottom-up synthesis, as illustrated in Figure 1.4.

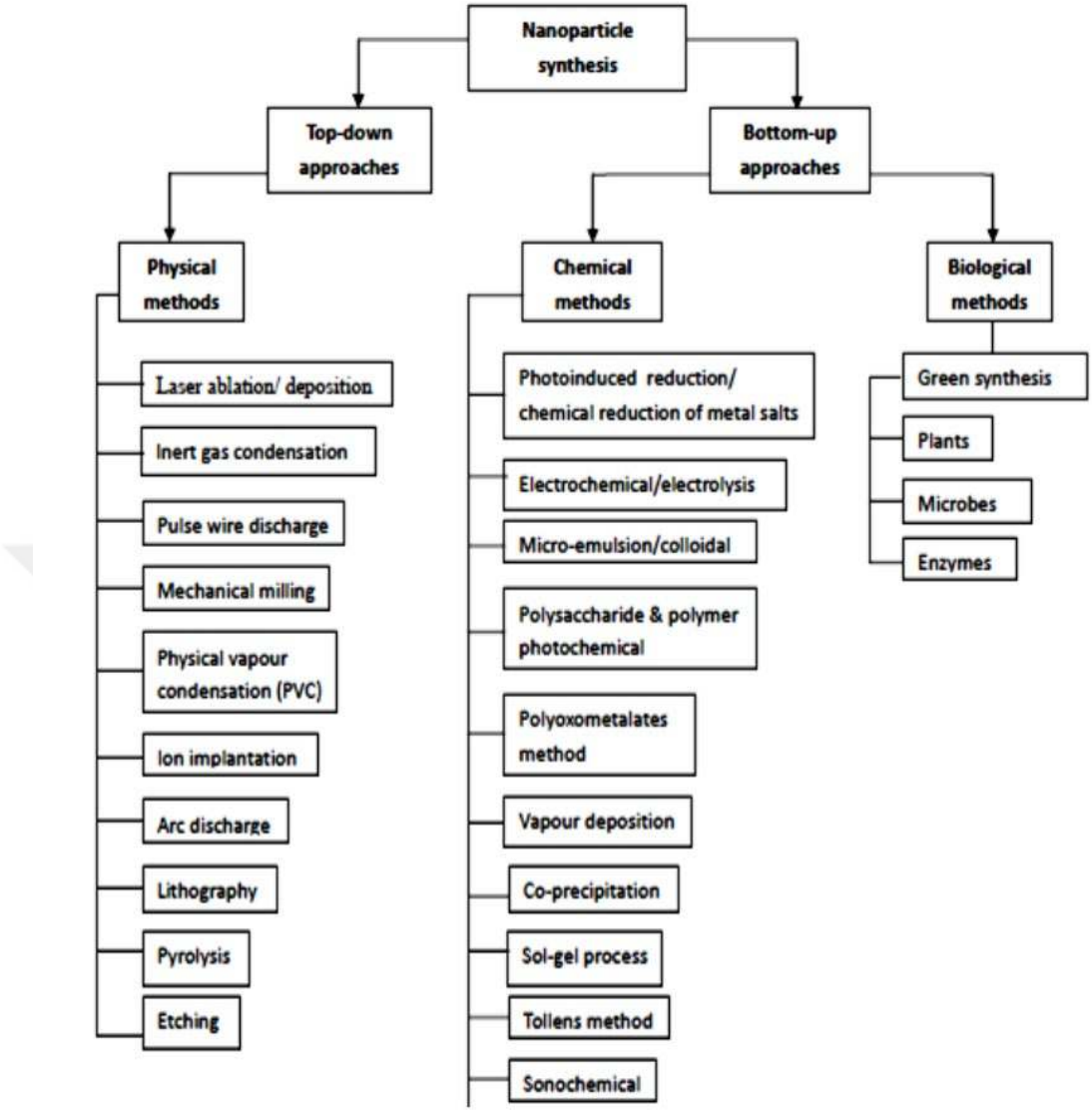

Figure 1.4 Different methods for synthesis of metal nanoparticles (Rajoriya, 2017).

Metal nanoparticles are produced by converting metal salts into zero-valent metal atoms, a process known as reduction. The size of these nanoparticles is affected by several critical factors, including the chemical environment during synthesis, the nature of the reducing agent, the type of stabilizing agent used to prevent aggregation, and the reaction temperature (Zhang et al., 2016). However, conventional physical and chemical approaches are gradually being supplanted by biogenic synthesis techniques due to concerns regarding the release of harmful chemicals, over high energy consumption, the challenging synthesis conditions and the intricacy of equipment requirements (Ying et al., 2022). Moreover, green synthesis presents extensive

opportunities for fabricating nanoparticles of various metals and metal oxides, including silver (Ag), gold (Au), iron (Fe), copper (Cu), copper oxide (CuO), titanium dioxide (TiO_2), aluminium (Al), cadmium (Cd), cobalt (Co), lead (Pb), zinc (Zn), among others (Khandel et al., 2018; Salavati-Niasari, et al., 2008). Recently, there has been considerable attention devoted to the biologically mediated fabrication of silver nanoparticles (AgNPs) with controlled sizes, utilizing diverse living entities encompassing plant extracts and other lower groups like bacteria and fungi as well as macromolecules derived from biological sources such as amino acids, proteins, secondary metabolites, vitamins and similar substances (Zhang et al., 2016).

Thanks to their unique properties, AgNPs find applications across various fields (Figure 1.5). They are utilized as antibacterial agents, integrated into healthcare products, household, and industrial, incorporated into medical device coatings, consumer goods, and optoelectronic sensors (Kumar et al., 2020). AgNPs also play crucial roles in orthopaedics, diagnostics, pharmaceuticals, cosmetics, the food industry, and drug delivery, where they enhance the efficacy of anticancer drugs by augmenting tumour-killing effects (Gurunathan et al., 2009; Rai et al., 2014).

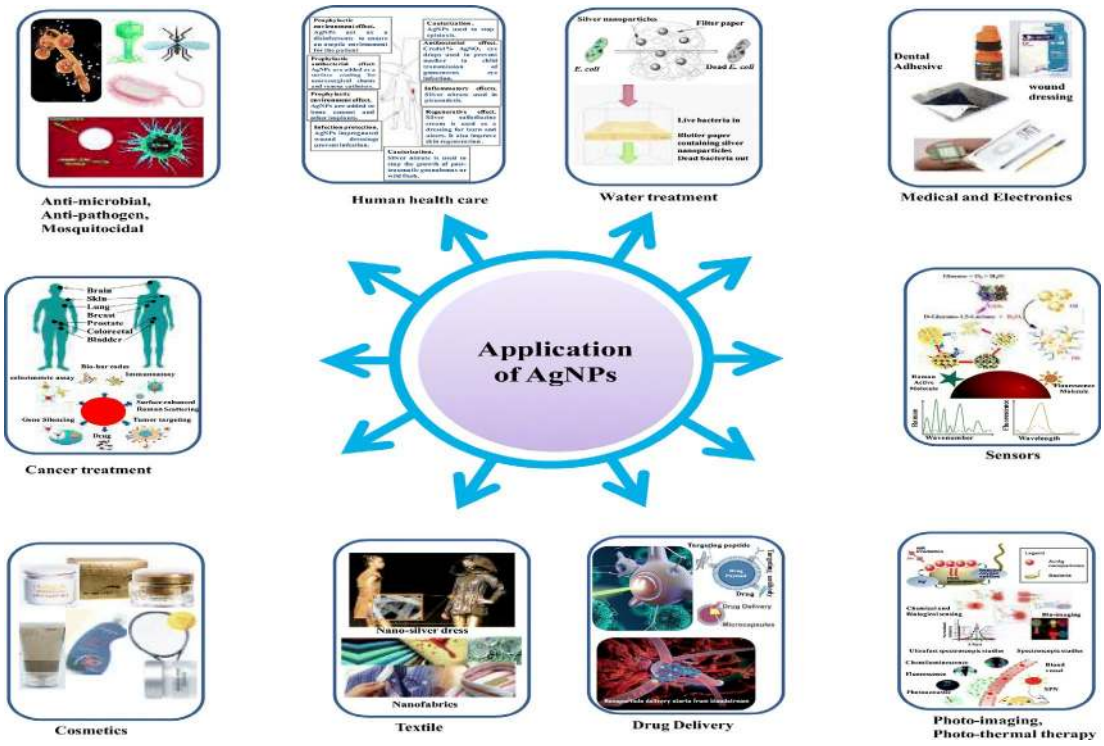

Figure 1.5 Application of AgNPs (Kumar et al., 2020).

Silver nanoparticles demonstrate substantial antibacterial properties through mechanisms such as membrane disruption, attachment to and penetration of bacterial cell walls, disruption of cellular functions, and generation of reactive oxygen species (ROS) (Jahan et al., 2024). These reactive species have the ability to damage bacterial cellular components, resulting in cell death. Furthermore, silver and other metallic nanoparticles can release ions that disrupt bacterial cellular processes (Zhang et al., 2016).

Metallic nanoparticles such as AgNPs show promise in cancer treatment by facilitating targeted delivery of anticancer drugs directly to cancer cells, thereby reducing side effects. They also convert light into heat, which can selectively destroy cancer cells upon irradiation. Furthermore, metal nanoparticles have the capability to deliver genetic material for modifying the expression of genes associated with cancer (Cho et al., 2008; Peer et al., 2020).

2. MATERIALS AND METHOD

2.1 Chemicals and Regents

The selection and utilization of reagents and chemicals in the experimental phase of a research study significantly impact the outcome of the experiment. These materials encompass a wide array of substances, including organic and inorganic compounds, solvents, substrates, and various other materials. All chemicals were used as commercially obtained.

2.2 Step by Step of Kefir Production

The art and science of kefir production lies in the meticulous crafting of kefir, beginning with the careful selection of ingredients and continuing through the fermentation and storage stages. While traditional kefir is typically made from cow's milk, it can also be crafted using cow's milk, sheep's milk, goat's milk, or even plant-based alternatives (Yaman, 2011). In the present study, both cow milk and goat milk were commercially obtained from local diary markets in Mardin and used as they were. At the heart of kefir production are the kefir grains, small clusters resembling cauliflower, which play a vital role in the fermentation process. These grains contain a symbiotic mixture of LAB and yeast as well as acetic acid bacteria, essential for creating the characteristic flavours and textures of kefir (Magalhães et al., 2011; Nielsen et al, 2014).

To initiate the kefir production process, a few basic pieces of equipment are necessary:

- Glass jars or containers for fermenting.
- A non-metallic stirring utensil, such as wood, should be used.
- A fine mesh strainer for separating kefir grains from the finished product.

The kefir was subjected to three consecutive production cycles to validate the findings and was analysed over a continuous period of eight days. (González-Orozco et al., 2022; Kim et al., 2018).

2.2.1 Obtaining kefir grains and activation them

The kefir grains utilized in this study were sourced from a well-known company that markets products in Turkey *via* Trendyol (electronic market). The yeast was thoroughly washed with distilled water using a strainer, placed in a glass jar, and combined with 250 mL of milk per 15 g of yeast. The jar was covered and stored in a dark place at 28 °C for 2 h to activate the yeast. This method was repeated three times to ensure the yeast was activated.

2.2.2 Obtaining and processing cow and goat milk

Goat milk and cow milk were procured from local livestock farms in Mardin, Turkey. The milk had an approximate pH of 6.5. Standard milk samples were heated at 90 °C for 25 min and then allowed to cool to 28 °C, the temperature suitable for grain incubation (Hamouda and Salunke, 2024).

2.2.3 Fermentation

After the yeast was activated and the commercially obtained cow and goat milks reached a temperature of 28 °C, 2 g of kefir grains were added to 500 mL of milk placed in glass jars. Four samples were prepared, two from cow milk and two from goat milk. The jars were loosely closed and placed away from light at a temperature of 28 °C for 24 h, then moved to a temperature of 8 °C for another 24 h, resulting in a total fermentation period of 48 h (Benzer Gürel et al., 2021). The kefir samples were prepared in triplicate using cow and goat milk.

2.2.4 Straining and separating of kefir grains

Once the fermentation process was completed, the kefir was strained using a fine mesh strainer to separate the grains from the liquid. The liquid obtained was the end product, kefir, ready for consumption.

2.2.5 Refrigeration

The prepared kefir samples were stored in the refrigerator to slow down the fermentation process. This ensured that the drink remained fresh and safe for

consumption. Kefir can be kept for several weeks, but the flavour may continue to develop over time.

2.3 Chemical Analysis of Prepared Kefirs

The protein content of goat and cow milk kefir samples was determined by the Micro-Bradford's method, while the dry matter content was determined using the gravimetrically. The titratable acidity was determined as a percentage of lactic acid by titration method. The pH value was determined by using a Mettler Toledo pH meter (Cemeroğlu, 2007).

2.3.1 Measurement of pH values

The pH of kefir samples made from goat milk and cow milk was measured with a calibrated digital pH meter. Changes in pH were recorded over eight consecutive days at a temperature of 25 °C. The pH meter was calibrated using standard buffer solutions with pH values of 4.01 and 7.00. Monitoring the pH of kefir over this period may reveal various changes influenced by the dynamic microbial and chemical processes during fermentation (Garrote et al., 2001).

2.3.2 Titratable acidity determination

Following the preparation of solutions, 10 mL of cow and goat milk kefir was dispensed into three separate samples for each type. Two drops of phenolphthalein indicator were added to each sample, followed by titration with sodium hydroxide until the kefir's samples colour changed to pink. The volume of hydroxide consumed for each sample was meticulously recorded throughout the titration process (Gul et al., 2015).

To determine the titratable acidity of kefir samples:

- Representative samples of goat milk kefir and cow milk kefir were taken, ensuring thorough mixing for homogeneity.
- An accurate volume of the kefir sample (e.g., 10 mL) was measured using a pipette.

- A few drops of phenolphthalein indicator were added to the kefir sample. Phenolphthalein changes colour in the presence of acidic solutions.
- The kefir sample was titrated with sodium hydroxide solution. NaOH was added drop by drop while stirring until the colour changed from the initial acidic hue to a faint pink, marking the endpoint of the titration.
- This process was repeated for eight consecutive days, using fresh kefir samples each day.
- Calculated Titratable acidity content following aquation:

$$\text{Titratable acidity (\%A)} = \frac{(\text{N} \times \text{V} \times 0.009)}{\text{m}} \times 100$$

A: Titratable acidity value (percent by mass) in terms of lactic acid; V: Amount of NaOH used in titration (volume) (mL); N: Normality of NaOH solution; m: Sample weight (g).

2.3.3 Total dry matter analysis

Representative samples of cow and goat milk kefir, approximately 2 g each, were taken daily for eight consecutive days. The samples were ensured to be well-mixed:

- An empty, clean, and dry weighing glass dish was weighed on the weighing scale. The weight was recorded as "W_1".
- A known volume of the kefir sample was transferred into the weighing dish. The weight of the dish with the kefir sample was recorded as "W_2".
- The weighing dishes with the kefir samples were placed in a drying oven (BINDER, ED 115 incubator) at a low temperature (e.g., 105 °C) to evaporate the water content.
- The samples were dried until a constant weight was achieved, which typically took 2 to 3 h.
- The dried kefir samples in the weighing dishes were weighed. The weight was recorded as "W_3".
- This process was repeated for eight consecutive days, using fresh kefir samples each day.

