TOKAT GAZİOSMANPAŞA UNIVERSITY

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DETECTION OF BIOGENIC AMINES IN KEFIR

MASTER'S THESIS

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A thesis submitted to Tokat Gaziosmanpaşa University's graduate school of bioengineering.

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ETHICS OF CONTRACT

I declare that the Master thesis prepared according to the thesis writing guide of Tokat Gaziosmanpaşa University Graduate Education Institute, named as "DETECTION OF BIOGENIC AMINES IN KEFIR", prepared under supervision of Bilge Hilal ÇADIRCI EFELI is an original work in accordance with scientific ethical values and rules, and I will accept all kinds of legal sanctions if the opposite is determined.

21/07/2022

Mustafa Nooraldeen Salman

COMMITTEE ACCEPTANCE and APPROVAL

The thesis study titled "**Detection of Biogenic Amines In Kefir**" prepared by **Mustafa Nooraldeen Salman** was held on the date of the defense exam on 21-07-2022 and it has been accepted as a Master's Thesis by the Majority of Votes in Tokat Gaziosmanpasa University Graduate Education Institute Department of Bioengineering.

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ABSTRACT

DETECTION OF BIOGENIC AMINES IN KEFIR

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There are numerous causes of foodborne illness and food poisoning, including bacteria, viruses, parasites, mold and chemicals. Because biogenic amines (BA) are found in a wide variety of foods, including fish, meat, vegetables, wine and dairy products, they are followed by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). BAs formed due to microbial decarboxylation of amino acids can cause allergic reactions in consumers such as difficulty in breathing, itching, rash, vomiting, fever and hypertension. Among the BAs, which are low molecular weight organic bases with aliphatic, aromatic or heterocyclic structures, the most common in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine and agmatine.

In this study, the effect of adding starter cultures to help reduce the BA concentration that may occur during kefir making was investigated. In this context, *L. brevis*, *L. parabrevis* and *L. plantarum* strains were added during the addition of kefir granules to milk, and the content of tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine were analyzed by HPLC. On the other hand, the study also showed the effect of pasteurization on BA reduction. During the study, it was discovered that pasteurized milk increased the histamine rate by 882% and the tryptamine rate by 719%. It also reduced spermine by 67% and cadaverine by 44%. Supplementing milk with *L. brevis* increased the tyramine ratio by 631% and decreased spermine by 72%. *L. plantarum* increased tyramine by 31% and decreased histamine by 95%. *L. parabrevis* increased spermatozoa by 32% and decreased histamine by 84%, while combining the three bacterial strains resulted in a 121% increase in tyramine and a 93% decrease in hitamine.

Key words: Biogenic Amines; Kefır; Probiotic Bacteria

ÖZET

KEFİR'DE BİYOJENİK AMİNLERİN TESPİTİ

Mustafa Nooraldeen SALMAN

Yüksek lisans, Biyomühendislik anabilim dalı

Tez Danışmanı: PROF. DR. Bilge Hilal ÇADIRCI EFELİ

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Gıda kaynaklı hastalık ve gıda zehirlenmesinin bakteriler, virüsler, parazitler, küf ve kimyasallar gibi sayısız nedeni vardır. Biyojenik aminler (BA), balık, et, sebze, şarap ve süt ürünleri dahil olmak üzere çok çeşitli gıdalarda bulunduğundan, Gıda ve İlaç Dairesi (FDA) ve Avrupa Gıda Güvenliği Otoritesi (EFSA) tarafından takip edilmektedir. Amino asitlerin mikrobiyal dekarboksilasyonu nedeniyle oluşan BA'ler, tüketicilerde nefes almada zorluk, kaşıntı, kızarıklık, kusma, ateş ve hipertansiyon gibi alerjik reaksiyonlara neden olabilir. Alifatik, aromatik veya heterosiklik yapılara sahip düşük moleküler ağırlıklı organik bazlar olan BAlerden gıdalarda en yaygın bulunanları, histamin, tiramin, kadaverin, 2-feniletilamin, spermin, spermidin, putresin, triptamin ve agmatin BA'lardır.

Bu çalışmada kefir yapımı esnasında oluşabilecek BA konsantrasyonunun azaltılmasına yardımcı olmak için başlangıç (starter) kültürlerinin eklenmesinin etkisi araştırılmıştır. Bu bağlamda kefir granüllerinin süte eklenmesi esnasında *L. brevis, L. parabrevis ve L. plantarum* soyları da ilave edilmiş, oluşan BA’lerden, triptamin, 2-feniletilamin, putresin, kadaverin, histamin, tiramin, spermidin, spermin içeriği HPLC ile analiz edilmiştir. Öte yandan, çalışma pastörizasyonun BA azaltımı üzerindeki etkisini de göstermiştir. Çalışma sırasında pastörize sütün histamin oranını % 882, triptamin oranını % 719 artırdığı keşfedilmiştir. Aynı zamanda spermini %67, kadaverini ise %44 düşürmüştür. Sütü *L. brevis* ile desteklemek tiramin oranını %631 artırırken, spermini %72 oranında azaltmıştır. *L. plantarum,* tiramin oranını %31 artırırken, histamini %95 oranında azaltmıştır. *L. parabrevis* spermini %32 oranında artırırken, histamini %84 oranında azaltırken, üç bakteri suşu birleştirildiğinde, tiraminde %121 artış ve hitaminde %93 azalma elde edilmiştir.

Anahtar sözcükler: Biyojenik Aminler; Kefir; Probiyotik Bakteriler

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ABBREVIATIONS and SYMBOLS

Symbol Explanation

Aw: Water Activity

MRS: de Mann Rogosa Sharp Broth

µL: Micro Liter

BA: Biogenic Amine

BAs: Biogenic Amines

BC: Before Christ

°C: Celsius Degree

CO_2: Carbon Dioxide

DAD: Diode Array Detector

DNA: Deoxyribonucleic Acid

e.g.: Example

EC: European Commission

EFSA: European Food Safety Authority

EU: European Union

FDA: Food and Drug Administration

G: Gram

HPLC: High Performance Liquid Chromatography

LAB: Lactic Acid Bacteria

LDL: Low Density Lipoprotein

M: Molarity

MAO: Monoamine Oxidase

MAOI: Monoamine Oxidase Inhibitor

mg: Milligram

mg/L: Milligram per liter

Min: Minute

mL: Milliliter

mm: Millimeter

MPa: Megapascal

NaCl: Sodium Chloride

Nm: Nanometer

OD: Optimum Density

pH: Potential Hydrogen

Ppm: Part per Million

RPM: Revolutions per Minute

UV: Ultraviolet Light

1. INTRODUCTION

Biogenic amines (BA) are nitrogenous, organic substances that occur naturally in a variety of fermented foods, including cheese, sausage, fermented vegetables, wine, and fish. The primary reason for interest in BAs is their potential toxicity to people, as well as their use as indicators of food quality in the food industry. In most circumstances, intestinal amine oxidases are capable of detoxifying even trace amounts of BAs. It is possible that excessive BA ingestion will weaken or completely block the detoxifying capacity of the enzyme amine oxidase, resulting in serious health consequences. Numerous studies have established that histamine is the causative agent for the source of scombroid food poisoning, which can occur as a result Consumption of fish or fish products containing high levels of histamine greater than 1000 parts per million(Doeun et al., 2017). Due to its potential toxicity, the BA content of numerous foods has been extensively researched. BAs, such as tyramine and b-phenylethylamine, have been implicated in the development of hypertensive crises and dietary-induced migraine in certain patients. Histamine, another amine, has been involved in multiple instances of food poisoning. A dose of 8–40 mg, 40–100 mg, or more than 100 mg of histamine may cause mild, moderate, or severe poisoning, respectively (Parente et al., 2001). Dietary poisoning is a possibility, especially when aggravating variables such as monoamine oxidase inhibitor (MAOI) medicines, alcohol, gastrointestinal illnesses, and other food amines are present. Histaminic poisoning, hypertension generated by the combination of food and MAOI antidepressants, and food-related migraines are the most often reported adverse responses associated with the ingestion of foods high in BAs. (Hernández-Jover et al., 1997). Dairy products constitute a significant part of the diet in developed countries, as seen by the EU's 2010 cheese output of approximately 7.7 million tons (Eurostat, EU). In addition to fish and wine, dairy products, particularly cheese, can accumulate significant amounts of BA. Polyamines are the most abundant constituents of the raw material (milk). Tyramine, histamine, putrescine, and cadaverine are all discovered in the end product at varying amounts, as well as 2-phenylethylamine and tryptamine, which are present at lesser levels. BA buildup in food requires the presence of bacteria with decarboxylase activity, as well as the presence of amino acid substrates and environmental conditions that support their growth and the activity of this enzyme, in addition to the presence of bacteria with decarboxylase activity. Because of this, dairy products have been involved with foodborne intoxications due to contamination with preformed toxins of microbiological origin, including bacterial exotoxins, mold mycotoxins, and BAs, among other substances. The presence of these later hazardous chemicals at high quantities in many types of dairy products, particularly ripened cheeses, continues to be a source of concerns, leading to higher public knowledge of their actual or potential negative health effects. Furthermore, the fact that BAs are produced not only by microbial dairy contaminants of various origins, but also by the technological microbiota used in the fermentation and/or ripening of dairy products, such as lactic acid bacteria LAB yeasts, and molds, makes their control by conventional methods more difficult to accomplish (Benkerroum, 2016).

Kefir is a fermented food which is made from kefir grains, which are flat or cauliflower-like granules that are around the size of a pea or a walnut and are composed of a white to yellowish, hard and gelatinous substance, with the hard mass being the most common. Kefir is high in protein and free amino acids, both of which may be utilized by decarboxylase-positive microbes in the fermentation process. It is possible that a variety of BAs will be formed during the process. As a result, the current study sought to determine the amount of BAs present in various kefir samples.

2. LITERATURE REVIEW

2.1. Food Safety

Food is necessary for survival; therefore, food safety is a fundamental human right. Hundreds of millions of people around the world are at risk of consuming contaminated food. Every year, millions of people fall ill and hundreds of thousands of people die as a result of consuming contaminated food. As a result, safe food helps to save lives. Individual and public health are improved when food is prepared safely. Food safety practices and improvements help to boost the economic growth of the regions where they are implemented and improved. A safe food supply is essential for sound scientific research and equitable law enforcement. Because of technology advancements, new laws must be created to ensure a continuous supply of food products that are safe and wholesome for people's health and wellness.