- The total dry matter content was calculated using the following equation:

$$\text{Total Dry Matter Content (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

- The initial weight ($W_2 - W_1$) represented the water content of the kefir sample.

Variability in drying time can occur based on factors such as sample volume, oven temperature, and the water content of the kefir. Monitor changes in total dry matter content over eight days. The results were given as % (w/w) (Benzer Gürel et al., 2021).

2.3.4 Total free amino acid content analysis

The total free amino acid content of the samples was determined following a method described in the literature with minor adjustments (Folkertsma and Fox, 1992). Thermo Fisher Scientific, Heraus Fresco 21 was used for lyophilization, and the absorbance was measured at 507 nm using a Perkin–Elmer Lambda 25 UV–vis spectrophotometer.

To determine the free amino acid content of kefir samples:

- A combined of 30 mL of hydrochloric acid (HCl) and 30mL of kefir were homogeneously mixed.
- The final blend was stirred using a magnetic stirrer for 15 min, followed by incubation at 40 °C.
- The mixture was centrifuged at 460×g for 30min using a cooling centrifuge.
- The aqueous phase was filtered, and 0.30 mL of the resulting supernatant was diluted to a specified volume using ultrapure water.
- To the diluted solution, 4 mL of cadmium-ninhydrin reagent (prepared by mixing 80mL of ethanol, 10mL of glacial acetic acid, 1 mL of 1 g/mL $CdCl_2$ solution, and 0.8 g ninhydrin) was added.
- The mixture was incubated at 84 °C for 5 min, followed by cooling.
- The cooled mixture was centrifuged, and the absorbance was recorded at 507 nm using a spectrophotometer.
- Standard solutions of known amino acid concentrations were prepared for calibration using L-serine.

- The absorbance of kefir samples was compared with the standard curve to quantify the amino acid content.
- The analysis was repeated for each day and each type of kefir.
- Trends or variations in amino acid content over the eight-day period were analysed (Winters et al., 2002).

2.4 Water-Soluble Protein Extraction of Kefir

After taking 25 mL of cow and goat milk kefirs separately for each day for eight consecutive days, the samples were centrifuged for 20min at 14,000 × g. The samples were filtered to separate the kefir serum from the sediments. The serum was taken and frozen with liquid nitrogen, then placed in a freeze dryer (Labconco Co., Kansas City, United States) to ensure that all samples became completely dried powders. The obtained cow milk kefir water-soluble protein extract (CK-WSPE) and goat milk kefir water-soluble protein extract (GK-WSPE) were stored at +4 °C until use (Figure 2.1).

A **B**

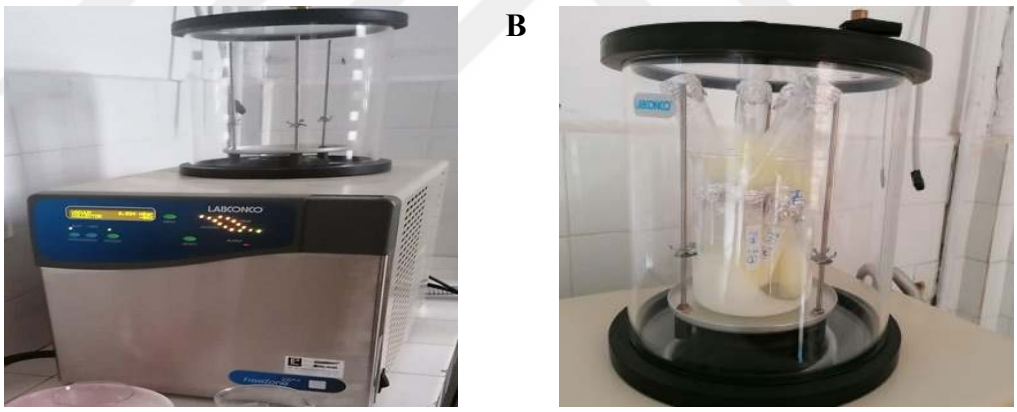

Figure 2.1 Image of (**A**) freeze dryer and (**B**) freeze-drying processes of CK-WSPE and GK-WSPE samples.

2.4.1 Protein content of water-soluble protein extract from kefirs

Total water-soluble protein amounts of cow and goat milk kefirs were determined by Micro-Bradford's method using BSA (bovine serum albumin) as the standard for calibration curve (Bradford, 1976).

2.4.2 Electrophoretic analysis of water-soluble protein extract of kefirs

Protein profiling and molecular weight analysis of the lyophilized CK-WSPE and GK-WSPE were conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to modified Laemmli method (Gaspar et al., 2017; Laemmli, 1970; Yavuz et al., 2014). The lyophilized samples were dissolved to a final protein concentration of 2 mg/mL in a buffer containing 0.0625M Tris (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 0.0002% (w/v) bromophenol blue and 5% (v/v) β-mercaptoethanol. The samples were then heated at 100 °C for 10 min and centrifuged at 5000 × g for 5 min at 18 ± 2 °C. A 10 μL aliquot of the supernatant and protein marker were applied to the gradient gel for SDS-PAGE (Gallagher, 2012).

To determine protein profiling of the WSPE of kefirs:

- Prepared 0.5M Tris pH 8.8 (9.0825g Trizma Base + 40 mL H_2O + 4 mL 4 M HCl, the total value = 50 mL).
- Prepared 1.5M Tris pH 6.8 (3.0275g Trizma Base + 40 mL H_2O + 4 mL 4 M HCl, the total value = 50 mL).
- Prepared 1 L reservoir buffer (3 g Trizma base + 15 g Glycine +1 g SDS)
- Prepared Laemmle sample buffer (16 mL 2× Laemmle Sample Buffer; 1.0 mL 20% SDS + 1.2 mL 0.5M Tris pH 6.8 + 0.5 mL β-mercaptoethanol + 1.9 mL glycerol + 0.8 mL 1% bromophenol blue + 10.4 mL H_2O).
- Prepared 10% APS (ammonium persulfate) (1 g APS + 10 mL H_2O).
- Prepared mixture of acrylamide/bis-acrylamide at 30%/0.8% w/v (14.5 g Acrylamide + 0.5 g *N,N′*-methylenebis(acrylamide) + 50 mL H_2O).
- Prepared 15% separating gel (4.4 mL H_2O + 5.2 mL 1.5 M Tris pH 8.8 +10.0 mL 30%/0.8% w/v acrylamide/bis-acrylamide + 200 μL 10% APS + 200 μL 10% SDS + 20 μL TEMED).
- Prepared 5% Stacking Gel (5.5 mL H_2O + 1.0 mL 0.5M Tris pH 6.8 +80 mL 30%/0.8% w/v acrylamide/bis-acrylamide + 80 mL 10% SDS + 80 μL 10% APS + 8 μL TEMED).
- Prepared staining solution: 125 mL MeOH + 25 mL AcOH + 100 mL H_2O + 0.25% Coomassie Brilliant Blue (0.625g)).

- Distaining Solution: (75 mL MeOH + 50 mL AcOH + 375 mL H_2O).

15 µL protein samples and 8 µL molecular weight markers were loaded into separate wells. The running buffer solution was added to the tank. The gel apparatus was connected to the power supply, and the gel was run at a constant voltage of 60 V for 15 min, followed by 80 V until the tracking dye reached the bottom of the gel, which took approximately 3 h (Figure 2.2).

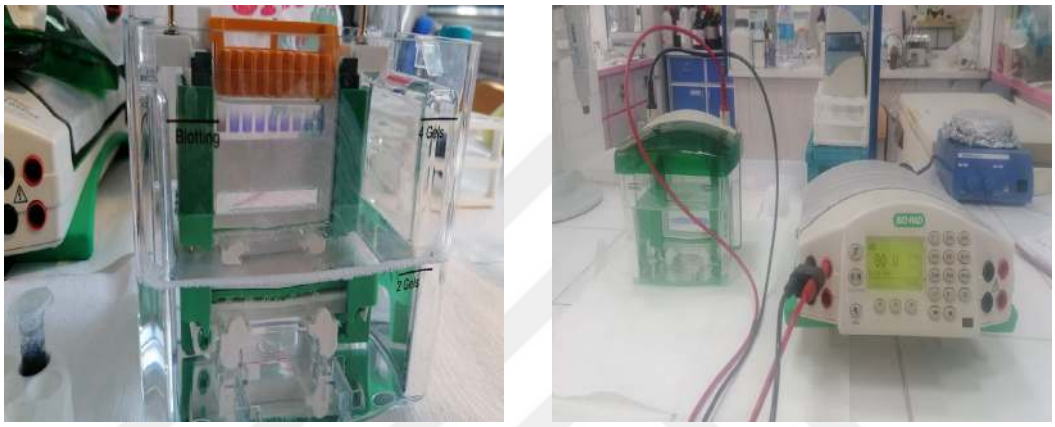

Figure 2.2 Image of electrophoretic analysis of water-soluble protein extract of kefirs (SDS-PAGE).

2.5 Morphological Characterization of B-AgNPs and WSPE with SEM and TEM

Scanning electron microscopy (SEM) analysis was carried out on kefir grains, the crude water-soluble protein extracts from cow and goat milk kefirs, and their B-AgNPs additive formulations to investigate the structural forms of the prepared samples. The morphology and microstructure of these samples were examined using a Quanta 250 field-emission gun scanning electron microscope (FEI Quanta 250 FEG-SEM). SEM images were captured at different magnifications to provide detailed insights into the structural characteristics of the samples. Particle size of B-AgNPs and morphological characterizations of kefir WSPE and B-AgNPs combined with CK-WSPE and GK-WSPE in the 1:2 mixtures were carried out using a transmission electron microscope (JEOL JEM 1010, TEM) connected to a camera (GATAN 782 ES500 W Erlangshen).

2.6 Biological Evaluation of Water-Soluble Protein Extract of Kefirs

The microbiological activities and cytotoxicity of the crude water-soluble protein extract obtained from cow and goat milk kefirs, as well as their biogenic silver nanoparticles (B-AgNPs) additive preparations, were studied in this thesis.

2.6.1 Enhancement antimicrobial activity

To assesses the *in vitro* antimicrobial properties of the goat and cow milk kefir extracts, lyophilized CK-WSPE and GK-WSPE and their B-AgNPs formulations, standardized test microorganisms are required. In this study, *Enterobacter cloacae* ATCC 23355, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, and *Staphylococcus aureus* ATCC 25923 were used as control strains from the American Type Culture Collection (ATCC). Additionally, clinical isolates of *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*, obtained from the Biochemistry Laboratory at the Faculty of Science of Dicle University, were also tested. Using standardized microorganisms ensures the reliability and reproducibility of the antimicrobial activity tests (Appelbaum et al., 1998). By including both standard strains and clinical isolates, the study offers comprehensive insights into the potential antimicrobial applications of the water-soluble protein extract of Kefirs and their biogenic silver nanoparticles (B-AgNPs) additive properties a diverse range of microorganisms.

2.6.2 Enhancement cytotoxicity with biogenic silver nanoparticles

A cell proliferation assay kit (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT reagent) was used to measure changes in cell viability of cells treated with the samples (Telli et al., 2023). For the MTT assay, human foreskin fibroblast (BJ, CRL-2522) healthy cell was seeded into 96-well plates and incubated until they reached confluence with normal morphology (Figure 2.3). CK-WSPE and GK-WSPE samples at concentrations ranging from 15.625 µg/mL to 500 µg/mL (were prepared, while B-AgNPs samples at concentrations of 7.8125, 15.625, 31.25, 62.5, 125, and 250 µg/mL were prepared, and were added to the wells, and the cell culture plates were incubated in a CO_2 incubator at 37 °C for 24 h. After incubation with lyophilized CK-WSPE and GK-WSPE and their B-AgNPs formulations at various concentrations, the

cells were washed to remove the culture medium. The MTT assay was performed on both cell lines according to standard procedures. The dose-dependent cytotoxicity of five different samples, i.e., CK-WSPE, GK-WSPE, B-AgNPs, B-AgNPs/CK-WSPE (1:2) and B-AgNPs/GK-WSPE (1:2) was presented as a graph of cell viability relative to the control (untreated) cells.

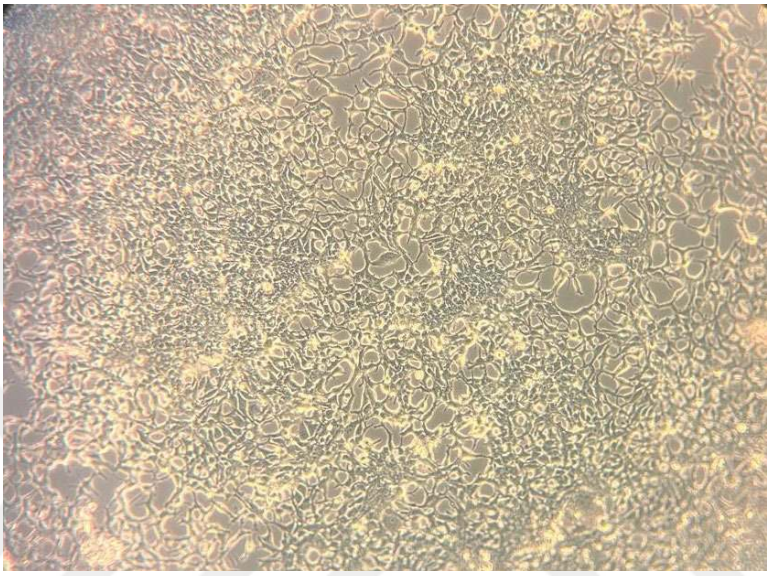

Figure 2.3 Microscopic image of normal foreskin fibroblast (BJ, CRL-2522) cells.

3. RESULTS AND DISCUSSIONS

Fermented beverages and foods play a crucial role in the human diet, providing essential nutrients and contributing to disease prevention. Acetic acid bacteria, lactic acid bacteria (LAB) and yeasts are the primary microorganisms involved in fermentation, with certain strains, known as probiotics, offering specific health benefits. This study focuses on kefir production through the fermentation of cow and goat milk, providing an in-depth analysis of their nutritional profiles. Figures 3.1 and 3.2 were shown laboratory-prepared cow and goat milk kefirs, as well as kefir grains.

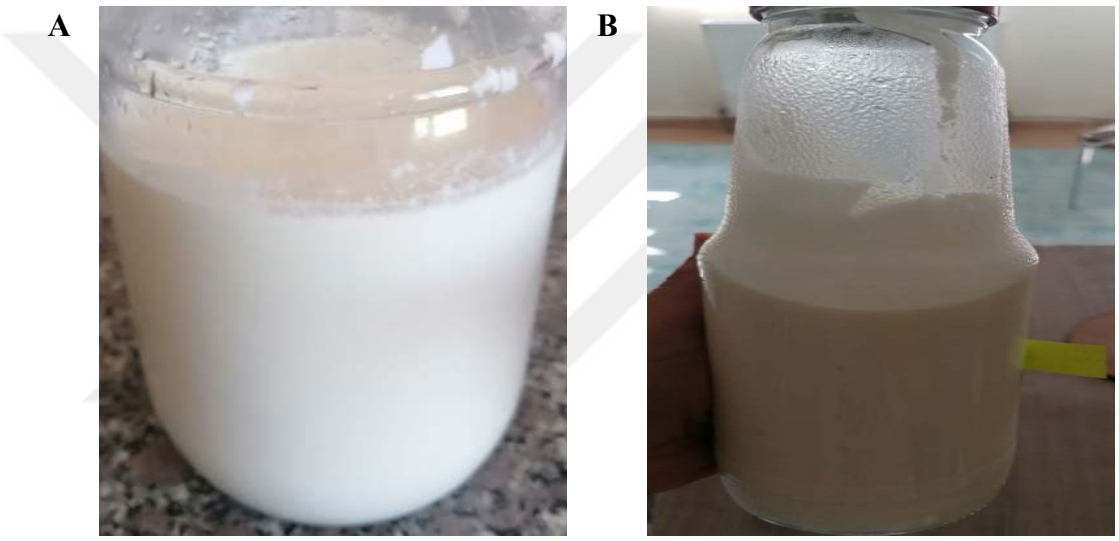

Figure 3.1 Laboratory-crafted (**A**) cow milk and (**B**) goat milk kefirs of exceptional quality.

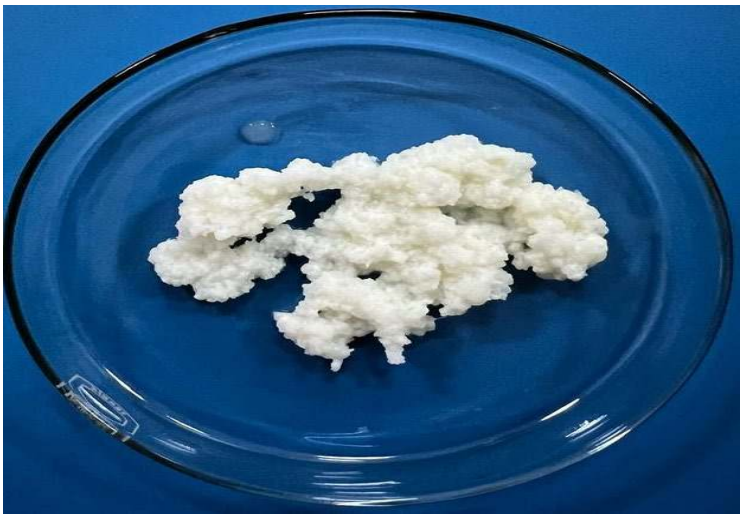

Figure 3.2 Magnified view of laboratory-crafted kefir grains.

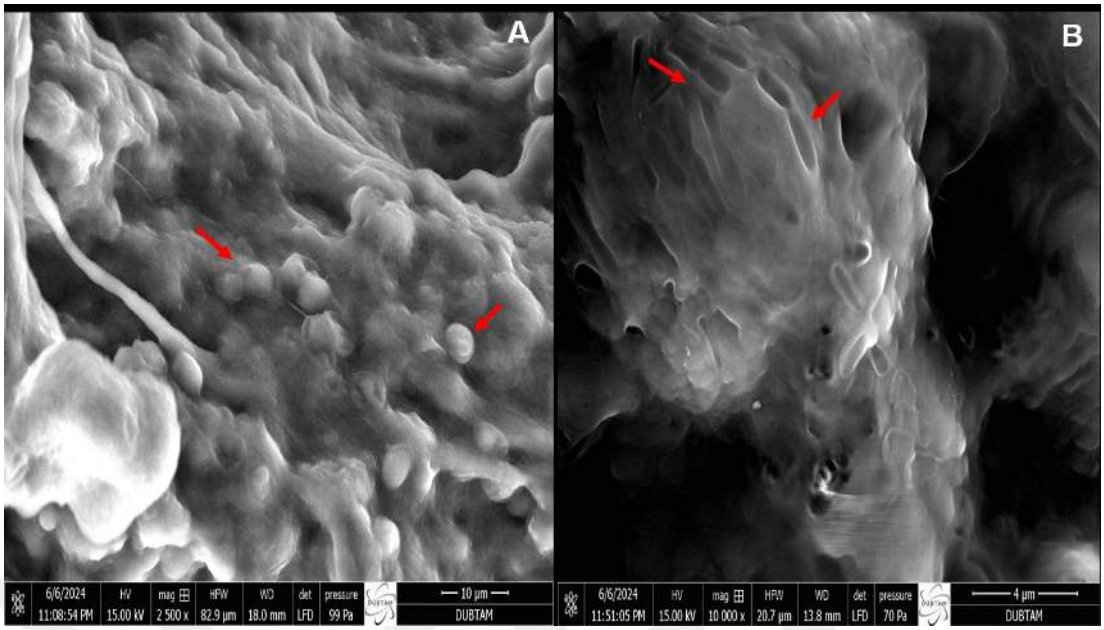

Figure 3.3 SEM image of kefir grains at 10 µm and 4 µm. Arrows indicate the presence of fungal spores (**A**) and rode shaped bacteria (**B**).

The SEM images in Figure 3.3 reveal fungal spores (A) and rod-shaped bacteria (B) within kefir grains, essential for fermentation. These microorganisms play crucial roles in converting sugars, enhancing flavour, and contributing to kefir's nutritional value. The images underscore the microbial diversity crucial for kefir production and its significance in food science research (Ottogalli et al., 1973).

We aim to provide valuable insights into the distinct characteristics of goat and cow milk kefir by examining their pH values, titratable acidity, total free amino acid content, and total dry matter over 8 days. Additionally, this research focus on protein profiling using the SDS-PAGE electrophoresis technique to reveal the intricate details of the protein composition in lyophilized CK-WSPE and GK-WSPE samples.

Beyond analytical pursuits, our emphasis on protein profiling serves as a foundational platform for investigating the cytotoxicity of treated proteins. To enhance the biocompatibility and cytotoxicity of these proteins, we introduce an innovative approach using biogenically synthesized silver nanoparticles (B-AgNPs). Known for their unique properties and applications, these nanoparticles refine the kefir's water-soluble protein structure. This pioneering methodology not only broadens the scope of

kefir research but also aligns with the growing interest in the biomedical applications of nanotechnology.

Through this multifaceted study, we aim to expand our understanding of the nutritional dynamics of goat and cow milk kefir and contribute to the evolving landscape of biologically synthesized nanomaterials in enhancing the health attributes of fermented dairy products. The intersection of traditional fermentation practices and cutting-edge nanotechnology promises valuable insights for both the dairy industry and the broader scientific community.

3.1 Determination of pH Values for Cow and Goat Milk Kefirs

Lactic acid is the primary acid produced during the fermentation of lactose by LAB, with acetic acid also being produced by certain strains. The accumulation of these acids leads to a decrease in pH. The LAB in kefir grains metabolize lactose in milk, producing lactic acid during the early stages of fermentation. This initial increase in acidity causes a significant drop in pH as lactic acid accumulates.

Temperature has a notable impact on the activity of microorganisms involved in fermentation. Warmer temperatures can expedite fermentation, resulting in a swifter pH decline, whereas cooler temperatures may slow down the process. Therefore, maintaining a temperature of 8 °C has been identified as the optimal choice.

When fermented at 8 °C, there was a decrease in pH values from 4.16 to 4.36 for goat milk kefir and from 4.54 to 4.28 for cow milk kefir, as depicted in Figure 3.4 and Table 3.1. This indicates that maintaining a controlled temperature effectively facilitates the fermentation process while preserving the quality and stability of the kefir. Additionally, our findings in this study show that there is a decrease in pH during the storage period and it is in line with previous studies conducted in fermented milk products as the pH level of lactose during storage is affected by bacteria, as mentioned by Irigoyen et al (2005).

Table 3.1 Changes in the pH values of goat and cow milk kefirs during unfermented (day 0) and fermented days from 1 to 8.

Fermentation Period (Days)	Cow milk kefir		Goat milk kefir	
	pH	± SD	pH	±SD
0	6.453	0.004	6.322	0.021
1	4.541	0.218	4.357	0.002
2	4.486	0.163	4.327	0.023
3	4.436	0.140	4.267	0.020
4	4.385	0.116	4.230	0.024
5	4.363	0.112	4.215	0.022
6	4.323	0.088	4.202	0.038
7	4.309	0.091	4.194	0.029
8	4.282	0.038	4.159	0.071

Values are the mean ± standard deviation (SD) of the data (n = 3)

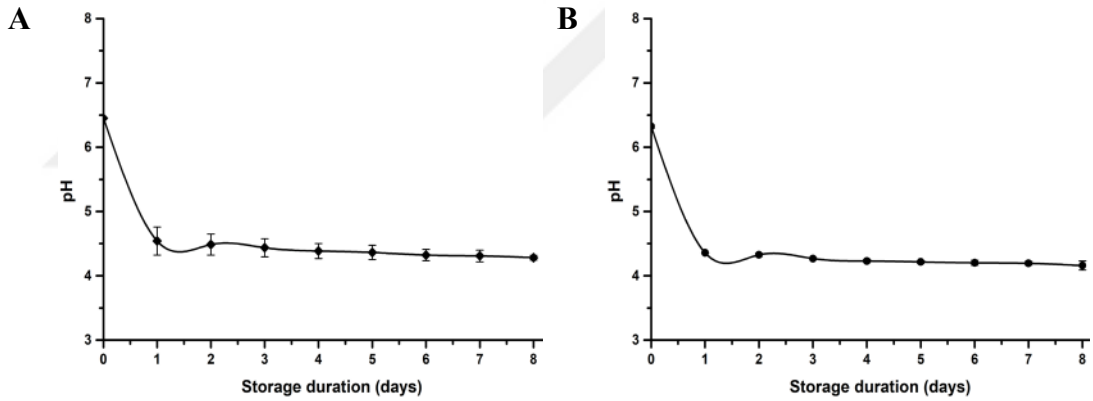

Figure 3.4 pH values of laboratory-crafted (**A**) cow milk and (**B**) goat milk kefirs during the storage at +8 °C for 8 days.

3.2 Determination of Titratable Acidity for Cow and Goat Milk Kefirs

The reduction in lactose content led to rise in acidity and decrease in pH was observed during milk fermentation indicate the metabolic activity of kefir grain microorganisms. These microorganisms produce CO_2, organic acids (such as citric acid, lactic acid and pyruvic acid), alcohol (primarily ethanol), as well as aromatic and volatile compounds. Our findings align with those of Azizkhani et al. (2018), who noted similar changes in lactose content, pH, and acidity during the cold storage of kefir samples made from sheep, goat, cow, camel, and ewe milk over 20 days. Additionally, de Lima et al.

(2020) reported a decrease in pH (by 0.91) and an increase in acidity in sheep milk fermented by kefir grains, consistent with our data. The production of organic acids, particularly lactic acid, and other metabolites by LAB serves as an inhibitory agent against foodborne pathogenic microorganisms and spoilage.

As it shown in Table 3.2 and Figure 3.5, the titratable acidity decreased from 0.92 to 0.69 and from 0.95 to 0.81 for cow milk kefir and goat milk kefir, respectively.

Table 3.2 Titratable acidity value (lactic acid equivalent) of goat and cow milk kefirs during unfermented (day 0) and fermented days from 1 to 8.

Fermentation Period (Days)	Cow milk kefir		Goat milk kefir	
	Titratable acidity (g/100 g, lactic acid)	± SD	Titratable acidity (g/100 g lactic acid)	±SD
0	nd	nd	nd	nd
1	0.92	0.023	0.95	0.045
2	0.90	0.000	0.89	0.009
3	0.86	0.000	0.86	0.000
4	0.81	0.031	0.86	0.045
5	0.74	0.023	0.81	0.045
6	0.69	0.014	0.81	0.000
7	0.74	0.023	0.86	0.000
8	0.79	0.023	0.87	0.014

nd: Not determined. Values are the mean ± standard deviation (SD) of the data (n = 3)

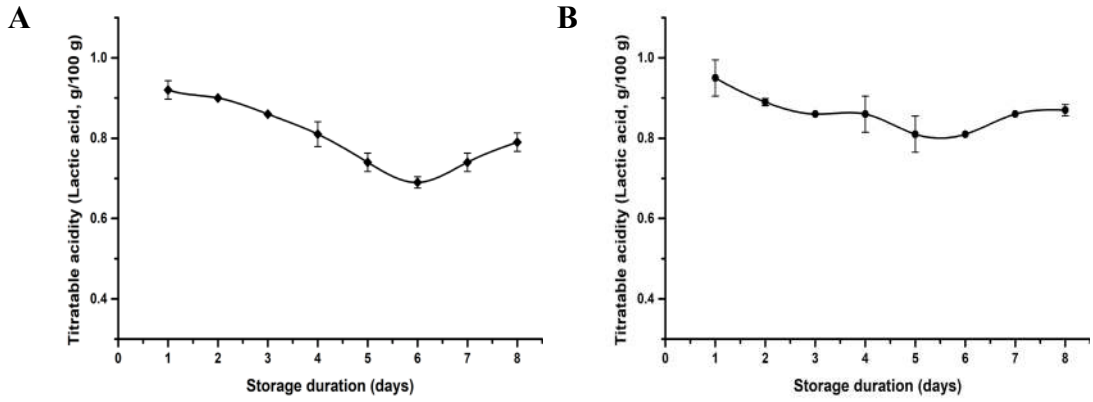

Figure 3.5 Titratable acidity value (lactic acid equivalent) of laboratory-crafted (**A**) cow milk and (**B**) goat milk kefirs during the storage at +8 °C for 8 days.

3.3 Total Dry Matter Analysis of Cow and Goat Milk Kefirs

The laboratory produces kefir's dry matter content measurements were made during 8 days period. The dry matter content changes over the eight-day period due to fermentation and changes in the composition of the kefir. The protein content may increase as a result of the breakdown of proteins in the milk, while the fat and sugar content may change based on the fermentation and nutritional factors available to the microbes.

The activity of microorganisms during fermentation can influence the overall composition of the kefir, including changes in dry matter content. Changes in microbial populations can affect the breakdown of components in kefir, potentially leading to variations in dry matter.

The dry matter values of cow milk kefir ranged between (10.37-17.32) and for goat milk kefir between (12.07-17.15), as shown in the Figure 3.6 and Table 3.3, that is, the value of the dry matter decreased during storage, and these finding are constituent with other studies (Irigoyen et al., 2005).

Table 3.3 Total dry matter content (lactic acid equivalent) of goat and cow milk kefirs during unfermented (day 0) and fermented days from 1 to 8.