Despite improvements in the standard of living, concerns about food safety and potential contaminants will continue to be a significant public health concern. Consumers demand high quality and safety in the products they buy. Food, in the form of energy and nutrients, is required to support life on the planet. In general, consumers rely on the government to verify that all food products are not only safe but that they are also sold as containing the ingredients that they claim to. Consider the case of a bottle of fresh juice branded as 100 percent natural, which must include exactly what is stated on the label.

Chemical, biological, personal hygiene, and environmental accidents are all examples of challenges and tragedies in food safety. It has long been known that food products can become polluted with industrial contaminants. This has been thoroughly recorded throughout history. There have been occasions where hundreds of thousands of people have been sickened or killed in Japan, Iraq, the United States, and other countries.

Food safety concerns have not decreased in the twenty-first century. Because of the rapidity and width of product distribution, local epidemics can quickly escalate into international crises. Foodborne disease outbreaks of a serious kind have happened on every continent. In China alone, the 2008 melamine poisoning of infant formula resulted in the hospitalization of 51,900 newborns and young children and the death of six children. Other potential risks, such as cancer or growth retardation in the future, have been mentioned, in addition to kidney injury. Unsafe food poses a threat to public health around the world. The young, the old, and the sick are particularly at risk of becoming victims. If food sources are insecure, the population shifts to less nutritious diets and eats more unsafe foods which are foods that have chemical, microbiological, and other hazards that can be harmful to human's health. Increased healthcare costs result, which in turn depletes the nation's financial resource.

2.2. Biogenic Amines

Basic nitrogenous chemicals known as biogenic amines BAs can be found in a wide variety of meals, including fish and fishery products, meat and animal products, cheeses, wine, beer, and other fermented foods and drinks. Biogenic amines have a complex chemical structure. They are able to materialize as a result of the decarboxylation of amino acids that takes place as a result of the interaction of bacterial microorganisms.

At the level of the cell, BAs have an effect on the synthesis of alkaloids, proteins, and hormones. Additionally, BAs have an effect on the replication of DNA as well as the permeability of cell membranes. In addition to this, they play a role in the control of the temperature of the body, the pressure in the blood vessels, and the activity in the brain. On the one hand, BAs are essential for the continued viability of cells and the efficient operation of a large number of metabolic activities. BAs can also have toxic or carcinogenic consequences due to the fact that they are precursors of cancer-causing compounds.

In terms of chemical composition, BAs are classified into three groups: aliphatic e.g. spermidine, spermine, cadaverine, or putrescine, aromatic e.g. phenylethylamine or tyramine, and heterocyclic e.g. tryptamine or histamine. Consuming a big quantity of foods that are rich in these amines may create serious adverse effects on one's health. Nevertheless, the toxicity of BAs in low levels is considered to be minor. The digestive tract efficiently metabolizes even low concentrations of BAs into less bioactive metabolites when the amount is high enough. The enzymes monoamine oxidase, diamine oxidase, and polyamine oxidase are the ones responsible for the body's process of detoxifying amines.

Because of this, contamination with any biogenic amines can result in food poisoning and even the development of other symptoms such as allergies. Histamine, putrescine, and tyramine are the BAs that are accountable for the most severe and widespread poisonings. The eventual harmful effect is determined by a number of different elements, including the dose of histamine that was ingested, the presence of additional amines, the level of activity of amino oxidases, and the individual's intestinal physiology. Symptoms of histamine poisoning include urticaria, a drop-in blood pressure, tachycardia, nausea, vomiting, diarrhea, headache, and convulsions, and they reveal themselves within a few hours after eating food that contains histamine. A headache, palpitations, a feeling of nausea and vomiting, an increase in blood pressure, profuse sweating, and stiffness in the neck are all symptoms that can be brought on by consuming an excessive amount of tyramine (Agata Durak-Dados et al.)

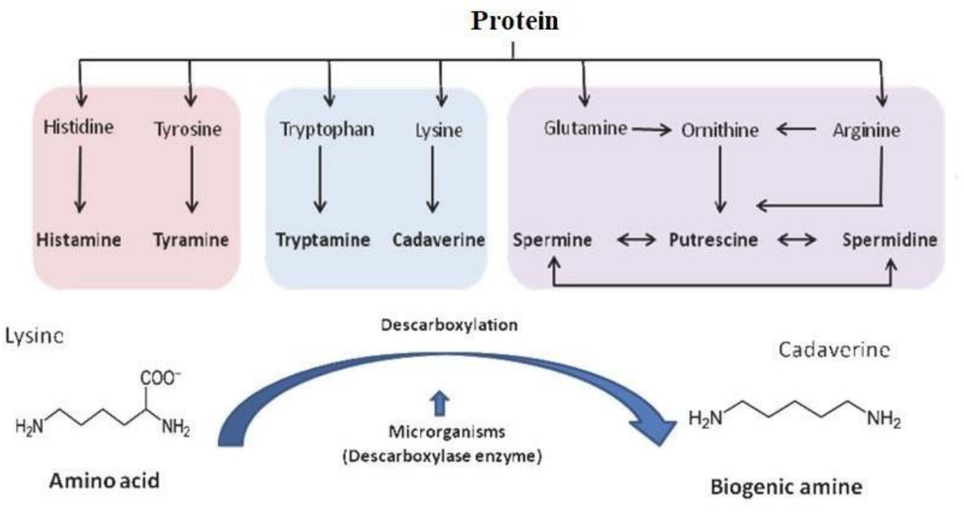

Figure 1. Decarboxylation of amino acids

2.2.1. Biogenic amines in food

There is a high concentration of BAs in diets that are high in protein across the board. In some circumstances, BAs improve the organoleptic properties of food, such as while maturing cheese. BAs are indications of the freshness and acceptability for ingestion of some foods, such as fish. The levels of BAs is also a marker of the sanitary quality of food. A high level of BAs indicates that hygiene criteria were not adhered to during the processing of raw materials, which results in the presence of a large number of bacteria that are not desired. Histamine and tyramine are believed to play a role in the majority of cases of BAs brought on by food poisoning. Histamine's vasoactive nature makes it capable of causing a flushing sensation, as well as abdominal cramping, headache, and high blood pressure.

The BAs that are most frequently produced from seafood include histamine, tyramine, cadaverine, and putrescine. Histidine has been discovered in its natural state in the muscles of numerous fish species that are members of the family *Scombridae*. Histamine may be produced by histidine decarboxylase bacteria at any point in time after harvesting the plant material. In most cases, the production of BAs in fish products is primarily influenced by factors such as the length of storage and the temperature. According to the findings of Klausen and Lund, the quantities of histamine, cadaverine, putrescine, and spermidine in herring and mackerel held at a temperature of 10°C are two to twenty times higher than those in samples stored at a temperature of 2°C for the same amount of time. Furthermore, (Rogers et al., 1997). found that BAs could accumulate at any time, including during capture or handling of the fish. Histamine was found in high concentrations in a variety of fish products, including fish sauce, fish paste, shrimp paste, fermented anchovies, dried fish, and lightly cured horse mackerel. The FDA established a limit of 50 ppm for the amount of histamine that can be present in fish products, while the EC established a limit of 200 ppm.

In the fermentation of sausage, starter cultures such as LAB, coagulase negative cocci, yeast, and mold have the potential to generate BAs. Tyramine and putrescine are the BAs that are found in dry sausages in the greatest quantities. 48 percent of the LAB and 13 percent of the staphylococci that were isolated from sausages that spontaneously fermented were able to decarboxylate amino acids, (Latorre-Moratalla et al., 2012). The levels of BAs that are present in sausages might vary greatly from product to product and manufacturer to manufacturer. The biological quality of the raw materials, the components, and the additions, as well as the diameter of the sausage and the method of ripening, are the main features involved with such variations.

The conditions that are created during the production of cheese are just suited for the development of BAs. During the process of manufacturing cheese, the amino acid decarboxylase activities of bacteria present in the raw material could form BAs. During the ripening process of cheese production, in which casein is degraded and free amino might be turned into BAs by microbial decarboxylases, there is a possibility that BAs will form in the cheese. The use of starter culture and enzymes, the treatment of milk, pH, the amount of proteolysis, temperature and the length of time it is allowed to ripen, the presence of oxygen, water activity AW, relative humidity, and the availability of microorganisms are all possible factors that could have contributed to the formation of BAs. There is a possibility that cheese could have more than 2000 ppm of BAs at its highest level.

Since soy sauce is made by hydrolyzing soybean protein, it naturally includes significant concentrations of BAs such histamine and tyramine. According to (J.-H. Kim et al., 2003) findings, fermented soybean products from Korea include not just tyramine but also histamine, putrescine, cadaverine, spermine, spermidine, b-phenylethylamine, agmatine, and tryptamine as well.

2.3. Microorganisms with Biogenic Amines Producing Ability

It has been determined that several different microbial groupings are capable of producing decarboxylase. It has been demonstrated that some types of microbes, such as *Lactobacilli*, *Pseudomonads*, *Enterobacteriaceae*, and *Enterococci*, which are found in meat products have the ability to produce decarboxylase. *Staphylococcus xylosus*, which was isolated from salted semi-preserved anchovies, *Morganella morganii*, *Hafnia alvei*, and *Klebsiella pneumoniae*, which were all isolated from tuna, and *Aeromonas hydrophila*, which was isolated from mackerel, have all been verified as histamine producers. Other bacterial species that have been isolated from seafood include *Aeromonas hydrophila*, are capable of producing BA. *Streptococcus thermophilus* is responsible for the production of BAs, including tyramine and histamine, in yogurt. A number of researches have shown that fermented soybean products contain bacteria that produce histamine. These investigations have identified the bacteria as *Staphylococcus pasteuri*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis.*

2.3.1 The factors that contribute to the production of biogenic amines in food

In addition to the presence of particular bacterial strains and the quality of the raw material, there are many other factors that can influence the formation of BAs. Some of these factors include temperature, pH, the availability of substrates (amino acids), Aw, the amount of sodium chloride present, and redox potential.