Fermentation Period (Days)	Cow milk kefir		Goat milk kefir	
	Total dry matter content (g/100 g)	± SD	Total dry matter content (g/100 g)	±SD
0	nd	nd	nd	nd
1	13.02	0.744	15.14	0.309
2	13.59	1.712	15.89	0.138
3	16.99	2.472	16.73	2.402
4	17.32	2.408	17.15	2.254
5	16.11	3.013	16.95	2.024
6	12.50	0.743	14.72	0.103
7	10.37	2.443	13.75	0.542
8	10.37	2.436	12.07	2.142

nd: Not determined. Values are the mean ± standard deviation (SD) of the data (n = 3)

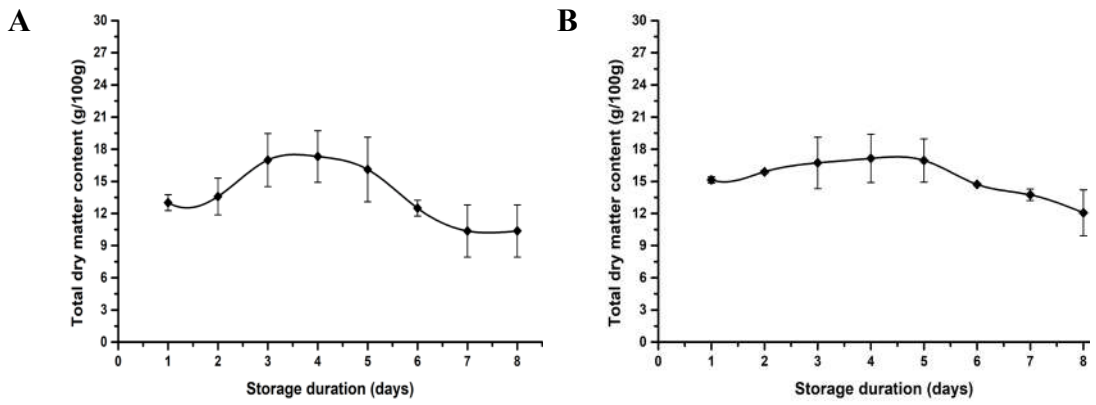

Figure 3.6 The effect of time on the total dry matter content of laboratory-crafted (**A**) cow milk and (**B**) goat milk kefirs during the storage at +8 °C for 8 days.

3.4 Total Free Amino Acid Content Testing of Cow and Goat Milk Kefirs

The total free amino acid content is relatively low at the beginning of the fermentation process, reflecting the composition of the fresh milk and any remaining amino acids present in the kefir grains.

The amount of free amino acids in CK and GK produced during the eight-day fermentation process was calculated as equivalent to L-serine (Figure 3.7). By eight days the total free amino acid content reaches a peak, indicating that the fermentation process has progressed considerably. According to Table 3.4 and Figure 3.8, the final measurement showed a significant increase compared to the initial baseline, reflecting the accumulation of free amino acids during fermentation.

In general, the results prove that the proteins in milk are converted into free amino acids through the fermentation process, which enhances the nutritional value and flavour of kefir, although there are slight differences.

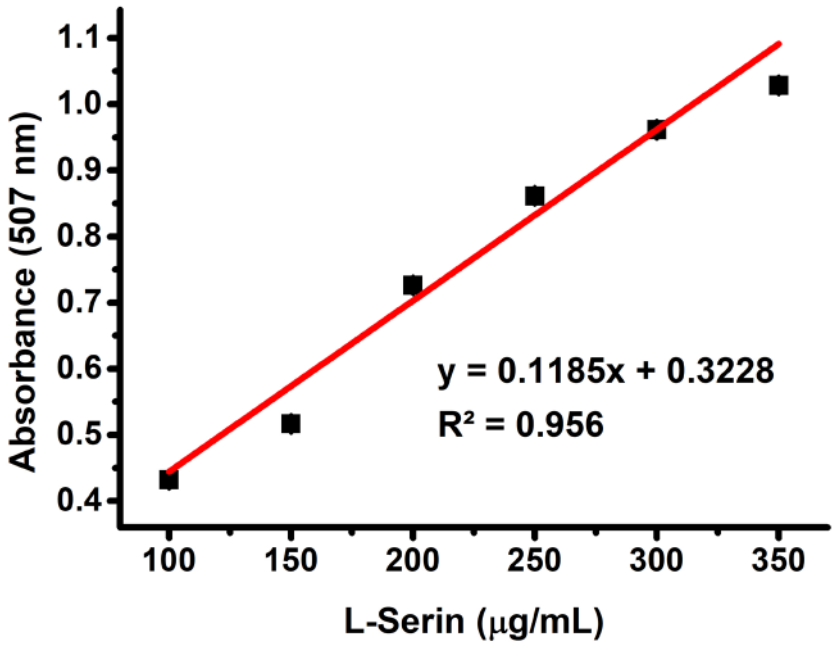

Figure 3.7 Calibration curve of L-serine for total free amino acid content testing of cow and goat milk kefirs.

Table 3.4 Total free amino acid content (L-serine equivalent) of goat and cow milk kefirs during unfermented (day 0) and fermented days from 1 to 8.

Fermentation Period (Days)	Cow milk kefir		Goat milk kefir	
	Total free amino acid content (µg/mL)	± SD	Total free amino acid content (µg/mL)	±SD
0	nd	nd	nd	nd
1	1.35	0.203	1.65	0.212
2	1.04	0.066	1.45	0.054
3	1.09	0.030	1.79	0.316
4	1.04	0.157	1.96	0.318
5	1.74	0.125	2.20	0.107
6	2.36	0.024	2.63	0.239
7	2.44	0.010	3.54	0.119
8	2.45	0.211	3.98	0.131

nd: Not determined. Values are the mean ± standard deviation (SD) of the data (n = 3)

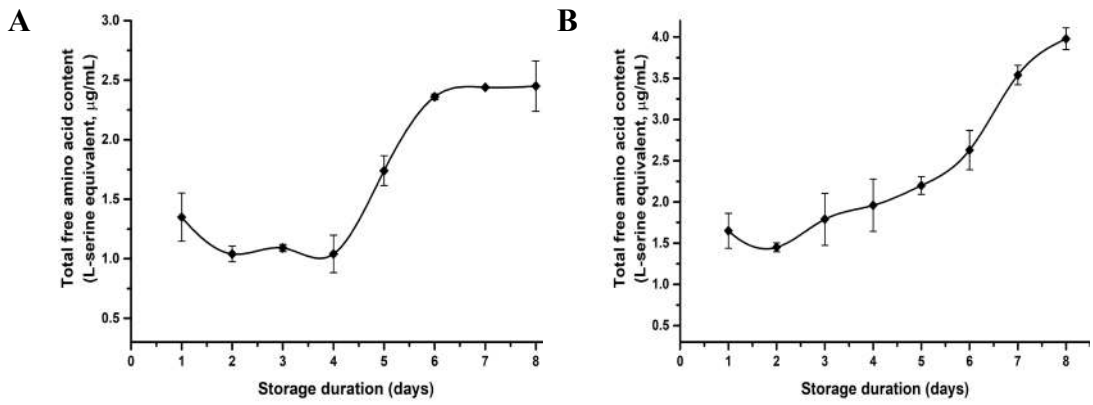

Figure 3.8 Changes of total free amino acid content of laboratory-crafted (**A**) cow milk and (**B**) goat milk kefirs during the storage at +8 °C for 8 days.

As the fermentation process progressed, there was an increase in the total free amino acid content each day. This increase is due to the activity of microbial cultures in kefir grains, which break down the proteins in milk into amino acids. The rate of increase may vary depending on factors such as pH, temperature, and health of the kefir grains. This enhancement contributes to the nutritional value and flavour of the kefir (Nielsen et al., 2014).

3.5 Water-Soluble Protein Extraction of Kefir

In this study, lyophilized water-soluble proteins were successfully isolated from kefir samples obtained from goat and cow milk in a laboratory environment. These CK-WSPE and GK-WSPE were treated with B-AgNPs (biogenic silver nanoparticles) to prepare nanotechnological formulations for evaluation in terms of both antimicrobial activity and cytotoxic activity.

These prepared nanotechnological formulations potentially offer new biomedical applications for the treatment of microbial infections and the eradication of cancer cells. This process, by investigating the synergistic effects of proteins derived from kefir and silver nanoparticles, may contribute to the development of innovative therapeutic methods in the healthcare field.

3.5.1 Protein content of water-soluble extracts of kefir

The Bradford method, also known as the Bradford protein assay, is a common technique used to quantify the concentration of proteins in a solution (Noble et al., 2009). It relies on the interaction between a dye called Coomassie Brilliant Blue G-250 and protein molecules. The intensity of the colour change is proportional to the concentration of proteins present, allowing for their quantification (Manzoor et al., 2021). Bradford assay considered as fast and sensitive (Bradford, 1976). The protein contents of the crude water-soluble protein extracts from CK and GK were evaluated using the modified Bradford method, with BSA used as a standard protein (Figure 3.9).

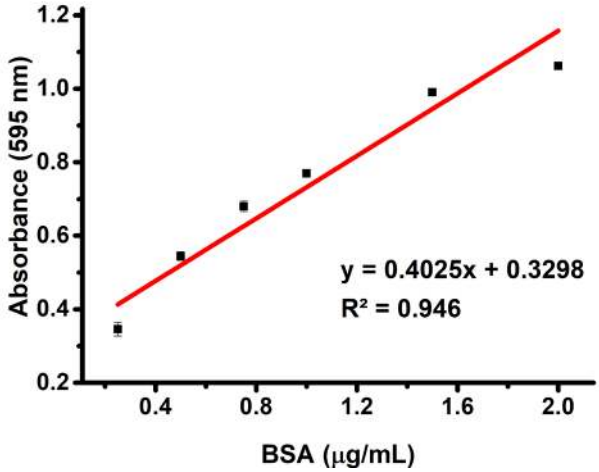

Figure 3.9 Calibration curve of bovine serum albumin for total protein content of cow and goat milk kefirs extracts.

Table 3.5 Total protein content of the CK-WSPE and GK-WSPE during unfermented (day 0) and fermented days from 1 to 8.

Fermentation Period (Days)	Cow milk kefir		Goat milk kefir	
	Total protein content (µg/mg WSPE)	± SD	Total protein content (µg/mg WSPE)	±SD
0	nd	nd	nd	nd
1	1.19	0.007	0.97	0.081
2	1.27	0.087	1.10	0.007
3	1.18	0.015	1.03	0.017
4	1.20	0.073	1.09	0.026
5	1.08	0.015	1.21	0.049
6	1.19	0.009	1.27	0.017
7	1.25	0.006	1.28	0.061
8	1.21	0.015	1.30	0.027

nd: Not determined. Values are the mean ± standard deviation (SD) of the data (n = 3)

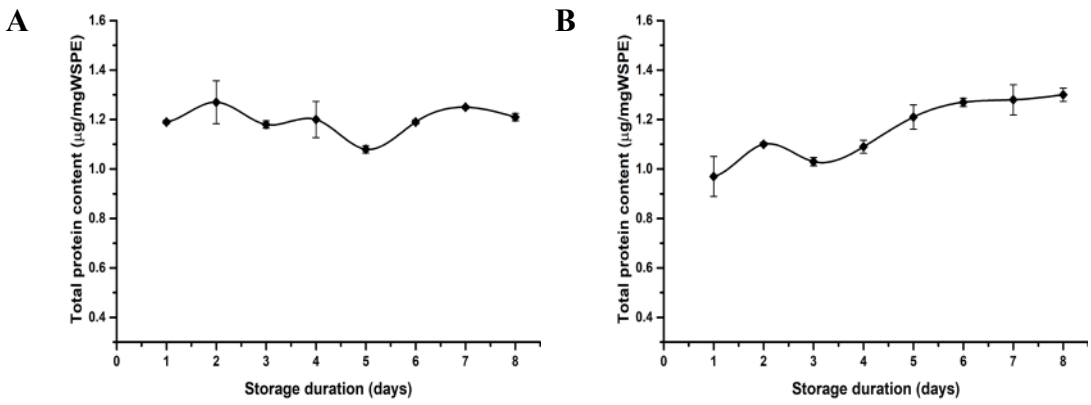

Figure 3.10 Changes of total protein content of laboratory-crafted (**A**) CK-WSPE and (**B**) GK-WSPE during the storage at +8 °C for 8 days.

When measuring water-soluble proteins in kefir using the Bradford method, the fermentation process increases these proteins by breaking down large proteins like casein into smaller peptides and amino acids. As shown in Table 3.5 and Figure 3.10, the protein contents of both CK-WSPE and GK-WSPE were similar. While they did not exhibit significant changes throughout the 8-day fermentation period, they did show a limited increase. This increase is due to the activity of yeasts and bacteria in kefir grains, with no significant difference observed between CK and GK. Research by Azizkhani et al. (2018) and Irigoyen et al. (2005) confirms that fermentation enhances the amount of soluble protein.

3.5.2 Protein profiling of kefirs water-soluble proteins with SDS-PAGE

The SDS-PAGE results demonstrate that both CK and GK contain a variety of water-soluble proteins with distinct profiles. The differences in protein band patterns between CK-WSPE and GK-WSPE suggest that the source of milk influences the protein composition of kefir. It was obtained from cow and goat milk kefir extracts which contain water soluble protein were showed two major band on the gels. According to SDS-PAGE profiling, these extracts showed small molecular mass nearly 25 and 10 kDa (Figure 3.11).

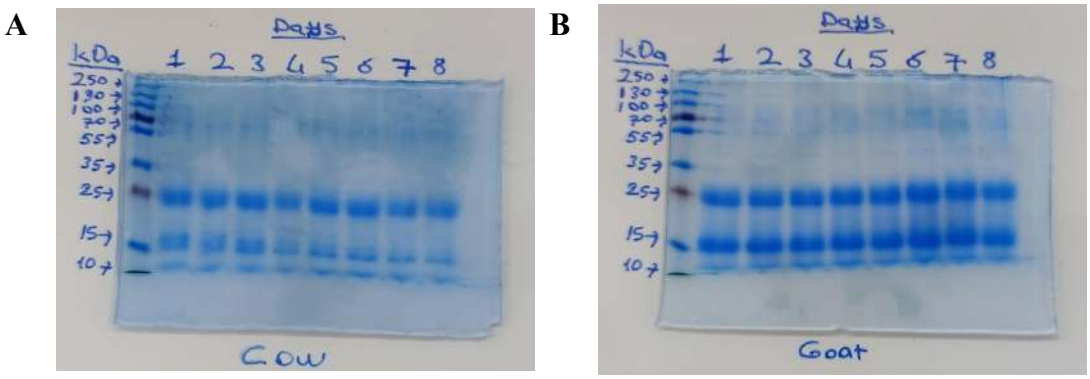

Figure 3.11 SDS-PAGE of (**A**) CK-WSPE and (**B**) GK-WSPE during the storage at +8 °C for 8 days. Lanes 1 to 8; The lyophilized water-soluble extract of kefir samples during the storage at +8 °C for 1st to 8th days, respectively. lane 0; Protein marker (250kDa 130kDa, 100kDa, 70kDa, 55kDa, 35kDa, 25kDa, 15kDa and 10kDa).

The SDS-PAGE results show that both CK and GK contain a variety of water-soluble proteins with distinct profiles. The differences in protein band patterns between CK-WSPE and GK-WSPE indicate that the source of milk influences kefir's protein composition. Three major bands were observed in both extracts, with molecular masses around 25 kDa, 15 kDa and 10 kDa, suggesting the presence of specific proteins characteristic of kefir from different milk sources. These findings similar with previous research by Simova et al. (2002).