2.3.2. Temperature

It has been demonstrated that temperature plays an important role in the production of BAs in food (Suzzi & Gardini, 2003). It has been proven by a number of authors that the concentration of BAs rises both with increasing temperature and with increasing time spent in storage (Halász et al., 1994), (Karovičová, J. 2005). Although there was no correlation between the temperature of the anchovy and the amount of tyramine that was generated in the fish (Santos-Buelga et al., 1986) It has been demonstrated that keeping sardines at a low temperature during storage is insufficient to prevent the development of BAs like histamine (Ababouch et al., 1991).

2.3.3. pH

Because the activity of amino acid decarboxylases is higher in acidic conditions, specifically at pH 4.0–5.5, acidity (pH) is another highly important element that influences the synthesis of amines. This is due to the fact that amino acid decarboxylases seem to be more stable in acidic environments (Teodorovic et al., 1994). At a pH of approximately 4.0, *Klebsiellapneumoniae* that was isolated from tuna had the highest level of activity in converting histidine to histamine; however, at a pH in the range of 6.0, this activity reduced to 70 percent (Baranowski et al., 1985). In mackerel with a lower pH level, researchers discovered greater quantities of the histamine (Santos-Buelga et al., 1986). The level of acidity found in cheeses with a pH of 5.0 is ideal for the production of tyramine (Diaz-cinco et al., 1992). In the case of meat products, the weaker generation of histamine and putrescine was the result of the addition of glucono-deltalactone to sausages. This substance lowers the pH, and as a result, the number of *enterococci* and coliform bacteria in the sausages decreased (Maijala et al., 1993). According to (Bover-

Cid et al., 2006), a rapid and sharp reduction in the pH of sausages inhibits the growth of amine-positive microbes, especially Enterobacteriaceae, and protects the meat product from the generation of BAs.

2.3.4. Water activity (Aw) and NaCl content

The difference in the amount of water present as well as the salt-to-water ratio that occurs throughout the fermentation process and subsequent storage of fermented sausages has a significant impact on the multiplication of microorganisms. When the level of salt in the medium was increased from 0 percent to 6 percent, the rate of amines synthesis by *L. delbrueckii subsp. bulgaricus* was drastically cut down (Chancier et al., 1989). (Chin & Koehler, 1986) demonstrated that the generation of histamine can be inhibited by using doses of NaCl ranging from 3.5 percent to 5.5 percent. The synthesis of BAs is significantly impacted by the presence of certain chemicals that are utilized in the creation of raw maturing meat products. In research that was carried out (Gardini et al., 2001), it was shown that sodium chloride has an effect that is inhibitory on the synthesis of BAs. After 72 hours of incubation, a high concentration of NaCl was able to successfully reduce the amine synthesis in the medium (Gardini et al., 2001). It was demonstrated by (Santos', 1996) that the addition of salt to mackerel that had been preserved at 25 degrees Celsius will suppress the formation of histamine, and that the inhibitory impact will be proportionate to the increase in salt content in the fish brine. (Gök et al., 2008) and (Kaban, 2013) found that the ripening period had a considerable effect on the Aw of fermented meat. As an illustration of this, the Aw values of fermented beef matured for 2 months were significantly lower than those for fermented beef aged for 21 days. The Aw of samples that were aged for 2 months had a mean value of 0.936, while the Aw of samples that were aged for 36 months had a mean value of 0.906. Aw significantly declined with age. According to (Wójciak & Solska, 2016) research, the reduction in Aw in fermented meat is caused by the increasing proteolysis of proteins, which results in the formation of free amino acids.

2.3.5. Biogenic amine reduction (Packaging)

According to (Özogul & Özogul, 2006) research, the presence of carbon dioxide in modified atmosphere packaging has a crucial role in extending the shelf life of food by limiting the microbiological growth of histamine-forming bacteria. According to the findings of (Yassoralipour et al., 2012), the presence of CO_2 in MAP at concentrations ranging from 75 to 100 percent can dramatically lower the amount of BA found in barramundi fillets. In addition, the use of vacuum packing that was kept at a temperature of 4 degrees Celsius has demonstrated a reduced amount of BAs when compared to packages that were not preserved in a vacuum but instead were kept open to the air (Özogul & Özogul, 2006). When compared to air packaging, the packaging that the Seer fish is kept in when oxygen is not present or when oxygen is removed by oxygen scavengers can result in reduced amounts of BAs, and the shelf life is increased (Mohan et al., 2009). When compared to air packaging, the chicken that had been precooked and stored in MAP at 4 degrees Celsius for up to 23 days showed a reduction in the development of tyramine and putrescine (Patsias et al., 2006). After being kept at a temperature of 2 degrees Celsius for 16 days, (Özogul & Özogul, 2006) found that the amount of histamine in MAP was 197 parts per million, whereas the level was 284 parts per million in vacuum packaging, and 396 parts per million in air packaging.

2.3.6. Additives

It has been observed that the use of preservatives and additives in food can suppress the synthesis of BAs. Mackerel that has been held at 25 degrees Celsius for ten days with the addition of Dsorbitol, succinic acid, malic acid, and ascorbic acid can block the activity of decarboxylase and prevent the generation of histamine (Shalaby, 1996). Citric acid at a concentration of one percent can reduce the amount of BAs produced by fermentation in pickled cabbage, according to research conducted by (Yücel & Üren, 2007). Potassium sorbate and ascorbic acid are two more substances that have the potential to significantly cut down on the accumulation of BAs in sausages (Bozkurt & Erkmen, 2004). In addition to this, (González-Fernández et al., 2003a) found that the concentration of BA in fermented sausage fell dramatically when the starter culture was chosen to contain either 0.5 or 1 percent sugar. According to the findings of (Mah et al., 2009), the incorporation of 5 percent garlic extract into fermented anchovy resulted in a reduction of around 8.7 percent of BA. According to research conducted by (Roseiro et al., 2006), adding 6 percent salt to sausages has the potential to lower levels of tyramine, putrescine, cadaverine, and phenylethylamine.

2.3.7. Hydrostatic pressure

The population of bacteria and the amount of BA that accumulates in raw material and finished food products is lowered when there is a high hydrostatic pressure (Naila et al., 2010), (Lanciotti et al., 2007) have demonstrated that cheese produced from milk that has been subjected to high pressure treatment has a level of BA that is approximately 2.5 times lower than that of raw milk. The growth of enterobacteria can be stopped by subjecting meat to pressure of 200 MPa for ten minutes at a temperature of 17 °C (Latorre-Moratalla et al., 2007). Likewise, it was found by (Ruiz-Capillas et al., 2007) that the development of putrescine, cadaverine, and tyramine in frankfurter packed with vacuum and hydrostatic pressure treatment is greatly suppressed. In addition, research conducted by (Halász et al., 1994) and colleagues has demonstrated that a high hydrostatic pressure treatment provided to sausage can prevent the creation of cadaverine and putrescine, while simultaneously encouraging the development of spermine and tyramine. In most cases, the effectiveness of BA reduction is directly proportional to the amount of pressure that is applied (Calzada et al., 2013).

2.3.8. Irradiation

Irradiation can be used for several purposes in the processing of food, including the decrease of BA levels. It has been found that subjecting blue jack mackerel to gamma irradiation lowers the levels of histamine, tyramine, cadaverine, and putrescine in the fish (Mendes et al., 2000). (J. H. Kim et al., 2005) found that the levels of BAs in irradiated sausages were lower compared to those in non-irradiated sausages, and that the levels of BAs in ripened sausages that had been irradiated declined (Rabie et al., 2010). In addition, poultry, beef, and pork that have been subjected to irradiation have demonstrated a significant decrease in the levels of BA content when compared to untreated control samples (Min et al., 2007). When compared to the amount of BA found in the control group, the quantity of BA found in blue cheese that has been irradiated with gamma during the storage process is significantly reduced (Rabie, Siliha, et al., 2011)

2.3.9. Smoking

When compared to the levels found in non-smoked samples, the levels of histamine, tyramine, cadaverine, putrescine, spermine, and spermidine found in smoked samples were shown to have been successfully reduced by smoking (Křížek et al., 2011a). The smoking process can result in items with aseptic qualities, which lowers the amount of BA in the substance by preventing the growth of bacteria that break down amines into carboxylates (Martuscelli et al., 2009). In comparison to traditionally fermented sausages, smoked cooked sausage has significantly lower levels of BAs (Rabie, Elsaidy, et al., 2011).

2.3.10. Pasteurization

It has been hypothesized by (Gennaro et al., 2003) that the process of pasteurization can also prevent the development of BAs in processed food products. There was a noticeable drop in BA concentration after subjecting milk to higher temperatures and pasteurization prior to cheese production (Gennaro et al., 2003). In addition to lowering BA concentrations, the process of pasteurization can also significantly reduce the number of BA-producing strains. It has been demonstrated that pasteurization of carp has a beneficial influence on sensory quality, the extension of shelf life, and the reduction of BA (Křížek et al., 2011).

2.3.11. Starter culture

Starter cultures are employed in the vast majority of industrial dairy fermentations to ensure that the end products meet the required quality standards. There is a possibility that certain LAB, which are typically used as starter cultures, contain specific amino acid decarboxylase activities. As a result, these LAB have the capacity to produce BA, which may then become deposited in dairy products. Lactococci, lactobacilli, and streptococci are all members of this category of bacteria.

By utilizing well-characterized starter cultures and removing any strains that have the unfavorable potential to produce BA, the danger of integrating strains that produce BA can be reduced, hence minimising the impact of introducing BA-producing bacteria. Several strains of Streptococcus thermophilus (Ladero et al., 2010), (la Gioia et al., 2011), Lactobacillus brevis and Lactobacillus curvatus (Linares et al., 2011), and Lactococcus lactis (Linares et al., 2011) acquired from dairy sources have been proven to be producers of histamine, tyamine and putrescine.