3.5.3 Characterization of B-AgNPs additive water-soluble kefir extracts with SEM and TEM

SEM generates high-resolution images by scanning a focused electron beam across a sample's surface and detecting the resulting secondary or backscattered electron signals. Additionally, an energy dispersive X-ray analyser (EDX) is employed to identify elements and provide quantitative compositional data. Both SEM and SEM-EDX analysis results confirm the formation of B-AgNPs and its 1:2 lyophilized kefir WSPE formulations (Figure 3.12). TEM profile of B-AgNPs and its WSPE formulation have been morphology spherical or globular in shape with the distribution range of 3.36 nm to 49.97 nm in diameter (Figure 3.13).

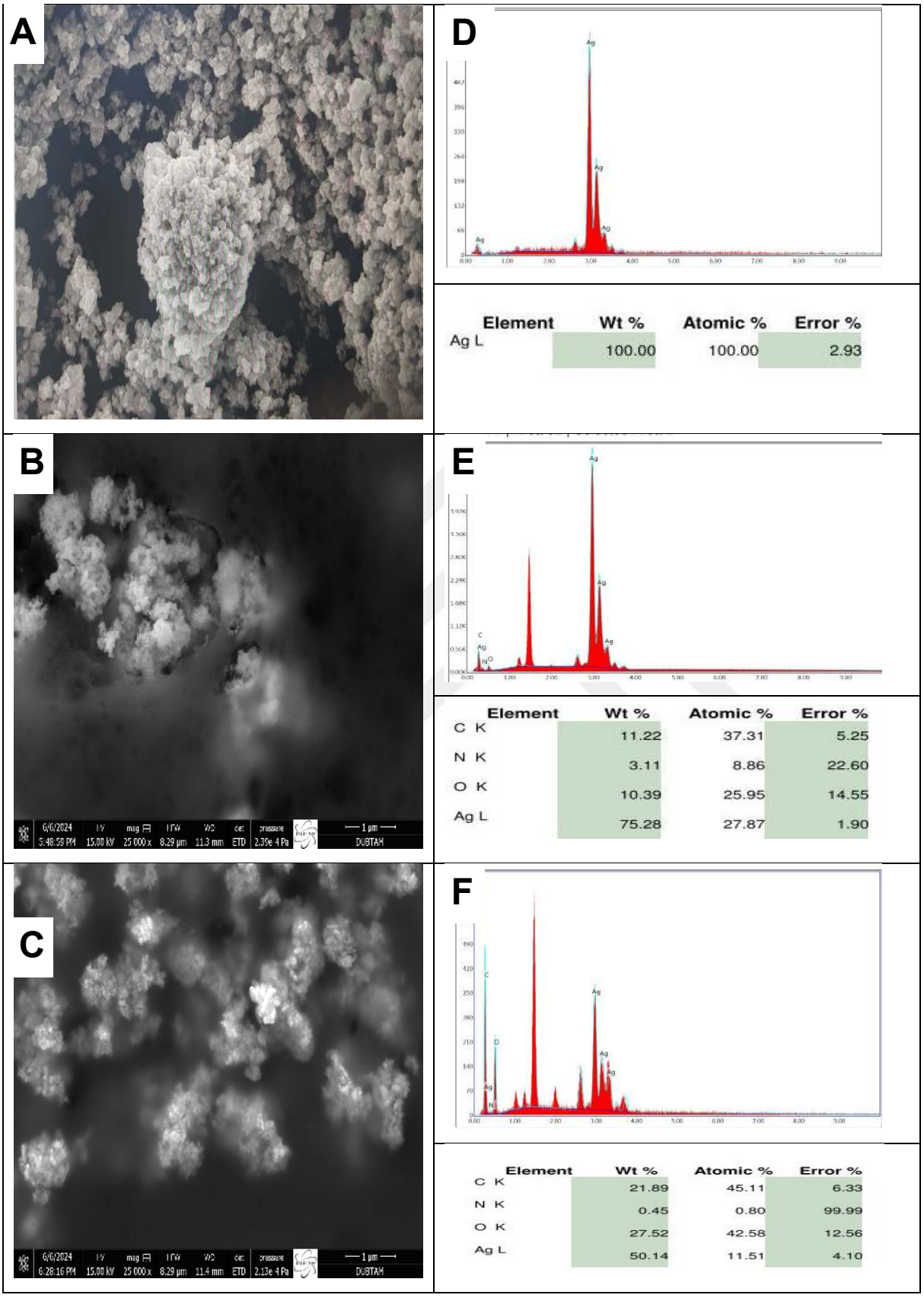

Figure 3.12 SEM image of (**A**) B-AgNPs, (**B**) cow milk kefir WSPE/B-AgNPs, (**C**) goat milk kefir's WSPE/BAgNPs and (**D-E-F**) their EDX analysis.

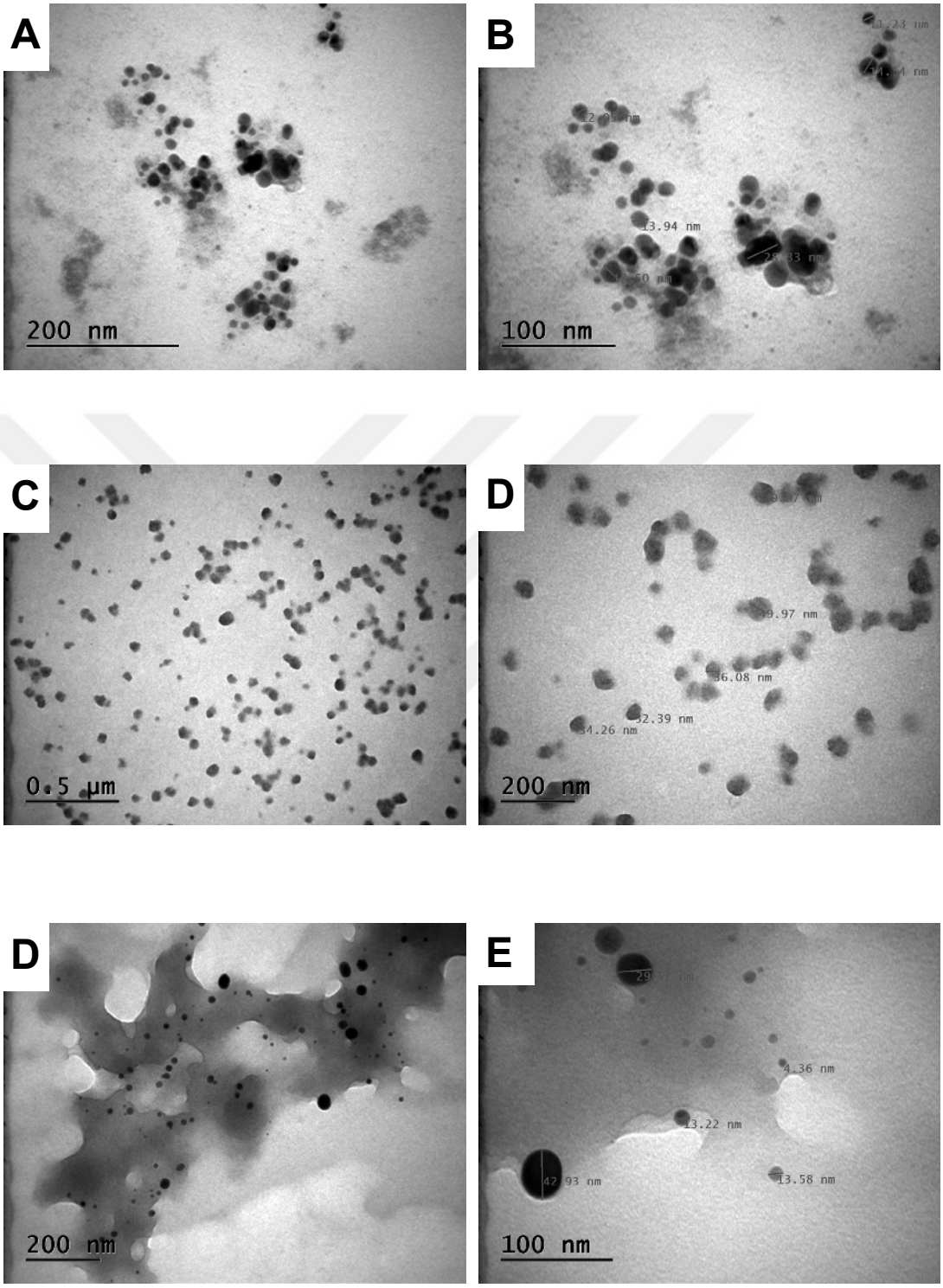

Figure 3.13 TEM image of (**A, B**) B-AgNPs, (**C, D**) cow milk kefir WSPE/B-AgNPs, (**E, F**) goat milk kefir's WSPE/BAgNPs.

3.6 Biological Evaluation of the CK-WSPE and GK-WSPE

3.6.1 Enhancement antimicrobial activity of B-AgNPs additive water-soluble kefir extracts

The disk diffusion test technique was used to examine the antibacterial properties of CK-WSPE and GK-WSPE and their B-AgNPs treated preparate. However, no inhibition zones were found for CK-WSPE and GK-WSPE (Table 3.6 and Figure 3.14). The activities exhibited by standard antimicrobial agents vary as seen in Figure 3.15. Additionally, neither cow nor goat milk kefir samples, after centrifugation, also showed no inhibition zones. As shown in the Figure 3.16, when kefir was tested directly, surprisingly a small inhibition zone was observed with the goat milk kefir, particularly with the kefir that was more than a year old, rather than the new one.

Table 3.6 Antimicrobial activities of standard antibiotics, and water-soluble protein extracts of cow and goat milk kefirs.

Test microorganisms	µg/6 mm paper disc	Zone of inhibition (mm)				
		Compounds		Standard antibiotics		
		Cow milk kefir	Goat milk kefir	OFX (5)[b]	E (15)[b]	N (60)[b]
Escherichia coli ATCC 25922	1000	–	–	28	10	nt
Escherichia coli[a] (Clinical isolate)	1000	–	–	25	–	nt
Enterobacter cloacae ATCC 23355	1000	–	–	33	–	nt
Bacillus subtilis ATCC 11774	1000	–	–	28	30	nt
Staphylococcus aureus ATCC 25923	1000	–	–	27	23	nt
Staphylococcus aureus (Clinical isolate)	1000	–	–	23	24	nt
Candida albicans ATCC 10231	1000	–	–	nt	nt	23
Candida albicans (Clinical isolate)	1000	–	–	nt	nt	23

[a]Clinical isolates; [b]µg/6 mm paper disc; nt: not tested.

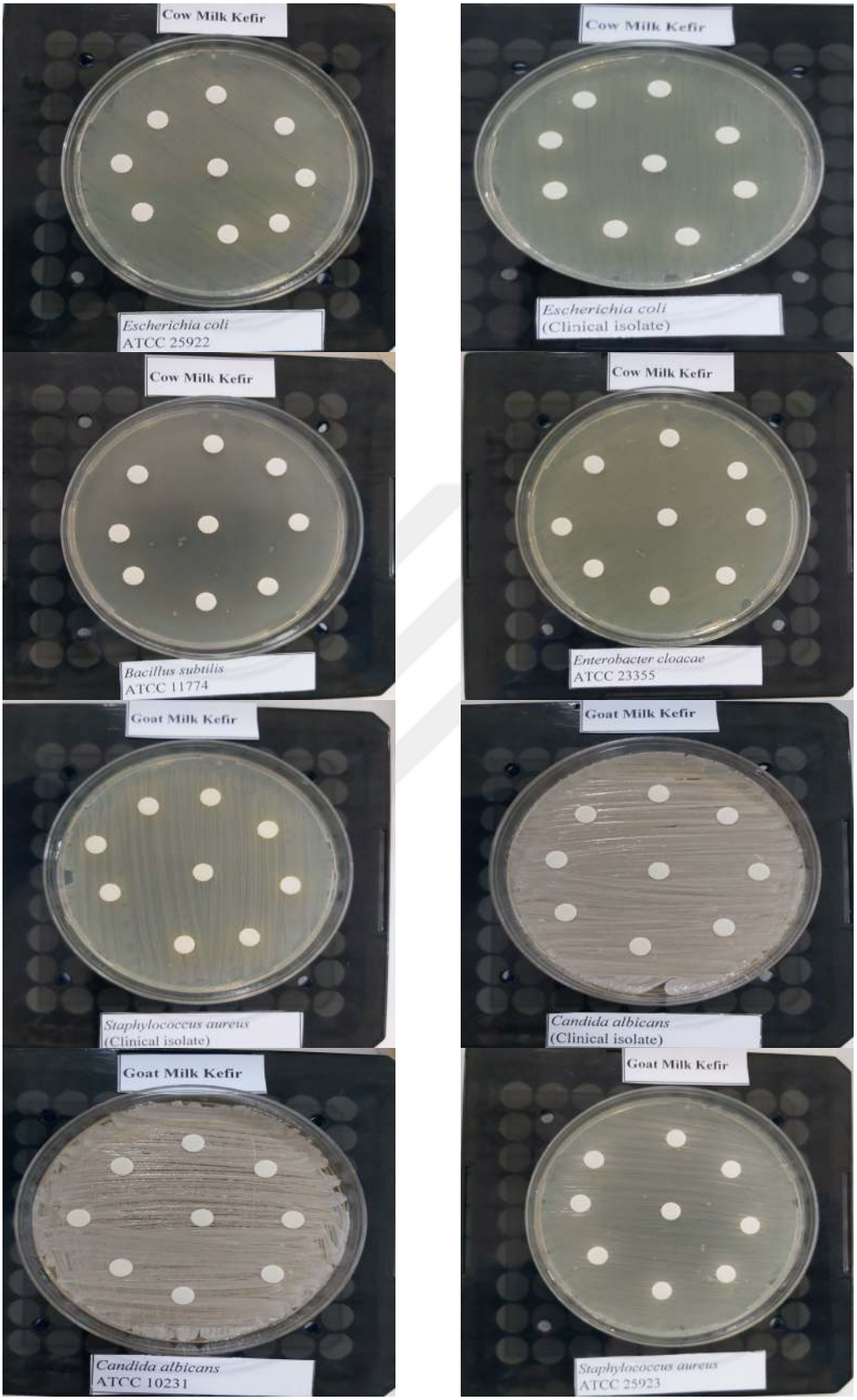

Figure 3.14 Inhibition zone of CK-WSPE and GK-WSPE against studied microorganisms.