There is also the possibility of using adjunct cultures that contain bacteria that are able to degrade BA (Naila et al., 2010). This strategy is similar to the one that has been proposed for the fermentation of fish sauce, sausage, or wine (Gardini et al., 2002; García-Ruiz et al., 2011), In the study of cheeses, Brevibacterium linens were used to catabolize histamine and tyramine during the production of Munster cheese, this was one of the methods detailed in the study of (Leuschner & Hammes, 1998)

As a consequence of this, the synthesis of BA might take place during the processing and storage of food as a result of the actions of bacteria that are capable of decarboxylating amino acids. The production of dairy products is not a sterile procedure, and it is likely that BA producers will make their way into the food chain as non-starter LAB that are native to the raw material (Linares et al., 2012)

The development of BA can be affected differently depending on the starter culture used. In addition to strains that are capable of generating BA, there are also strains that have negative decarboxylase activity or enzymes that have the potential to oxidize BAs found in food (la Gioia et al., 2011). In several products, such as sausage (Gardini et al., 2002), wine (García-Ruiz et al., 2011a), fish sauce (Zaman et al., 2011), and cheese fermentation (Nieto-Arribas et al., 2009), the use of a specified starting culture has prevented the formation of BAs. *L. sakei* has been shown to lower the levels of tyramine, cadaverine, and putrescine in chorizo dry sausages, as reported by (González-Fernández et al., 2003), (Ayhan et al., 1999). The digestive tract efficiently metabolizes even low concentrations of BAs into less bioactive metabolites when the amount is high enough. The enzymes (MAO), diamine oxidase, and polyamine oxidase are the ones responsible for the body's process of detoxifying amines.

Because of this, contamination with any BAs can result in food poisoning and even the development of other symptoms such as allergies. Histamine, putrescine, and tyramine are the BAs that are accountable for the most severe and widespread poisonings. The eventual harmful effect is determined by a number of different elements, including the dose of histamine that was ingested, the presence of additional amines, the level of activity of amino oxidases, and the individual's intestinal physiology. Symptoms of histamine poisoning include urticaria, a drop-in blood pressure, tachycardia, nausea, vomiting, diarrhea, headache, and convulsions, and they reveal themselves within a few hours after eating food that contains histamine. A headache, palpitations, a feeling of nausea and vomiting, an increase in blood pressure, profuse sweating, and stiffness in the neck are all symptoms that can be brought on by consuming an excessive amount of tyramine (Durak-Dados et al., 2020).

The utilization of starter cultures has been supported by a number of studies as a method to reduce the development of BAs during the fermentation of meat. However, not all investigations have been successful in demonstrating the beneficial influence of the starter on the formation of amines during the fermentation process of making fermented sausage (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001). The LAB is the most important type of organisms that can be used as starters. In order to speed up the process of fermentation and ripening, starter cultures are introduced to the mixture of meat. This helps to optimize and homogenize the organoleptic qualities as well as the sanitary quality of the final goods, and it also reduces the amount of time required.

Table 2.1. Effect of starter cultures on reduction of biogenic amines in some meat products.

Product	**Starter culture**	**% of reduction**	**Reference**
French fermented sausage	*L. sakei* + *S. succinus* + *S. equorum*	87% of TY, 35% of CA, and 38% of PU	Talon et al. (2008)
Salami	*L. plantarum* VLT 73 + Kokuriavarians MIAL	26% of CA and 27% of PU. No effect on TY and HI	Coloretti et al. (2008)
Greek fermented sausage	*L. sakei* 4413	13% of TY and 72% of PU. No effects on CA	Baka et al. (2011)

Finnish fermented sausage	*P. pentosaceus* + *S. carnosus* (Rudolf Müller and Co, Germany) *	41% of TY, 28% of HI, and 86% of CA. No effect on PU	Maijala et al. (1995)
Turkish Soudjoucks	*L. sakei* + *P. pentosaceous* + *S. carnosus* + *S. xylosus* (Bioback-K, Wiberg, Germany)	100% of PU. No effect on TY	Ayhan et al. (1999)
Turkish fermented sausage	*L. sakei* + *S. xylosus* B-FM (CHR-HANSEN, Germany) *L. plantarum* + S. carnosus TD-66 (CHR-HANSEN, Germany *L. curvatus* + *S. carnosus* + S. xylosus RM-10 (CHR-HANSEN, Germany)	54% of TY and 62% of PU 52% of TY and 61% of PU 55% of TY and 63% of PU	Gücükoglu and Küplülü (2010)
Thai fermented sausage	*L. plantarum* BCC9546 (BIOTEC, Thailand) *L. plantarum* BCC9546 + *L. brevis* BCC26756 (BIOTEC, Thailand)	94% of TY, 75% of CA, and 97% of PU 37% of TY, 75% of CA, and 99% of PU	Tosukhowong et al. (2011)

2.3.12. Lactic acid bacteria (LAB)

Foodborne pathogens pose a significant risk to the health of consumers since they are able to trigger a variety of diseases that are transmitted through ingesting contaminated food. Since they play an essential role in preventing bacterial growth and reducing the formation of BAs, LAB have received a considerable attention in the food biotechnology sector in order to eliminate the potential risk posed by those pathogens. This is because LAB have been shown to reduce the formation of BAs. The foodborne illnesses diarrhea, vomiting, and abdominal discomfort, etc. caused by those microbial pathogens are due to a number of different reasons. One of these reasons is connected to the decarboxylation of available amino acids, which results in the generation of BAs. The production of BAs in foods by pathogens can be the cause of a decline in both the nutritional and sensorial characteristics of the foods. The synthesis of BAs can also have toxicological effects and result in a variety of various forms of poisoning. It is important to keep an eye on the expansion of FBP and their BAs production in order to prevent or minimize the occurrence of problems like these. LAB has the ability to improve food safety by reducing the rate at which foods go rotten and increasing the length of time they may be stored. LABs are exploited by the food industry to make fermented products with their antibacterial actions as bio-preservative agents to produce fermented products with an extended storage term while maintaining their nutritional and gastronomic properties. LABs are responsible for the fermentation process, which adds flavor to meals, but they also produce a variety of antimicrobial compounds, such as organic acids, hydrogen peroxide, and bacteriocins.

LAB are gram positive creatures that require a complex growth medium (include amino acids, vitamins, nucleic acids, and minerals), (Mohankumar & Murugalatha, 2011). They are able to function well in anaerobic as well as aerobic environments (Pessione, 2012a). LAB are a large group that contain a significant number of bacterial genera, the most well-known of which are *lactobacilli*, which can be found in milk, meat, vegetables, and cereal; *lactococci*, which can be found in milk; *streptococci*, which can also be found in milk; *leuconostoc*, which can be found in vegetables and meat; and *pediococci*, which can be found in vegetables and meat (Klaenhammer et al., 2002). According to (König et al., 2017), LAB can be found in surroundings that have a plentiful supply of nutrients, such as fermented meat and fish, sourdough, pickled vegetables, and dairy products. They are also present in the genitourinary tract and gastrointestinal tract of humans and animals, where they are a part of the healthy microbiota of the gut and the gastrointestinal system. In addition to this, we classify them as probiotics due to the fact that they contribute to the health of the host by inhibiting the growth of pathogens that are found in the intestinal microflora and enhancing the mucosal immunity (Klaenhammer et al., 2002). Except for a limited few species, such as *Enterococcus*, which are known to be harmful to humans and animals, LAB are non-pathogenic organisms and are classed as GRAS (generally regarded as safe) (Mayo et al., 2010) (Pessione, 2012). LAB is responsible for the production of a wide range of antimicrobial substances. Some examples of these substances include lactic acid, acetic acid, ammonia, bacteriocins, ethanol, reuterin, hydrogen peroxide, and diacetyl. These substances are able to prevent the progression of foodborne diseases by inhibiting the growth of food spoilage and pathogenic organisms (Zamfir et al., 2000), (Mahrous et al., 2013), (Abo-Amer, 2010).

2.3.13. Biogenic amines production by foodborne bacterial pathogens and LAB

The ability to create BAs is shared by a wide range of bacteria. Gram-negative bacteria are prevalent in aquatic environments and arsse characteristic of the flora of fish. These bacteria belong to the genera *Pseudomonas, Moraxella, Acinetobacter, Shewanella, Flavobacterium, Vibrionaceae,* and *Aeromonadaceae*. Gram-negative bacteria are the most common type of microorganisms found in fish; however, Gram-positive bacteria such *Bacillus, Micrococcus, Clostridium, Lactobacillus, and coryneforms* have also been found in fish at varied quantities (Visciano et al., 2012). In a study conducted by (Gokdogan et al., 2012), the BAs generation of eight prevalent Gram-negative and Gram-positive foodborne pathogens was investigated in histidine decarboxylase broth. All of the bacteria that were tested, including *S. aureus, E. coli, K. pneumoniae, E. faecalis, P. aeruginosa, L. monocytogenes, A. hydrophila,* and *Salmonella parathypi* A, were able to produce a variety of BAs, including putrescine, cadaverine, spermidine, tyramine. The major histamine producers were *Hafnia alvei, Morganella morganii, Klebsiellapneumonia* in histidine decarboxylase broth.

Bacteria that create lactic acid, often known as LAB, are the primary source of BA in fermented food products. The presence of histamine in wine may be traced back to three different types of bacteria: *Oenococcusoeni, L. hilgardii,* and *Pediococcus parvulus*. In cheese and other fermented meat products, LAB strains of *Enterococcus* and *Lactobacillus* are the primary producers of tyramine, which is the BA with the highest concentration and the highest frequency of detection. Strains of the bacteria *Enterococcus faecalis, Enterococcus faecium,* and *Enterococcus durans* are regarded as being among the most potent tyramine makers. At the end of the cheese ripening process, histamine was found, and cadaverine has been reported as the most common BA in Brazilian cheeses (Russo et al., 2010). Putrescine can be produced by specific LAB strains, including *L. brevis* and *L. hilgardii,* which were recovered from wine; *L. curvatus, E. faecalis, L. fermentum,* and *L. paracasei,* which were isolated from cheese, beef, and sausage (Ladero et al., 2010) (Wunderlichová et al., 2014).

The use of LAB as a bio-preservative agent has the potential to assist restrict the growth of FBP, which would lead to a reduction in the synthesis of BAs by FBP. Because some of the LAB is able to increase the formation of BAs, particularly when combined with other FBPs, its important to choose LAB strains with care in order to prevent the growth of FBP and their BAs formation.

There is a potential for BA to accumulate in dairy products, particularly cheese, with concentrations ranging from just traces to more than 1000 mg Kg-1. Because of the toxicity of these substances, there was a widespread consensus that it should be prohibited for these substances to accumulate in food (Linares et al., 2011).

Table 2.2. The biogenic amine content of some food products.

Products Mg/kg	His	Tyr	Put	Cad	Spm	Smd	Phe	Tryp	References
Parmesan (cheese)	40.64	3.75	0.83	1.98	0.82	0.83	0.2	0.07	(Mayr & Schieberle, 2012)
Semi-hard Italian (cheese)	28.55	29.89	75.87	15.56	4.46	7.71	9.51	11.85	(Innocente et al.,2007)
Yogurt	ND	ND	0.03	0.38	0.17	0.47	0.001	ND	(Mayr & Schieberle, 2012)
Soy sauce	307	402.5	14.4	ND			52.1		Guidi and Gloria (2012)
Dry sausages	<1–200	3–320	<1.850	<1–790	19–48	<1–14	>1-48	<10–91	Eerola et al. 1997
Canned Tuna	0.33	0.06	0.35	0.07	11.25	7.6	0.03	0.02	(Mayr & Schieberle, 2012)

2.4. Kefir

Kefir is fermented milk made from grains that contains a special and complicated variety of bacteria and yeasts that live together in a symbiotic partnership. Kefir is produced by fermenting milk. The nutritional profile of kefir can change depending on the type of milk that is used, the microbial make-up of the grains that are fermented into it, the duration and temperature of the fermentation process, and the conditions under which it is stored. Kefir is thought to have originated in either the Caucasus or Tibet. Kefir has recently attracted more attention from the scientific world as a result of the multiple positive impacts it has on one's health. Kefir has been reported historically as a probiotic drink with great potential in health promotion. Currently, several scientific studies have supported the health benefits of kefir, as reported historically as a safe and inexpensive food that can be easily produced at home (Rosa et al., 2017a).

2.4.1. Perspective on the past

Kefir can be traced back to the Caucasus, Tibetan, or Mongolian mountains. In the mountains of the Caucasus, the grains that are used to make kefir were already being traditionally passed down from one generation to the next before the year 2000 BC. These grains were regarded as a source of family wealth at the time. The word "well-being" or "living well" comes from the Slavic language, and the word "kefir" comes from that language. The drink gives its consumers a general feeling of health and well-being, which is where the word "keif" comes from. (Farnworth E, 2005)

Kefir is distinct from other fermented products because it is made from kefir grains, which are made up of a specific and complex mixture of lactic acid- and acetic acid-producing bacteria, as well as lactose-fermenting and non-fermenting yeast, all of which live in a symbiotic association with one another. Kefir is produced from these grains. Other fermented products are made from bacteria alone (Rementeria et al., 2006).

2.4.2. Microbiological characteristics of kefir

Kefir is made up of yeasts, LAB, and acetic acid bacteria in its particle structure. (Karabıyıklı, 2016). The particles that are utilized in the production of kefir consist of an inert polysaccharide/protein matrix and a glucose-related structure. The matrix hosts bacteria, and the glucose-related structure hosts small, irregularly shaped yeasts that resemble cauliflower or popcorn. At least 25 percent of kefir particles are made up of kefiran, which is made up of between 30 and 34 percent casein, 45 to 60 percent saccharide, 3 to 4 percent fat, both living and dead bacteria, and equal amounts of glucose and galactose (Karatepe et al., 2012).

Kefir particles are made up of yeast (including *Kluyveromyces*, *Candida*, *Torulopsis*, and *Saccharomyces* spp.), lactobacilli (including *L. brevis, L. acidophilus, L. casei, and L. delbruckii*), streptococci (including *Streptococcus salivarus*), lactococci (including *L. lactis* ssp. *thermophilus, Leuconostoc mesenteroides* and *L. cremoris*). The LAB species capable of homofermentation are the most significant contributors to the bacterial flora. Recent research has identified a novel strain of lactobacillus called *L. kefiranofaciens*. These bacteria are responsible for producing the polysaccharide coating that coats the outside of kefir particles (Karatepe et al., 2012).

Kefir is made by adding LAB from kefir to milk in order to help start the fermentation process. These microbes convert lactose into lactic acid, which in turn lowers the pH level of the environment. LAB is responsible for many of the primary qualities that are associated with fermented milk, such as its flavor and its ability to last for a longer period of time. Genera belonging to the *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Lactococcus* families are utilized in the process of making fermented milk (Karatepe et al., 2012).

Yeasts play a significant part in the fermentation process of kefir because of the ethanol and carbon dioxide that they produce. Yeasts play a crucial role in the symbiosis that occurs between the microorganisms that live on kefir particles. This helps to improve the kefir's authentic flavor and scent. In general, kefir particles include both lactose-fermented yeasts (such as *Kluyveromyces lactis*, *Kluyveromyces marxianus*, and Torula kefir), as well as nonlactose-fermented yeasts (*Saccharomyces cerevisiae*). Yeasts are one of the most significant factors that contribute to the deterioration of dairy products. Kefir and kumis both undergo fermentation, and yeasts play a supporting role in this process (Karatepe et al., 2012)

2.4.3. Nutritional composition

Kefir is filled of not only the fundamental components of a healthy diet but also substantial quantities of amino acids, protein, phosphorus, and calcium (Ahmed et al., 2013). The following is a list of the chemical components that make up kefir: 90 percent water, 6 percent carbohydrates, 3.5 percent fat, 3 percent protein, and 0.7 percent ash are the components of this substance. In comparison to milk that has not been fermented, kefir has higher concentrations of the aminoacids serine, lysine, alanine, threonine, tryptophan, valine, methionine, phenylalanine, and isoleucine (Rosa et al., 2017).

The quality of the milk that is used, the bacteria that are present in the kefir particles, and the preparation procedure all have an effect on the vitamin content of kefir. Kefir is rich in vitamins. According to (Yazar et al., 2015), kefir includes thiamine, riboflavin, as well as vitamins C, A, and K, and carotenoids. During the fermentation process, the production of certain chemicals such pyridoxine, vitamin B12, folic acid, and biotin increases, but the quantities of riboflavin and thiamine decrease (Esmek & Güzeler, 2015).

Magnesium, calcium, and phosphorus are some of the minerals that can be found in adequate amounts in kefir. In addition, it has trace amounts of a variety of minerals, including zinc, copper, iron, manganese, and cobalt (Rosa et al., 2017). The type of milk that is used in the fermenting process has an effect on the amount of fat that is included in kefir (Rosa et al., 2017).

The production of lactic acid, carbon dioxide, and ethanol are the primary byproducts of the lactic fermentation process. In addition, aldehydes, isoamyl alcohol, and the acids formic, succinic, and propionic can be found in kefir. The ethanol concentration in kefir ranges anywhere from 0.5 percent to 2 percent, and the pH level can be anywhere from 4.2 to 4.6

Kefir is loaded with naturally occurring amines like tyramine, cadaverine, and putrescine that are created by the LAB in the product (Arslan, 2014). Kefir's flavor and mouthfeel are both diminished when there is a high concentration of the BAs. According to (Rosa et al., 2017)., the presence of these BAs in kefir is regarded as a key indicator of both the product's quality and its overall acceptability.

2.4.4. Manufacturing of kefir

There are a few different approaches that can be utilized to make kefir, but the most common and favored approaches are the traditional and industrial approaches. Kefir can be made from a variety of milks, including goat, cow, sheep, rice, and soy milk, among others (Otles & Cagindi, 2003).

The traditional method for making kefir involves adding kefir grains or particles to milk. The unpasteurized milk is heated or boiled. After cooling the milk kefir particles are added at a concentration ranging from 2 to 10 percent (often 5 percent), the mixture is allowed to ferment. Using a strainer, the kefir grains are extracted from the milk after 18–24 hours have passed. These kefir grains are kept at a temperature of 4 degrees Celsius till the subsequent inoculation (Otles & Cagindi, 2003).

The production of kefir on an industrial scale often makes use of a variety of processes; however, the fundamental principles remain consistent. Controls both microbiological and chemical are performed on the milk before to its utilization in the manufacturing of industrial kefir. After being homogenized, it is heated at temperatures between 90 and 95 degrees Celsius with an 8 percent dry content for either 5 or 10 minutes. After that, the temperature is lowered to between 18 and 24 degrees Celsius, and it is allowed to ferment with kefir particles at a concentration of between 2 and 8 percent. After the fermentation process, which lasts for 18–24 hours, the kefir is bottled, let to mature at temperatures ranging from 3–10 degrees Celsius, and then stored at 4 degrees Celsius (Otles & Cagindi, 2003). There are a few different ways that kefir particles can be stored. Among these are dehydration by air, lyophilization, storage in the freezer, and refrigeration for longer-term preservation. The processes of air drying and lyophilization allow the probiotic activity of traditional kefir particles to be preserved for a period of 12–18 months. If kept at a temperature of 20 degrees Celsius, kefir grains can keep their microbial activity for up to seven or eight months. However, after around ten days, there is a noticeable decline in their activity (Turan and Ilter, 2007).

Dried particles are able to maintain their activity for a period of 12–18 months. Washing the product too much or using it incorrectly might alter both the quality of the final product and the microflora of the particles. The particles of kefir can be frozen for long-term preservation, or they can be dried for 36–48 hours at ambient temperature, stored in a cold and dry place, and used later (Altay et al., 2013)

2.5. Health Effects of Kefir

Kefir has extensive physiological benefits on health, including those that are preventative, restorative, and many more. These benefits show themselves as a result of the various bioactive components that are created during the fermentation process and the extensive microbiota (Rosa et al., 2017). it also has a natural ability to fight germs and bacteria, Numerous antimicrobial compounds, including lactic acid, acetic acid, carbon dioxide, hydrogen peroxide, ethanol, diacetyl, and bacteriosins, are produced by kefir organisms (Nielsen et al., 2014). Due to these organic acids, hydrogen peroxide, carbon dioxide, bacteriosins, and acetaldehyde, kefir has an antimicrobial effect (John & Deeseenthum, 2015). Gram-negative and Gram-positive bacteria were suppressed by bacteriosin generated by *L. plantarum*, according to a study (Powell et al., 2007).