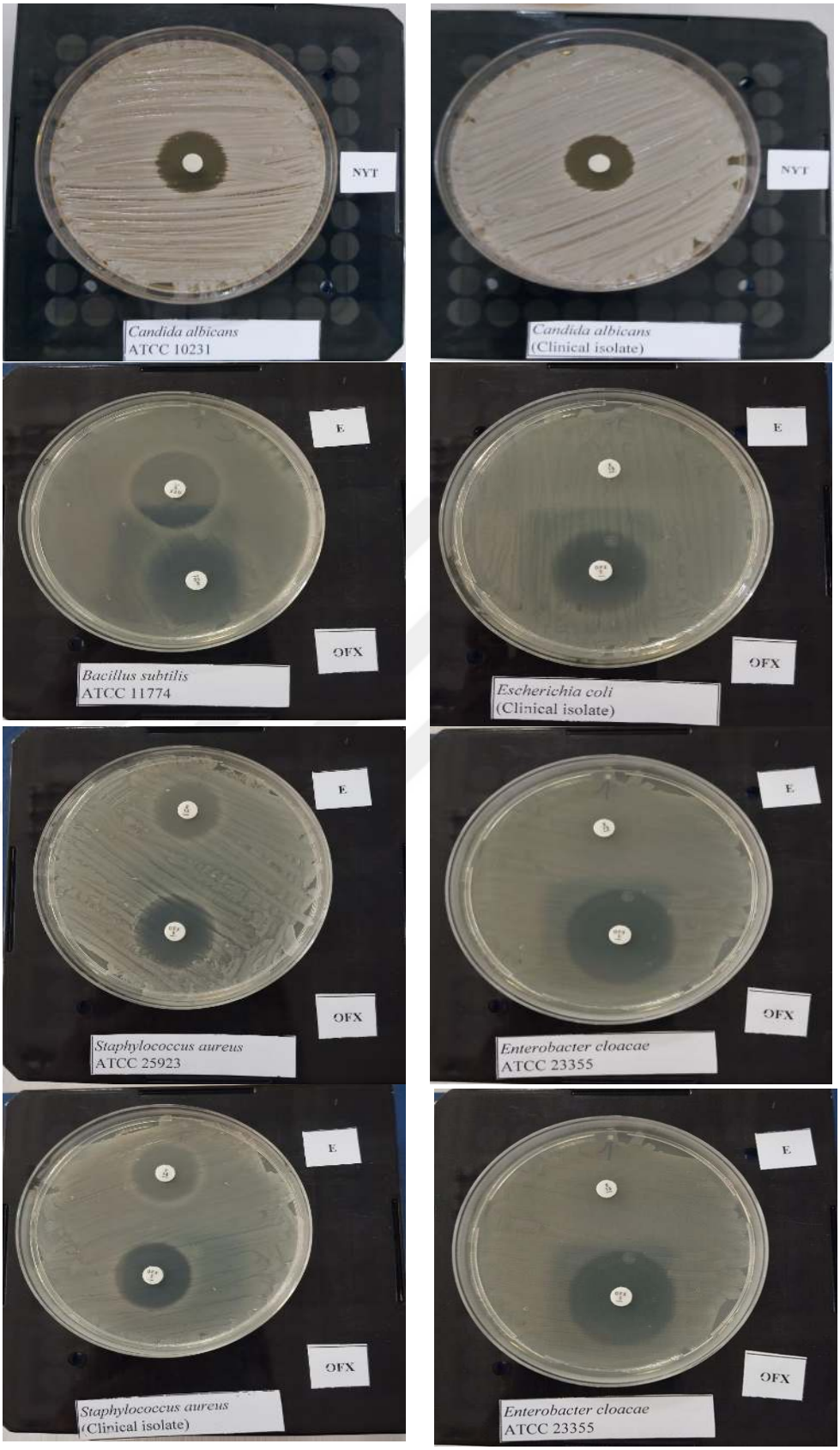

Figure 3.15 Inhibition zone of standard antibiotics against studied microorganisms.

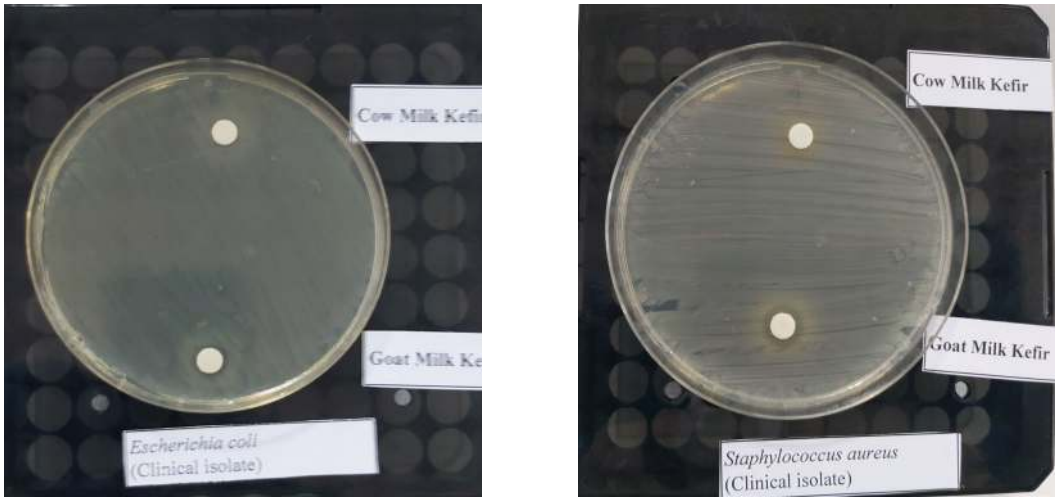

Figure 3.16 Inhibition zone of CK and GK against studied microorganisms.

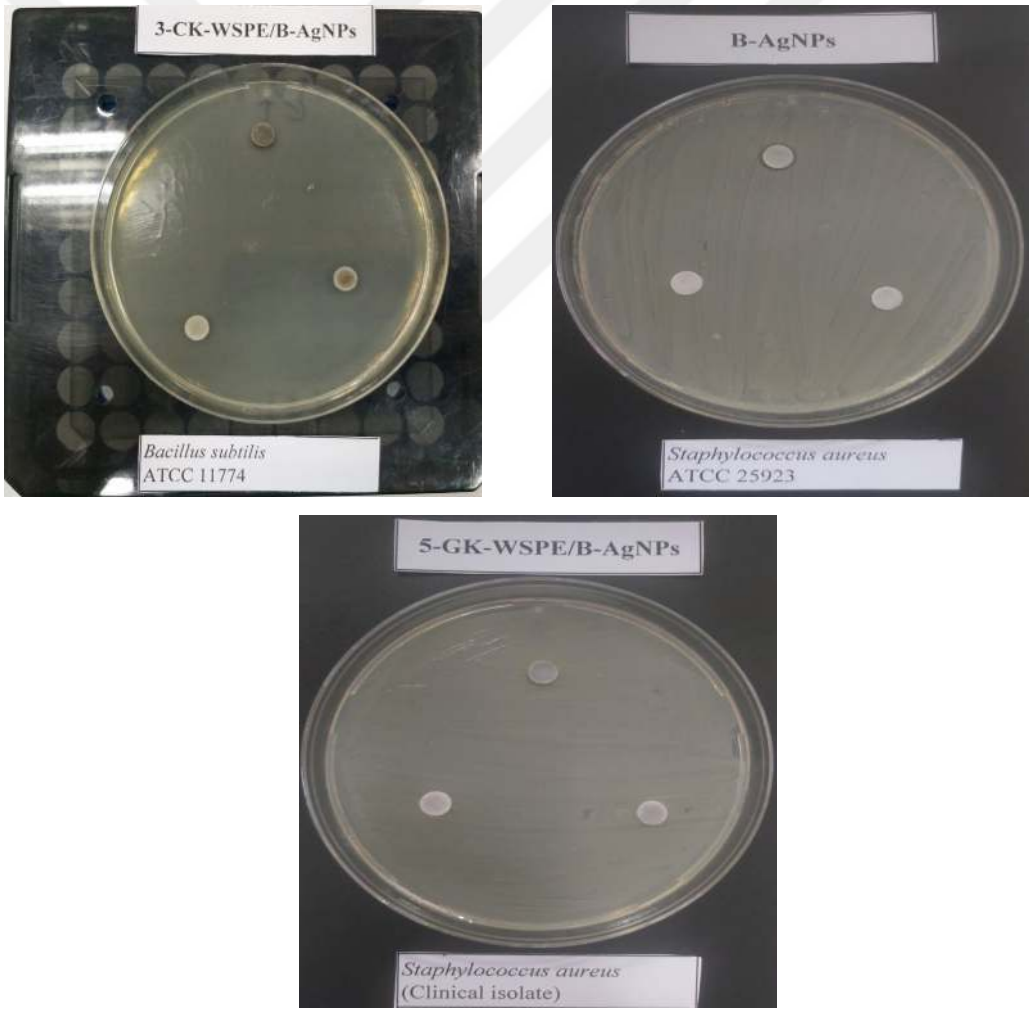

Figure 3.17 Antimicrobial activity of CK and GK WSPE/AgNPs against studied microorganisms.

As shown in Figure 3.17, nanoparticle additive preparations demonstrated some effects on Gram-positive bacteria such as *S. aureus* ATCC 25923 and *B. subtilis* ATCC 11774, as well as a clinical isolate of *S. aureus*. However, they showed no effect on Gram-negative bacteria such as *E. cloacae* ATCC 23355, *E. coli* ATCC 25922, and a clinical isolate of *E. coli* (Figure 3.18).

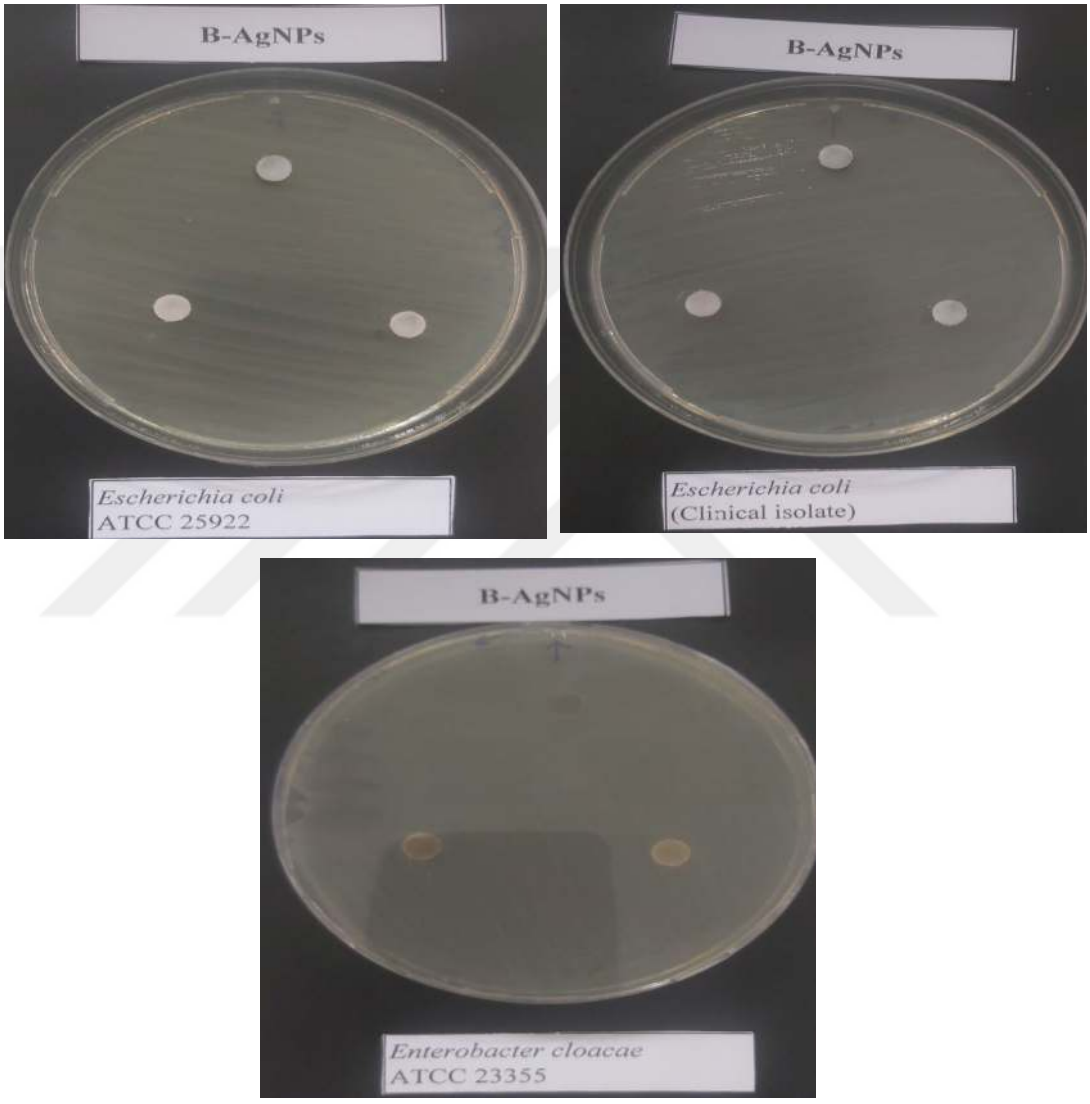

Figure 3.18 Antimicrobial activity of B-AgNPs against studied microorganisms.

Due to the nanotechnology as a therapeutic tool to combat microbial resistance, the therapeutic efficacy of B-AgNPs and its 1:2 CK-WSPE and GK-WSPE formulations was evaluated (Pelgrift and Friedman, 2013; Vanaja and Annadurai, 2013). B-AgNPs and its 1:2 lyophilized kefir WSPE formulations inhibition zone diameters against the

tested Gram-negative bacteria and Gram-positive bacteria were summarized in the Table 3.7. According to table, both B-AgNPs and the 1:2 lyophilized kefir WSPE formulations exhibited inhibition zones ranging from 7 to 12 mm particularly against the Gram-positive bacterial strains *S. aureus* ATCC 25923, *B. subtilis* ATCC 11774, and a clinical isolate of *S. aureus* (Figure 3.17).

Table 3.7 Antimicrobial activities of B-AgNPs and its additive water-soluble protein extracts formulations.

Test microorganisms	Zone of inhibition (mm)								
	B-AgNPs (µg/disk)			3-CK-WSPE/B-AgNPs (µg/disk)			5-CK-WSPE/B-AgNPs (µg/disk)		
	125	250	500	250/125	500/250	1000/500	250/125	500/250	1000/500
"	–	–	–	–	–	–	–	–	–
Escherichia coli[a] (Clinical isolate)	–	–	–	–	–	–	–	–	–
Enterobacter cloacae ATCC 23355	–	–	–	–	–	–	–	–	–
Bacillus subtilis ATCC 11774	9	11	12	8	9	11	8	9	11
Staphylococcus aureus ATCC 25923	–	7	8	–	7	7	–	–	7
Staphylococcus aureus (Clinical isolate)	–	8	9	–	7	8	–	–	8
Candida albicans ATCC 10231	–	–	–	–	–	–	–	–	–
Candida albicans (Clinical isolate)	–	–	–	–	–	–	–	–	–

[a]Clinical isolates

3.6.2 Enhancement cytotoxicity with biogenic silver nanoparticles of B-AgNPs additive water-soluble kefir extracts

Five different samples, i.e., CK-WSPE, GK-WSPE, B-AgNPs, B-AgNPs/CK-WSPE (1:2) and B-AgNPs/GK-WSPE (1:2) were used for evaluating cytotoxicity activity on normal foreskin fibroblast (BJ, CRL-2522) cells. CK-WSPE and GK-WSPE concentrations range from 15.625 µg/mL to 500 µg/mL were prepared, while concentrations of 7.8125, 15.625, 31.25, 62.5, 125, and 250 µg/mL were prepared for

B-AgNPs. A range of concentrations (3.90625:7.8125, 7.8125:15.625, 15.625:31.25, 31.25:62.5, 62.5:125, 125:250, 250:500 µg/mL) with a 1:2 ratio of biogenic silver nanoparticles (B-AgNPs) to water-soluble protein extracts (WSPE) from both cow and goat milk kefir were selected.