Gram-negative bacteria are killed by kefir's antibacterial properties. *Salmonella*, *Shigella*, *Staphylococcus*, *Helicobacter pylori*, *E. coli*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, and *Candida albicans* were also susceptible to this impact. In comparison to the antibacterial action of the kefir itself, the antibacterial activity of the kefir particles against Gram-positive cocci containing Gram-positive bacilli and staphylococci is significantly higher. It was discovered that fresh kefir had the ability to inhibit *Staphylococcus aureus* and *E. coli*, but it had no effect on *Saccharomyces cerevisiae* or *Candida albicans* (Rosa et al., 2017). *L. acidophilus* and *L. kefiranofaciens* varieties, in addition to the *S. thermophilus* that was isolated from kefir, exhibit antibacterial action against pathogenic organisms such *as E. coli*, *L. monocytogenes*, *S. aureus*, *S. typhimurium*, *S. enteritidis*, and *S. flexneri* (Bourrie et al., 2016)

2.5.1. Effects on blood cholesterol

A high serum cholesterol level is a major risk factor that contributes to cardiovascular illnesses, which are among the most common causes of mortality. The serum cholesterol level can be significantly influenced by changes in dietary intake. According to a number of studies, fermented milk may help lower the total cholesterol level in the serum (Bourrie et al., 2016). In addition, the consumption of probiotic milk products is recommended as an effective method for lowering cholesterol levels. Kefir contains an abundant amount of lactic acid, which, when bound to the hexogen cholesterol, inhibits the growth of bacteria in the small intestines. This lactic acid also has the potential to lower cholesterol levels by 33 percent, either directly or indirectly (Rosa et al., 2017). It was shown that a strain of *L. plantarum* that was isolated from kefir has the ability to lower total serum cholesterol, triglycerides, and LDL cholesterol in rats on a high cholesterol diet (Ahmed et al., 2013). During the fermentation process, kefir particles have the potential to lower the amount of cholesterol in milk (Bourrie et al., 2016).

2.5.2. Effects on cancer prevention

Kefir has been shown to have anticarcinogenic properties due to the presence of bioactive components in it. These bioactive components include peptides, polysaccharides, and sphingolipids. These bioactive components play significant roles in the regulation of various biological processes, including cellular proliferation, death, and transformation (Rafie et al., 2015). Kefir, when consumed on a regular basis, may have a beneficial effect on the makeup of the immune system as well as the microbiota in the intestinal tract. Kefir's anticancer properties are largely attributable to the lactobacilli that it contains (Ahmed et al., 2013).

2.5.3. Its influence on the immune system

Sphingomyelin, which can be separated from kefir's lipids, is an additional factor that contributes to the source of kefir's action in the immune system. Immune activities were seen in humans and other animals following the consumption of LAB present in kefir. It was shown that LAB improve the resistance to illnesses seen in both humans and animals. Kefir contains LAB. According to (Karatepe et al., 2015), the effect that LAB have on the immune system is readily observable following oral or parenteral treatment of the bacteria. Beneficial effects can be produced by these microorganisms either directly through the maintenance of the activity of microbial cells or indirectly through the production of metabolites. Kefir consumption results in a reduction in the rate of pathogenic activity carried out by macrophages in both the lungs and the peritoneum.

These reactions have the potential to influence the mucosal response in a variety of body regions. Microflora found in kefir have the potential to influence innate immunity by causing a change in cytokine response (Ahmed et al., 2013).

2.5.4. Its influence on plasma glucose

There is a correlation between diabetes and an increased risk of morbidity and mortality. If diabetes mellitus is not treated in a timely manner, serious complications such as nephropathy, retinopathy, and neuropathy could develop (Judiono et al., 2014). Kefir, according to the findings of several research, can improve insulin resistance and bring down hyperglycemia levels (Pereira et al., 2016). One study found that giving kefir to rats for an extended period of time enhanced their ability to tolerate glucose. Consumption of kefir led to improvements in proteinuria and azotemia, as well as reductions in diabetes-related symptoms such as increased thirst, increased hunger, and excessive urination (Punaro et al., 2014).

2.5.5. Its impact on the digestive system

There is evidence that consuming kefir can have beneficial effects on the digestive tract. Kefir acts as a barrier that stops potentially dangerous chemicals, such as food antigens, from entering the body. The consumption of fermented dairy products helps the body repopulate the small intestine with healthy lactic acid microflora while also preventing the growth of pathogenic germs (Ahmed et al., 2013). One of the requirements for a bacterium to be considered a probiotic is for it to be resistant to the strict requirements of the digestive system. These requirements include the presence of pH difficulties, bile salts, and digestive enzymes. In addition, the connection that probiotic bacteria make with intestinal epithelial cells, where they either compete with or take the place of pathogenic bacteria, provides an additional layer of defense against those germs. Milk kefir has the ability to moderate the pH level in the stomach, and as a result, it can buy more time for a large number of bacteria to go from the stomach and into the upper portions of the small intestine (St-Onge et al., 2002). Foods that contain probiotic substances have the potential to alter the intestinal microbiota and provide beneficial effects for the host. These effects can be achieved either by ensuring the introduction of new species into the digestive system or by promoting the increased growth of beneficial bacteria (Bourrie et al., 2016). Consumption of kefir resulted in a rise in the number of helpful bacteria, such as lactobacilli and bifidobacteria, as well as a reduction in the number of dangerous bacteria, such as *Clostridium perfringens*, in an experiment conducted on animals (Hamet et al., 2016). *C. difficile* is a type of bacterium that is anaerobic and Gram-positive, and it is responsible for a variety of issues in the gastrointestinal tract, including diarrhea. There has been a growth in the number of cases of diarrhea that are attributed to *C. difficile*, kefir may protect against enterocolitis brought on by *C. difficile*, according to the findings of a study conducted on rats (Bolla et al., 2013).

2.5.6. Effects of kefir on bones

Insufficient calcium consumption worsens bone mass loss and raises the risk of osteoporosis in premenopausal women. In situations of bone mass loss caused by decreased intestinal calcium absorption and postmenopausal estrogen deficiency, calcium intake should be increased. Vitamins, proteins, and amino acids are food components that affect calcium absorption and encourage bone growth, hence preventing osteoporosis. Good providers of calcium, dairy products promote bone growth, prevent bone loss, and reduce bone resorption (Tu et al., 2015). Because it is packed with calcium, protein, and several probiotic ingredients, kefir promotes the mineralization and production of bone while simultaneously inhibiting bone resorption.

3. MATERIAL and METHOD

3.1. Microorganisms

Because the bacterial strains (*L. brevis, L. parabrevis, L. plantarum*) were stored in deep freezing at temperatures below 80 degrees Celsius for an extended period of time, it is necessary to doubly activate the bacteria before adding them to kefir samples. The bacteria are grown in MRS broth and then kept in an incubator for 24 hours at a temperature of 30 degrees Celsius. By performing the first step once more, a second activation is carried o0ut in order to boost their level of activity. After that, the culture is centrifuged at 7000 RPM for 5 minutes in order to separate the bacterial culture from the MRS broth. This gets the culture ready to be added to kefir.

the plate pour method was used to count the quantity of viable bacteria in the sample. The process was completed by selecting 1 ml of live bacteria from the second activation and diluting it in a tube containing 9 ml of distilled water. This allowed the bacteria to be used in further steps. Then, one milliliter is extracted from the first tube, and it is added to another tube that already has nine milliliters of distilled water in it. The procedure is carried out a total of six times for every microorganism. In the final step, one milliliter of each bacterium's final dilution is taken from the final tube and added to plates containing MRS agar. At a temperature of 30 degrees Celsius, the samples are left in incubator for 24 hours. The results are shown in table 4.1

3.2. Kefir Powder

In this experiment, single-use kefir powders from a commercial source are used in place of the more traditional kefir grains. The powders are made in specialized labs and distributed to markets where they are sold in compact boxes at a storage temperature ranging from 2 to 8 degrees. Each box has four individual packets, each containing one gram of kefir culture. The following is comprised of the items that can be found inside each package: Maltodextrin, lactose, starter cultures (*Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. cremoris, Streptococcus thermophilus, Kluyveromyces marxianus subsp. marxianus, Debaryomyces hansenii*). The number of live bacteria in 1.0 g of product is at least $1x10^9$.

Figure 3.1. Commercial kefir powder

3.3. Production of Kefir

Six liters of raw cow milk are obtained from the market in order to prepare six samples, each measuring one liter in volume and containing a different type of bacteria but the same quantity of kefir powder. After being heated to 70 °C for twenty minutes during the pasteurization process, each of the five milk samples was allowed to cool to room temperature, while one sample left raw. The next step is to put 0.5g of commercial kefir powder into each of the six bottles. After that, a concentration of LAB equal to 5 percent is added to three of the samples, while the last sample will include a mixture of all of the bacteria, also equal to 5 percent, as will be detailed below. The next step is to leave all six bottles at room temperature to ferment for 24 hours.

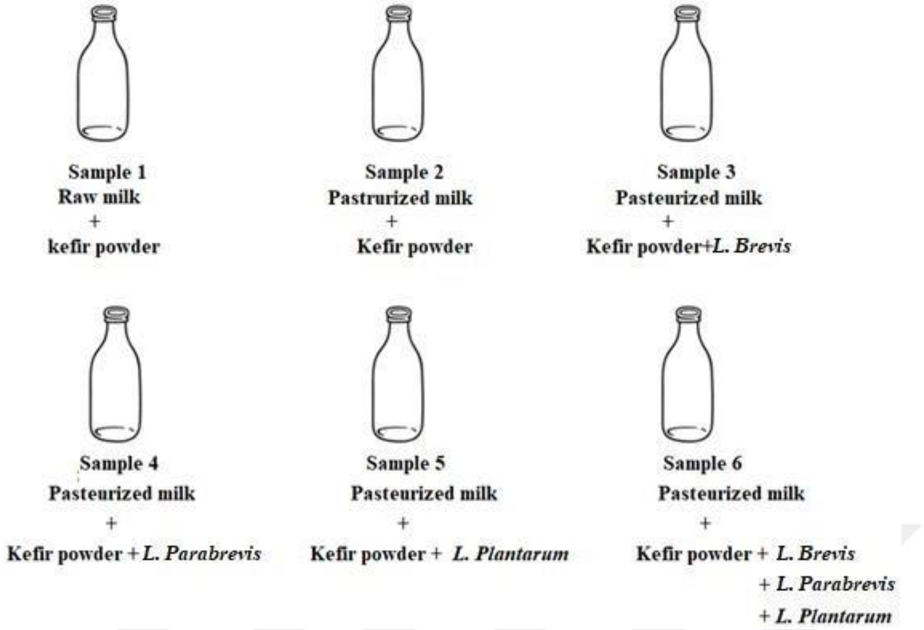

Figure 3.2. The prepared Kefir samples

The starter culture for the sixth sample is prepared by combining three different bacteria with 5%, making a total volume of 50 mL of the mixture. (16 mL) from *L. parabrevis*, *L. brevis* (17 mL), and *L. plantarum* (17 mL).