The result showed that CK-WSPE and GK-WSPE did not show any toxic effect, and their proliferative concertarations were determined as 62.5 and 500 µg/mL, respectively (Figure 3.19). In case of B-AgNPs, only significant non-toxic concentraton was determined at 7.8125 µg/mL (López-García et al., 2024).

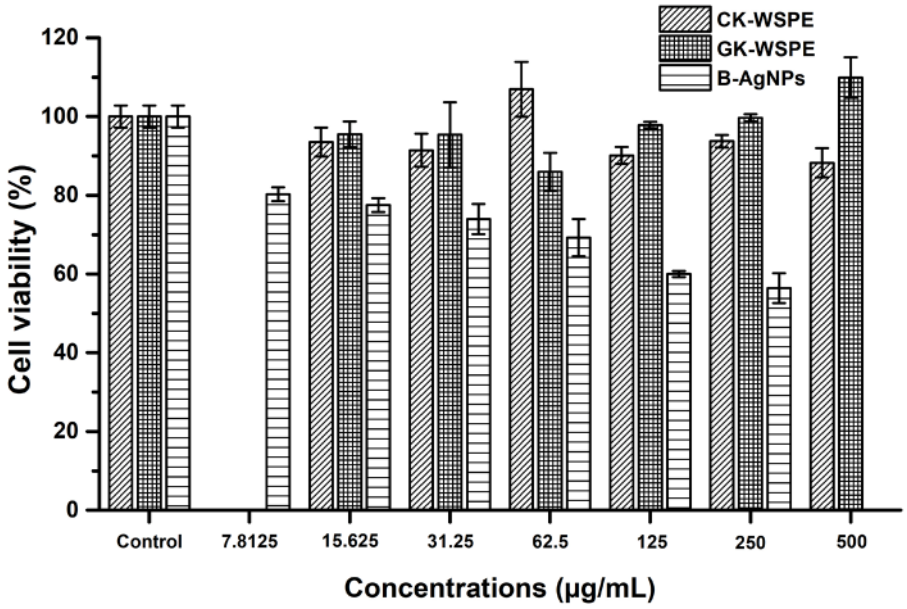

Figure 3.19 Cytotoxic effects of CK-WSPE, GK-WSPE, B-AgNPs on normal foreskin fibroblast (BJ, CRL-2522) cells.

Interestingly, 1:2 ratios of B-AgNPs to WSPE from both cow and goat milk kefir reduced the toxicity of B-AgNPs (Figure 3.20). Specifically, in the study, a concentration of 250 µg/mL of B-AgNPs was found to be toxic when combined with B-AgNPs/CK-WSPE (1:2 ratio). Conversely, a concentration of 62.5 µg/mL of B-AgNPs was determined to be toxic when mixed with B-AgNPs/GK-WSPE in a 1:2 ratio.

For the B-AgNPs/CK-WSPE (1:2), the proliferative concentration of CK-WSPE was determined to be 15.625 µg/mL, which is lower than the concentration required when CK-WSPE is used to treat the cells separately (Figure 3.19 and 3.20). However, no proliferative effect of GK-WSPE was observed for the B-AgNPs/CK-WSPE (1:2) which may be due to the toxic effect on B-AgNPs on GK-WSPE.

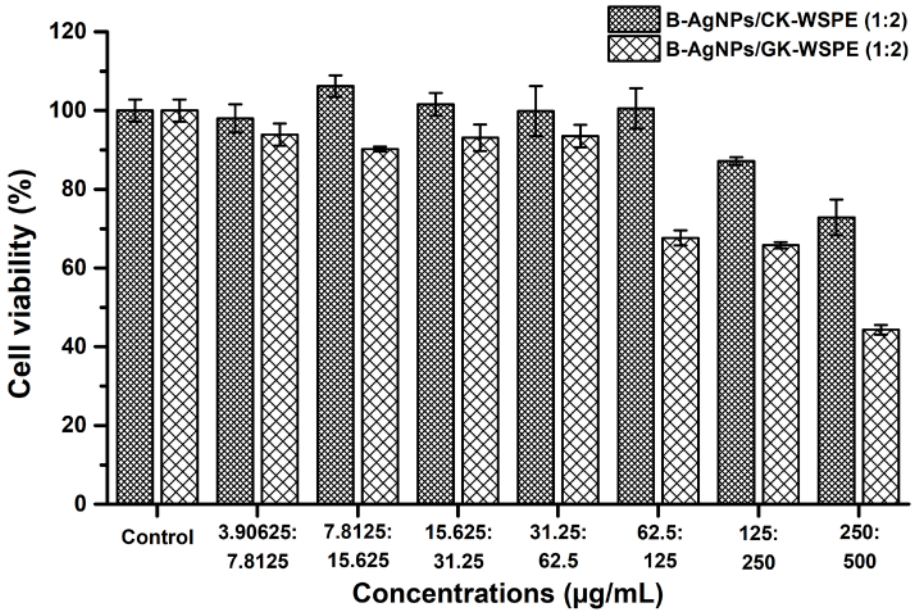

Figure 3.20 Cytotoxic effects of B-AgNPs/CK-WSPE (1:2) and B-AgNPs/GK-WSPE (1:2) on normal foreskin fibroblast (BJ, CRL-2522) cells.

4. CONCLUSIONS

In this study, we conducted a comprehensive profiling of cow milk and goat milk kefir, analysing their nutritional composition and evaluating the cytotoxicity of their crude water-soluble proteins, particularly those evolved with biogenic amines. The findings offer valuable insights into the diverse bioactive compounds present in these kefir variants and their potential implications for human health.

Cow and goat milk kefir emerge as nutritionally rich fermented beverages with a complex array of bioactive compounds. While they present significant health benefits, the presence of biogenic amines and cytotoxic proteins suggests the importance of moderate consumption and further exploration of their therapeutic potentials. This study highlights the intricate balance between beneficial and potentially harmful components within fermented foods, underscoring the need for careful consideration in dietary recommendations.

Biogenic amines, although naturally occurring in fermented products, warrant ongoing monitoring due to potential health risks, especially among individuals sensitive to these compounds.

Furthermore, this methodology aims not only to expand the scope of kefir research but also to align with the growing interest in biomedical applications of nanotechnology. By deepening our understanding of the nutritional dynamics of goat and cow milk kefir, this study contributes to the emerging field of biologically synthesized nanomaterials aimed at enhancing the health attributes of fermented dairy products. The integration of traditional fermentation practices with advanced nanotechnology holds promise for providing valuable insights and innovations beneficial to both the dairy industry and the broader scientific community.

This research not only sheds light on the nutritional and therapeutic aspects of kefir but also paves the way for future studies exploring the synergistic effects of fermentation and nanotechnology. The potential to develop new functional foods with enhanced health benefits is significant. Continued interdisciplinary collaboration will

be key to unlocking the full potential of these innovative approaches in food science and technology.

REFERENCES

Adesulu, A. T. and Awojobi, K. O. (2014). Enhancing sustainable development through indigenous fermented food products in Nigeria. *African Journal of Microbiology Research*, 8(12), 1338-1343. Doi: 10.5897/ajmr2013.5439.

Ahmed, Z., Wang, Y., Ahmad, A., Khan, S. T., Nisa, M., Ahmad, H. and Afreen, A. (2013). Kefir and health: A contemporary perspective. *Critical Reviews in Food Science and Nutrition*, 53(5), 422-434. Doi: 10.1080/10408398.2010.540360.

Altay, F., Karbancıoğlu-Güler, F., Daskaya-Dikmen, C. and Heperkan, D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process, and quality characteristics. *International Journal of Food Microbiology*, 167, 44-56. Doi: 10.1016/j.ijfoodmicro.2013.06.016.

Appelbaum, P. C., Clark, C. L. and Jacobs, M. R. (1998). Antipeumococcal activities of levofloxacin and clarithromycin as determined by agar dilution, microdilution, E-test, and disk diffusion methodologies. *Journal of Clinical Microbiolog*, 36(12), 3579-3584. Doi: 10.1128/jcm.36.12.3579-3584.1998.

Araujo, F., das Neves, J., Martins, J. P., Granja, P. L., Santos, H. A. and Sarmento, B. (2017). Functionalized materials for multistage platforms in the oral delivery of biopharmaceuticals. *Progress in Materials Science*, 89, 306-344. Doi: 10.1016/j.pmatsci.2017.05.001.

Ayichew, T., Belete, A., Alebachew, T., Tsehaye, H., Berhanu, H. and Minwuyelet, A. (2017). Bacterial probiotics, their importances and limitations: A review. *Journal of Nutrition and Health Sciences*, 4(2), 202. Doi: 10.15744/2393-9060.4.202.

Azizkhani, M. and Parsaeimehr, M. (2018). Probiotics survival, antioxidant activity and sensory properties of yogurt flavored with herbal essential oils. *International Food Research Journal*, 25(3), 921-927.

Benzer Gürel, D., Ildız, M., Sabancı, S., Koca, N., Çağındı, Ö. and İçier, F. (2021). The effect of using cow and goat milk on antioxidant, rheological and sensory properties of kefir. *Turkish Journal of Agriculture - Food Science and Technology*, 9(1), 7-14. Doi: 10.24925/turjaf.v9i1.7-14.3330.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. Doi: 10.1006/abio.1976.9999.

Buzea, C., Pacheco, I. I. and Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases*, 2(4), MR17-MR71. Doi: 10.1116/ 1.2815690.

Caplice, E. and Fitzgerald, G. F. (1999). Food fermentations: Role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, 50(1-2), 131-149. Doi: 10.1016/S0168-1605(99)00082-3.

Ceballos, L. S., Morales, E. R., de la Torre Adarve, G., Castro, J. D., Martínez, L. P. and Sampelayo, M. R. S. (2009). Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. *Journal of Food Composition and Analysis*, 22(4), 322-329. Doi: 10.1016/j.jfca.2008.10.020.

Cemeroğlu, B. (2007). Gıda Analizleri. Gıda Teknolojisi Derneği Yayınları No: 34, Ankara.

Cho, K., Wang, X. U., Nie, S., Chen, Z. and Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research*, 14(5), 1310-1316. Doi: 10.1158/1078-0432.ccr-07-1441.

Coultate, T. P. (2016). Food: The chemistry of its components. 6th edition, Royal Society of Chemistry: Cambridge.

De Lima, R. A., Oliveira, A. A., Pitta, G. R., de Gasper, A. L., Vibrans, A. C., Chave, J., ... and Prado, P. I. (2020). The erosion of biodiversity and biomass in the Atlantic Forest biodiversity hotspot. *Nature Communications*, 11(1), 6347. Doi: 10.1038/s41467-020-20217-w.

Dhanker, R., Khatana, K., Verma, K., Singh, A., Kumar, R. and Mohamed, H. I. (2023). An integrated approach of algae-bacteria mediated treatment of industries generated wastewater: Optimal recycling of water and safe way of resource recovery. *Biocatalysis and Agricultural Biotechnology*, 54, 102936. Doi: 10.1016/j.bcab.2023.102936.

Dinç, A. (2008). Kefirin bazı mikrobiyolojik ve kimyasal özelliklerinin belirlenmesi (Yüksek Lisans Tezi). Ankara Üniversitesi Sağlık Bilimleri Enstitüsü Besin Hijyeni ve Teknolojisi Anabilim Dal: Ankara.

El-Rayes, A. M. H., Body, E. A., El-Kholie, E. M. and El-Bedawy, L. A. (2015). Nutritional characterizations of kefir milk. *Journal of Home Economics*, 25(3), 25-40. Doi: 10.21608/mkas.2015.172785.

Ezema, C. (2013). Probiotics in animal production: A review. *Journal of Veterinary Medicine and Animal Health*, 5(11), 308-316. Doi: 10.1080/ 17450399009428406.

Farag, M. A., Jomaa, S. A., Abd El-Wahed, A. and El-Seedi, H. R. (2020). The many faces of kefir fermented dairy products: Quality characteristics, flavour chemistry, nutritional value, health benefits, and safety. *Nutrients*, 12(2), 346. Doi: 10.3390/nu12020346.

Farnworth, E. R. and Mainville, I. (2003). Kefir: A fermented milk product. *Handbook of Fermented Functional Foods*, 2, 89-127.

Folkertsma, B. and Fox P. F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. *Journal of Dairy Research*, 59(2), 217–224. Doi: 10.1017/S0022029900030466.

Gallagher, S. R. (2012). SDS-polyacrylamide gel electrophoresis (SDS-PAGE). *Current Protocols Essential Laboratory Techniques*, 6(1), 7-3. Doi: 10.1002/9780470089941.et0703s06.

Garofalo, C., Ferrocino, I., Reale, A., Sabbatini, R., Milanović, V., Alkić-Subašić, M., ... and Osimani, A. (2020). Study of kefir drinks produced by backslopping method using kefir grains from Bosnia and Herzegovina: Microbial dynamics and volatilome profile. *Food Research International*, 137, 109369. Doi: 10.1016/j.foodres.2020.109369.

Garrote, G. L., Abraham, A. G. and De Antoni, G. L. (2001). Chemical and microbiological characterisation of kefir grains. *Journal of Dairy Research*, 68(4), 639-652. Doi: 10.1017/s0022029901005210.

Gaspar-Pintiliescu, A., Oancea, A., Cotarlet, M., Vasile, A. M., Bahrim, G. E., Shaposhnikov, S., ... and Oprita, E. I. (2020). Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. *International Journal of Dairy Technology*, 73(1), 108-116. Doi: 10.1111/1471-0307.12659.

Ghosh, S., Sarkar, T., Pati, S., Kari, Z. A., Edinur, H. A. and Chakraborty, R. (2022). Novel bioactive compounds from marine sources as a tool for functional food development. *Frontiers in Marine Science*, 9, 832957. Doi: 10.3389/ fmars.2022.832957.

González-Orozco, B. D., García-Cano, I., Jiménez-Flores, R. and Alvárez, V. B. (2022). Invited review: Milk kefir microbiota-direct and indirect antimicrobial effects. *Journal of Dairy Science*, 105(5), 3703-3715. Doi: 10.3168/jds.2021-21382.

Gul, O., Mortas, M., Atalar, I., Dervisoglu, M. and Kahyaoglu, T. (2015). Manufacture and characterization of kefir made from cow and buffalo milk, using kefir grain and starter culture. *Journal of Dairy Science*, 98(3), 1517-1525. Doi: 10.3168/jds.2014-8755.

Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S. R. K., Muniyandi, J., Hariharan, N. and Eom, S. H. (2009). Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloids and Surfaces B: Biointerfaces*, 74(1), 328-335. Doi: 10.1016/j.colsurfb.2009.07.048.

Hamouda, M. E. and Salunke, P. (2024). Changes in milk protein functionality at low temperatures and rennet concentrations. *Foods*, 13(3), 447. Doi:10.3390/foods13030447.

Infusino, F., Marazzato, M., Mancone, M., Fedele, F., Mastroianni, C. M., Severino, P., ... and d'Ettorre, G. (2020). Diet supplementation, probiotics, and nutraceuticals in SARS-CoV-2 infection: A scoping review. *Nutrients*, 12(6), 1718. Doi: 10.3390/nu12061718.

Irigoyen, A., Arana, I., Castiella, M., Torre, P. and Ibanez, F. C. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry*, 90(4), 613-620. Doi: 10.1016/j.foodchem.2004.04.021.

Izquierdo-González, J. J., Amil-Ruiz, F., Zazzu, S., Sánchez-Lucas, R., Fuentes-Almagro, C. A. and Rodríguez-Ortega, M. J. (2019). Proteomic analysis of goat milk kefir: Profiling the fermentation-time dependent protein digestion and identification of potential peptides with biological activity. *Food Chemistry*, 295, 456-465. Doi: 10.1016/j.foodchem.2019.05.178.

Jahan, I., Bekler, F. M., Tunç, A. and Güven, K. (2024). The effects of silver nanoparticles (AgNPs) on thermophilic bacteria: Antibacterial, morphological, physiological and biochemical investigations. *Microorganisms,* 12(2), 402. Doi: 10.3390/microorganisms12020402.

Joudeh, N. and Linke, D. (2022). Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *Journal of Nanobiotechnology*, 20(1), 262. Doi: 10.1186/s12951-022-01477-8.

Karimi, R., Mortazavian, A. M. and Da Cruz, A. G. (2011). Viability of probiotic microorganisms in cheese during production and storage: A review. *Dairy Science & Technology*, 91, 283-308. Doi: 10.1007/s13594-011-0005-x.

Khandel, P., Yadaw, R. K., Soni, D. K., Kanwar, L. and Shahi, S. K. (2018). Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. *Journal of Nanostructure in Chemistry*, 8, 217-254. Doi: 10.1007/s40097-018-0267.

Kim, D. H., Jeong, D., Song, K. Y. and Seo, K. H. (2018). Comparison of traditional and backslopping methods for kefir fermentation based on physicochemical and microbiological characteristics. *LWT-Food Science and Technology*, 97, 503-507. Doi: 10.1016/j.lwt.2018.07.023.

Kumar, D., Kumar, P., Singh, H. and Agrawal, V. (2020). Biocontrol of mosquito vectors through herbal-derived silver nanoparticles: Prospects and challenges. *Environmental Science and Pollution Research*, 27, 25987-26024. Doi: 10.1007/s11356-020-08444-6.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. Doi: 10.1038/227680a0.

Lanas, A. (2009). Nonsteroidal antiinflammatory drugs and cyclooxygenase inhibition in the gastrointestinal tract: A trip from peptic ulcer to colon cancer. *The American Journal of the Medical Sciences*, 338(2), 96-106. Doi: 10.1097/maj.0b013e3181ad8cd3.

Lebeer, S., Vanderleyden, J. and De Keersmaecker, S. C. J. (2008). Genes and molecules of lactobacilli supporting probiotic action. *Microbiology and Molecular Biology Reviews*, 72(4), 728-764. Doi: 10.1128/mmbr.00017-08.

Leser, T. D. and Mølbak, L. (2009). Better living through microbial action: The benefits of the mammalian gastrointestinal microbiota on the host. *Environmental Microbiology*, 11(9), 2194-2206. Doi: 10.1111/j.1462-2920.2009.01941.x.

López-García, J., Lehocký, M., Humpolíček, P. and Sáha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: Extent of cytotoxicity, cell viability and proliferation. *Journal of Functional Biomaterials*, 5(2), 43-57. Doi:10.3390/jfb5020043.

Lourens-Hattingh, A. and Viljoen, B. C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*, 11, 1-17. Doi: 10.1016/s0958-6946(01)00036-x.

Luque, R. and Clark, J. H. (2013). Valorisation of food residues: Waste to wealth using green chemical technologies. *Sustainable Chemical Processes*, 1, 10. Doi:10.1186/2043-7129-1-10.

Magalhães, K. T., Dragone, G., de Melo Pereira, G. V., Oliveira, J. M., Domingues, L., Teixeira, J. A., ... and Schwan, R. F. (2011). Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. *Food Chemistry*, 126(1), 249-253. Doi: 10.1016/j.foodchem.2010.11.012.

Manzoor, M., Singh, J., Ray, A. and Gani, A. (2021). Recent advances in analysis of food proteins. In A. Gani and B. A. Ashwar (Eds), *Food Biopolymers: Structural, Functional and Nutraceutical Properties*, (pp. 269-298), Springer Nature Switzerland AG. Doi: 10.1007/978-3-030-27061-2_12.

Martín, R. and Langella, P. (2019). Emerging health concepts in the probiotics field: Streamlining the definitions. *Frontiers in Microbiology*, 10, 1047. Doi: 10.3389/fmicb.2019.01047.

Medina, R. (2019). Fermentation technology. United Kingdom, ED-Tech Press.

Nielsen, B., Gürakan, G. C. and Ünlü, G. (2014). Kefir: A multifaceted fermented dairy product. *Probiotics and Antimicrobial Proteins*, 6, 123-135. Doi: 10.1007/s12602-014-9168-0.

Noble, J. E. and Bailey, M. J. (2009). Quantitation of protein. *Methods in Enzymology*, 463, 73-95. Doi: 10.1016/0076-6879(90)82008-p.

Ottogalli, G., Galli, A., Resmini, P. and Volonterio, G. (1973). Microbiological and chemical composition and ultrastructure of kefir grains. *Annali di Microbiologia ed Enzimologia*, 23(4-6), 109-121.

Pasinszki, T. and Krebsz, M. (2020). Synthesis and application of zero-valent iron nanoparticles in water treatment, environmental remediation, catalysis, and their biological effects. *Nanomaterials*, 10(5), 917. Doi: 10.3390/nano10050917.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R. and Langer, R. (2020). Nanocarriers as an emerging platform for cancer therapy. In L. P. Balogh (Ed.) *Nano-enabled medical applications* (pp. 61-91).

Pelgrift, R. Y. and Friedman, A. J. (2013). Nanotechnology as a therapeutic tool to combat microbial resistance. *Advanced Drug Delivery Reviews*, 65(13-14), 1803-1815. Doi: 10.1016/j.addr.2013.07.011.

Plessas, S., Nouska, C., Mantzourani, I., Kourkoutas, Y., Alexopoulos, A. and Bezirtzoglou, E. (2016). Microbiological exploration of different types of kefir grains. *Fermentation*, 3(1). Doi: 10.3390/fermentation3010001.

Rad, A. H., Mehrabany, E. V., Alipoor, B., Mehrabany, L. V. and Javadi, M. (2012). Do probiotics act more efficiently in foods than in supplements? *Nutrition*, 28(7-8), 733-736. Doi: 10.1016/j.nut.2012.01.012.

Rai, M., Kon, K., Ingle, A., Duran, N., Galdiero, S. and Galdiero, M. (2014). Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. *Applied Microbiology and biotechnology*, 98, 1951-1961. Doi: 10.1016/b978-0-12-415769-0.00004-x.

Rajoriya, P. (2017). Green synthesis of silver nanoparticles, their characterization and antimicrobial potential (PhD Thesis). Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, India. ID: 11PHBT108.

Ray, R. C., and Joshi, V. K. (2014). Fermented foods: Past, present and future. In R. C. Ray and M. Didier (Eds), *Microorganisms and Fermentation of Traditional Foods* (pp. 1-36). Boca Raton: CRC Press.

Richard, B. (2016). A whole new generation of milk kefir grains formed from freeze-dried starter cultures a fascinating insight into a hidden world (Doctoral dissertation, independent researcher), hal-01253250.

Rosa, D. D., Dias, M. M., Grześkowiak, Ł. M., Reis, S. A., Conceição, L. L. and Maria do Carmo, G. P. (2017). Milk kefir: Nutritional, microbiological and health benefits. *Nutrition Research Reviews*, 30(1), 82-96. Doi: 10.1017/ s0954422416000275.

Saarela, M., Mogensen, G., Fonden, R., Mättö, J. and Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology*, 84(3), 197-215. Doi: 10.1016/s0168-1656(00)00375-8.

Salavati-Niasari, M., Davar, F. and Mir, N. (2008). Synthesis and characterization of metallic copper nanoparticles via thermal decomposition. *Polyhedron*, 27(17), 3514-3518. Doi: 10.1016/j.poly.2008.08.020.

Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M. and Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, 61(1), 1600240. Doi: 10.1002/mnfr.201600240.

Savage, N. and Diallo, M. S. (2005). Nanomaterials and water purification: opportunities and challenges. *Journal of Nanoparticle Research*, 7, 331-342. Doi: 10.1007/s11051-005-7523-5.

Schoevers, A. (1999). Mass cultivation and activity of kefir grains (Doctoral dissertation), Stellenbosch: Stellenbosch University.

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K. and Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation*, 6(4), 106. Doi: 10.3390/fermentation6040106.

Silva, C. C., Verruck, S., Di Luccio, M., Pimentel, T. C., Silva, M. C., Esmerino, E. A. and da Cruz, A. G. (2023). Dairy probiotic products. In N. Chhikara, A. Panghal and G. Chaudhary (Eds), *Microbes in the Food Industry*, (pp. 139-215), Scrivener Publishing LLC. Doi: 10.1002/9781119776406.ch4.

Simova, E., Beshkova, D., Angelov, A., Hristozova, T. S., Frengova, G. and Spasov, Z. (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *Journal of Industrial Microbiology and Biotechnology*, 28, 1-6. Doi: 10.1038/sj/jim/7000186.

Soetan, K. O. and Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*, 3(9), 223-232. Doi: 10.5897/ajfs.9000293.

Soto, B. (2019). Fermentation Processes. ED-Tech Press, United Kingdom.

Steinkraus, K. H., Hui, Y. H., Meunier-Goddik, L., Hansen, A. S., Josephsen, J., Nip, W. K., ... and Toldra, F. (2004). Origin and history of food fermentations. *Food Science and Technology*, 1-8.

Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., ... and Hutkins, R. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety*, 19(1), 184-217. Doi: 10.1111/1541-4337.12520.

Tamang, J. P., Watanabe, K. and Holzapfel, W. H. (2016). Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology*, 7, 377. Doi: 10.3389/fmicb.2016.00377.

Tan, K. X., Chamundeswari, V. N. and Loo, S. C. J. (2020). Prospects of kefiran as a food-derived biopolymer for agri-food and biomedical applications. *RSC Advances*, 10(42), 25339-25351. Doi: 10.1039/d0ra02810j.

Telli, F. Ç., Yavuz, M., Denizaltı, S., Salman, Y. (2023). Study of radiotherapy properties and antimicrobial activity of glyconanoparticles (GNPs) generated from imidazolium salts. *ChemistrySelect,* **8**, e202203810. Doi: 10.1002/slct.202203810.

Terzi, G. (2007). Kefirin bileşimi ve beslenme açısından önemi. *Veteriner Hekimler Derneği Dergisi*, 78(1), 23-30.

Vanaja, M. and Annadurai, G. (2013). Coleus aromaticus leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. *Applied Nanoscience*, 3, 217-223. Doi: 10.1007/s13204-012-0121-9.

Wang, S. Y., Chen, K. N., Lo, Y. M., Chiang, M. L., Chen, H. C., Liu, J. R. and Chen, M. J. (2012). Investigation of microorganisms involved in biosynthesis of the kefir grain. *Food Microbiology*, 32(2), 274-285. Doi: 10.1016/j.fm.2012.07.001.

Winters, A. L., Lloyd, J. D., Jones, R. and Merry, R. J. (2002). Evaluation of a rapid method for estimating free amino acids in silages. *Animal Feed Science and Technology*, 99(1-4), 177-187. Doi: 10.1016/s0377-8401(02)00112-8.

Yaman, H. (2011). Kefir: A fermented milk product and production methods. *Kocatepe Veterinary Journal*, 4(1), 43-56.

Yasin, S., Liu, L. and Yao, J. M. (2013). Biosynthesis of silver nanoparticles by bamboo leaves extract and their antimicrobial activity. *Journal of Fiber Bioengineering & Informatics*, 6(1), 77-84. Doi: 10.3993/jfbi03201307.

Yavuz, M., Kaya, G. and Aytekin, Ç. (2014). Using *Ceriporiopsis subvermispora* CZ-3 laccase for indigo carmine decolourization and denim bleaching, *International Biodeterioration & Biodegradation*, 88, 199-205. Doi: 10.1016/j.ibiod.2013. 10.014.

Ying, S. X., Guan, Z. R., Ofoegbu, P. C., Clubb, P., Rico, C., He, F. and Hong, J. (2022) Green synthesis of nanoparticles: Current developments and limitations. *Environmental Technology & Innovation*, 26, 102336. Doi: 10.1016/ j.eti.2022.102336.

Yüksekdağ, Z. N. and Beyatlı, Y. (2003). Kefir mikroflorası ile laktik asit bakterilerinin metabolik, antimikrobiyal ve genetik özellikleri. *Orlab On-Line Mikrobiyoloji Dergisi*, 1(2), 49-69.

Zhang, X. F., Liu, Z. G., Shen, W. and Gurunathan, S. (2016). Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. *International Journal of Molecular Sciences*, 17(9), 1534. Doi: 10.3390/ijms17091534.

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DİCLE ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
TEZ BENZERLİK BİLDİRİMİ FORMU

Öğrencinin Adı, Soyadı	Rınas VALİ		
Öğrenci No	20803003		
Ana Bilim Dalı	Kimya		
Program Türü	Proje ☐	Yüksek Lisans ☒	Doktora ☐
Tez Danışmanı (Ünvanı, Adı, Soyadı)	Doç. Dr. Murat YAVUZ		
II. Tez Danışmanı (Ünvanı, Adı, Soyadı)	Dr. Öğr. Üyesi Israt JAHAN SIDDIK		
Tez Başlığı	The Nutrient Profiling of Cow and Goat Milk Kefir and Their Cytotoxicity Evaluation of Crude Proteins Evolved with Biogenic Silver Nanoparticles		
RAPOR BİLGİLERİ			
Raporlama Aşaması	Tez Savunma Sınavı Sonrası		
Sayfa Sayısı	71		
Raporlama Tarihi	27.06.2024		
Benzerlik Oranı (%)	14		

Yukarıda bilgileri verilen tez çalışmamın toplam 71 sayfalık kısmına ilişkin, 27/06/2024 tarihinde şahsım/tez danışmanım tarafından Turnitin isimli intihal tespit programından aşağıda belirtilen filtrelemeler uygulanarak alınmış olan intihal raporuna göre, tezimin benzerlik oranı %14 olarak tespit edilmiştir.

Uygulanan filtrelemeler:

☐ Başlangıç Bölümleri (Kabul ve Onay sayfası, Teşekkür sayfası, Özet/Abstract) hariç

☒ Kaynaklar hariç

☐ Alıntılar hariç/dâhil

☐ Diğer (Açıklayınız)

Tezimin benzerlik oranı, Dicle Üniversitesi Fen Bilimleri Enstitüsü İntihal Raporu Uygulama Esaslarında belirtilen üst sınır benzerlik oranını aşmamaktadır. Benzerlik oranım üst sınır benzerlik oranının altında olsa dahi aksinin tespit edilmesi durumunda her türlü yasal sorumluğu kabul ettiğimi ve hukuki sonuçlarına razı olduğumu bildirir,
gereğini arz ederim.

Öğrenci: Rınas VALİ
Tarih: 27.06.2024
İmza:

Danışman: Doç. Dr. Murat YAVUZ İmza:
Tarih: 27.06.2024

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Tarih: 27.06.2024