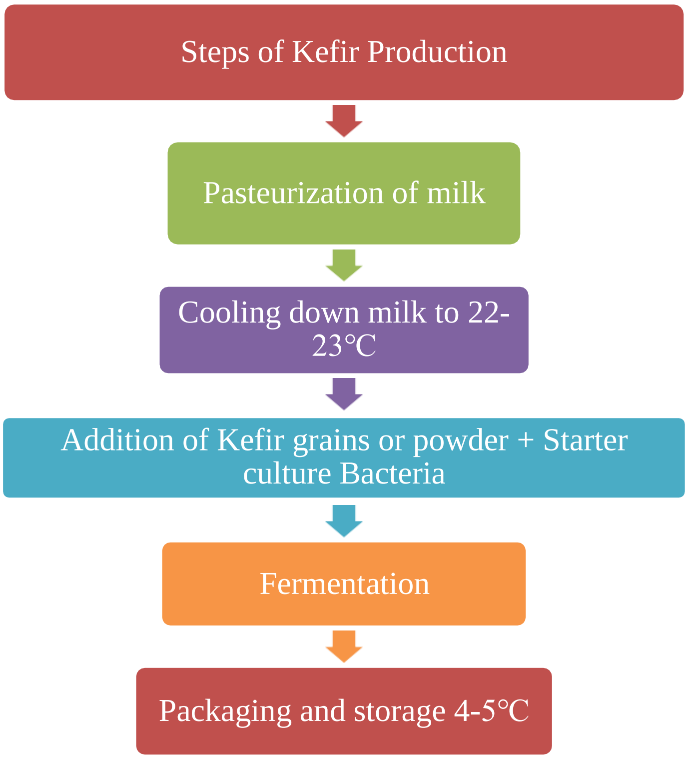

Figure 3.3. Steps of kefir production

3.4. Dry Matter Measurment

After the weight of the empty container has been determined, the procedure for determining the amount of dry matter in kefir continues with the addition of 10 mL of sample to the container (a petri dish), which is then placed in an oven preheated to 110 degrees Celsius for two hours. After noting the difference in weight caused by the sample, the sample is allowed to remain in the oven for a further sixty minutes in order to ensure that it has been thoroughly dried. The last step, but certainly not the least, is to weigh the sample and make a note of the total mass (Dry Matter Determination – DAIReXNET, 2019). And the calculation is performed by using equation below

$$\text{Total solids \%} = \frac{\text{Weight of residue}}{\text{Weight of milk sample}} \times 100$$

3.5. pH and acidity

After a period of fermentation that lasted for 24 hours at room temperature, each of the six samples of kefir had their pH determined with the use of a pH meter. Table 4.2 displays the outcomes of these measurements for your information.

To determine the acidity, titeration method is used according to (Indian Standards, 1960). The procedure involves of filling a burette with 0.1N sodium hydroxide and combining 10 milliliters of kefir sample with 10 milliliters of purified water. After the sample has been prepared, three to four drops of the phenolphthalein indicator are then added to the sample. The titration procedure is then initiated by allowing the drops of NaOH to combine with the solution. When the color of the solution below changes to a pale pink, the titeraion should be stopped. And the calculation is done by using the equation below.

$$\text{Lactic Acid\%} = \frac{\text{volume of NaOH} * 0.009}{\text{Volume of Kefir} * \text{Specific Gravity}} * 100$$

3.6. Biogenic Amines Analysis

After the fermentation process is complete, each and every kefir sample is subjected to BA testing. HPLC Chromatography is the method that accomplishes this goal. The samples were subjected to analyse content of (Tryptamine, 2-phenylethylamine, Putrescine, Cadaverine, Histamine, Tyramine, Spermidine, Spermine) by first having 5 mL of sample transferred to a 15-mL centrifuge tube, then having 10 mL of 0.4 M perchloric acid added to it, and finally having it mixed before being centrifuged at 3000 rpm for 10 minutes. The supernatant that was produced is filtered via a paper filter into a flask that has a capacity of 25 mL. The procedure is carried out once more by adding an additional 10 mL of perchloric acid at a concentration of 0.4 M to the residual. Using 0.4 M perchloric acid, the balloon's ultimate volume is brought up to 25 mL so that it can be used. During the stage of sample derivatization, 1 mL of extract was alkalized with 200 L of 2 N sodium hydroxide, and then 300 L of saturated sodium hydroxide is added to the mixture. Following the addition of 2 mL of dansyl chloride, it will be maintained at a temperature of 40℃ for 45 minutes, and at the conclusion of this time period, 100 µL of ammonia diluted to a concentration of 25 % will be added. The samples were centrifuged at 2500 rpm for 5 minutes after the total volume has been brought up to 5 mL with acetonitrile. 20 µL of the supernatant is injected into Nucleodur 100-5-C18 column (12.5X4 mm) on the HPLC (Shimadzu LC-20AT, Japan) system after being filtered through a 0.45 µL syringe tip membrane filter (Eerola et al., 1993; Genccelep et al., 2008). Chromatography was performed with 1 mL/min flow rate in the with the column temperature adjusted to 25℃. The peaks were analysed at 254 nm with Diode Array Detector (DAD).

4. RESULTS and DISCUSSIONS

4.1. Bacterial plate count

After the petri dishes had been incubated for 24 hours at a temperature of 30 degrees Celsius, the plate count was calculated by employing the technique of serial dilution, and the findings that follow in Table 4.1 are the direct consequence of this determination.

Table 4.1. The concentration of bacteria.

Microorganisms	**Number of bacteria (CFU/mL)**
L. brevis	5.85×10^9
L. parabrevis	7.8×10^9
L. plantarum	6.18×10^9

4.1.2. pH and Acidity

After fermentation for 24 hours at room temperature, each of the six samples of kefir had their pH determined with the use of a pH meter as shown in Table 4.2

Table 4.2. pH of the samples.

Sample	**pH**
Raw milk	4.6
Pasteurized Milk	4.7
Pasteurized *L. brevis*	4.6
Pasteurized milk + Kefir powder + *L. parabrevis*	4.7
Pasteurized milk + Kefir powder + *L. plantarum*	4.7
Pasteurized milk + Kefir powder + *L. brevis* + *L. parabrevis* + *L. plantarum*	4.6

As can be seen in the Table 4.2, the pH of the kefir is around 4.6-4.7. According to the findings of a research project that was carried out by (Özdestan & Üren, 2010), the pH of kefir ranges between (4.11 and 4.53). According to research conducted by (Guzel-Seydim et al., 2000), the initial pH of kefir samples before they were placed in the refrigerator for

21 days was 4.5, but it dropped to an average of 4.4 by the 21st day. Throughout the course of this research, the pH of kefir samples ranged anywhere from 4.6 to 4.7.

Titratable acidity is given as a percentage of lactic acid minimum (0.6 percent m/m), as specified by the Codex Standard for Fermented Milks, CODEX STAN 243-2003.

According to the findings of a study that was carried out by (Özdestan & Üren, 2010), the acidities of kefir samples ranged from 0.652 to 1.047 percent (as lactic acid, w/v).

In this study, the acidity test is done for sample 2 which is prepared from pasteurized milk on behalf of other kefir samples, when the burette was filled with 0.1 N NaOH for a total of five times, 50 mL of solution was used for each filling. The color of the sample began to shift to bright pink on the fifth repetition, when it was 31 mL, which indicates that 219 mL of 0.1 N of NaOH will be necessary to neutralize the kefir sample that is below. In addition, the calculation for determining the acidity yielded a figure of 1.97% once it was completed. Lactic Acid% for sample 2 (pasteurized milk+kefir powder) = 1.97

4.1.3. Dry Matter

In a study that was carried out by (Özdestan & Üren, 2010), , it was found that there were no significant correlations between the BA concentrations and the dry matter of the kefir, despite the fact that the total dry matter of their kefir sample was between 8.88 and 12.00 percent (w/w). while in this study the percentage of the dry matter was 13.60%.

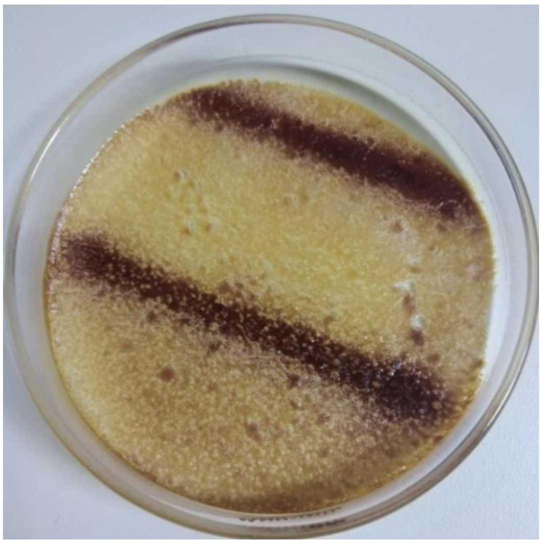

Figure 4.1. Dried Kefir Sample

4.2. Biogenic amine contents of kefir samples

A variety of LAB, including those utilized as starter cultures to increase technical and sensory qualities, and those that arise spontaneously during ripening or in spontaneous fermentations are capable of producing BAs in model or in real systems. The categories investigated were enterococci, lactobacilli, streptococci, lactococci, pediococci, oenococci and, the relevant amino acids are decarboxylated by bacteria, resulting in the exogenous BAs. This process is catalyzed by enzymes called decarboxylases. Histamine and cadaverine can be produced when histidine and lysine are decarboxylated by the enzymes histidine decarboxylase (HDC) and lysine decarboxylase (LDC), respectively. This process is known as the histamine and cadaverine synthesis. The enzyme tyrosine decarboxylase (TDC) is responsible for the conversion of tyrosine into tyramine. This same enzyme can also work on phenylalanine in order to produce 2-phenylethylamine (Bargossi et al., 2015; Pessione et al., 2009).

The potential for bacterial contamination is present in all foods that have been fermented. Despite the fact that LABs are classified as GRAS organisms (organisms that are generally regarded as safe), they are nonetheless capable of producing hazardous substances in the form of BAs. In particular, in foods that have been fermented (Özogul et al., 2018). Genetic research has shown that a good number of these strains include genes or operons that code for decarboxylating enzymes as well as other pathways that are involved in the manufacture of BA (Marcobal et al., 2012).

Using HPLC chromatography, a total of 6 samples representing a variety of conditions and 8 distinct BAs are analyzed. The following is what the research found:

4.2.1. Sample 1 Raw milk

According to the findings of the study carried out by Novella-Rodriguez et al., (2004) The highest microbial counts and levels of BA were found in cheese that had been made from raw milk and stored at 4 C for 48 hours. During the process of ripening, the levels of some microbial and BAs increased significantly as a result of the storage of milk in a refrigerated environment. Tyramine was found to be the most prevalent BA in the cheeses that were tested, followed by cadaverine. Whereas, in the current investigation as shown in Table 4.3, 2-phenylethylamine was found to have the highest concentration of any BA in kefir, which is produced from raw milk.

Table 4.3. The biogenic amine content of kefir made of raw milk without added starter culture.

Raw milk	**Conc. mg/L**
2-phenylethylamine	11.709
Spermidine	7.202
Cadaverine	4.885
Spermine	1.979
Putrescine	1.355
Tyramine	0.932
Tryptamine	0.225
Histamine	0.183

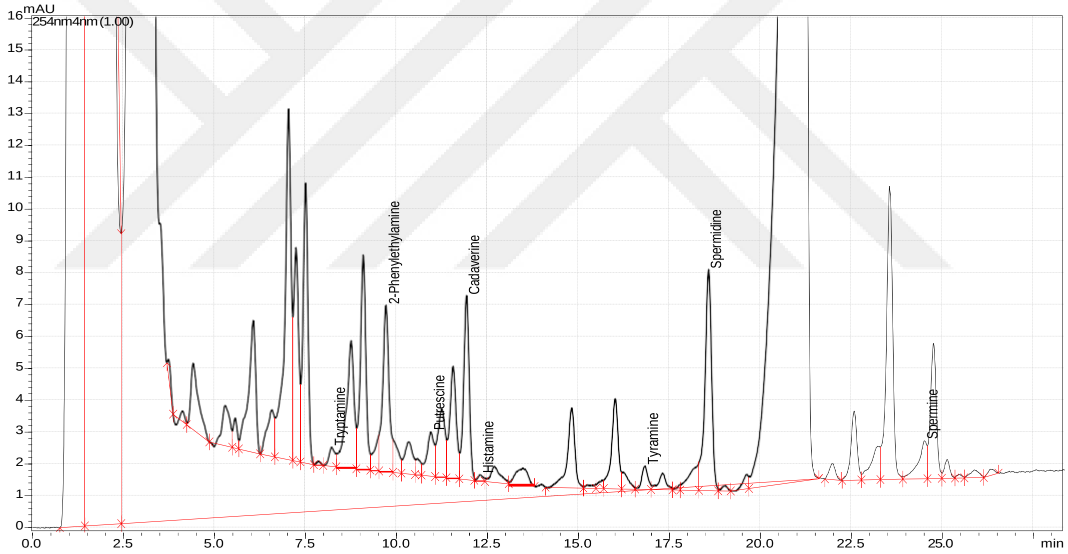

Figure 4.2. The chromatography peaks of BA content of kefir made with raw milk without starter culture.

According to the findings of a study that was carried out by Özdestan & Üren (2010), putrescine, cadaverine and spermidine were found in all of the samples. Tyramine was found in every single kefir sample with the exception of one. The most prevalent kind of BA was tyramine. The levels of tyramine in the kefir samples increased from undetectable levels to 12.8 mg/l. The total amount of BAs identified in the kefir samples ranged from 2.4 to 35.2 mg/l, however the 2-phenylethylamine content was the most telling BA in this investigation because it was present in all of the kefir samples in a relatively high concentration.

4.2.2. Sample 2: Pasteurized milk + Kefir powder

As in Table 4.4 According to the findings of the current research, 2-phenylethylamine is the predominant BA that may be discovered in kefir that is manufactured from pasteurized cow milk. According to the research carried out by Torracca et al. (2016), tyramine is the most abundant form of the BA that may be discovered in cheeses produced using milk that has been subjected to pasteurization. They further state that milk pasteurization effectively inhibited the development of BA both qualitatively and quantitatively.

Table 4.4. The biogenic amine content of kefir made of pasteurized milk without added starter culture

Pasteurized Milk	**Conc. mg/L**
2-phenylethylamine	11.219
Spermidine	7.236
Cadaverine	2.720
Putrescine	1.919
Tryptamine	1.839
Histamine	1.798
Tyramine	0.952
Spermine	0.656

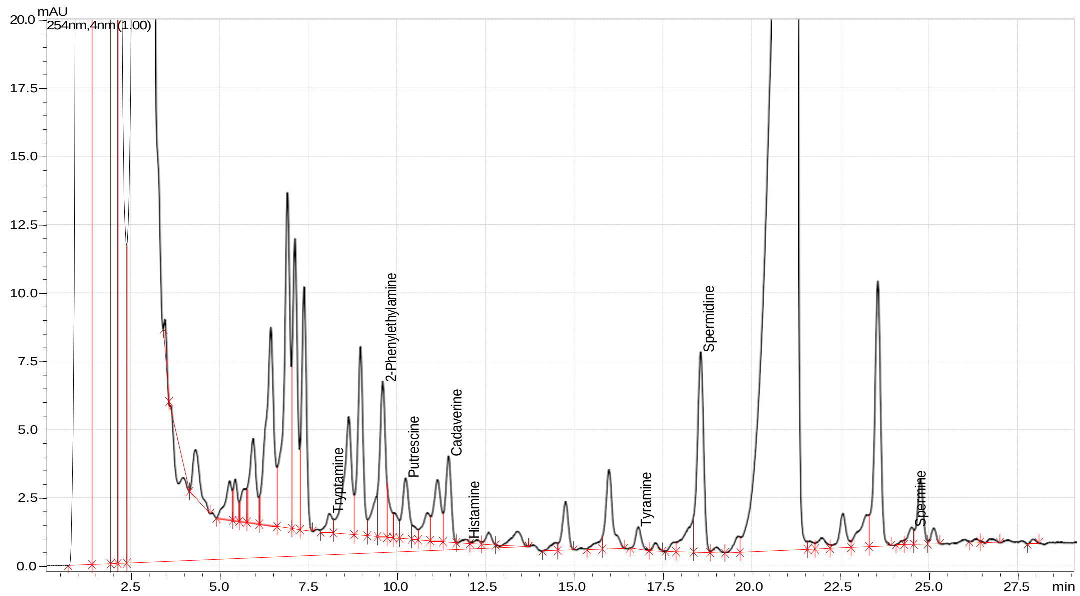

Figure 4.3. The chromatography peaks of BA content of kefir made with Pasturized milk without starter culture

4.2.3. **Sample 3: Pasteurized milk + Kefir powder + *L. brevis***

After adding 5 percent of *L. brevis* to a sample of pasteurized kefir, as shown in table 4.5, results show that 2-phenylethylamine is the greatest BA detected, and spermine was the BA that was detected the least.

Table 4.5. The biogenic amine content of kefir made of pasteurized milk supplemented with *L. brevis.*

Pasteurized *L. brevis*	**Conc. mg/L**
2-phenylethylamine	12.511
Spermidine	7.680
Tyramine	6.958
Cadaverine	2.918
Putrescine	1.703
Tryptamine	1.578
Histamine	1.215
Spermine	0.186

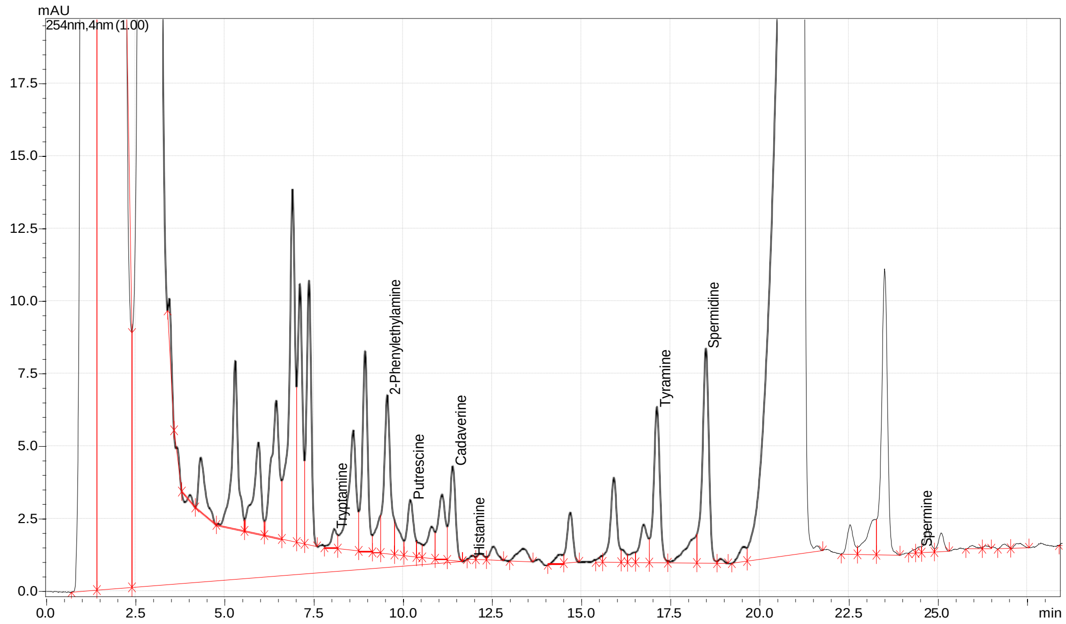

Figure 4.4. The chromatography peaks of BA content of kefir made of pasteurized milk supplemented with *L. brevis*

4.2.4. Sample 4: Pasteurized milk + Kefir powder + *L. parabrevis*

In the production of kefir, the addition of 5% *L. parabrevis* to pasteurized milk yielded the same result as the addition of *L. brevis*, which is that 2-phenylethylamine was the BA that was found in the highest concentration, and Histamine was the BA that was found in the lowest concentration as mentioned in Table 4.6.

Table 4.6. The biogenic amine content of kefir made of pasteurized milk supplemented with *L. parabrevis.*

Pasteurized *L. Parabrevis*	**Conc. mg/L**
2-phenylethylamine	11.646
Spermidine	7.284
Cadaverine	2.877
Putrescine	2.432
Tryptamine	1.379
Spermine	0.864
Tyramine	0.845
Histamine	0.292

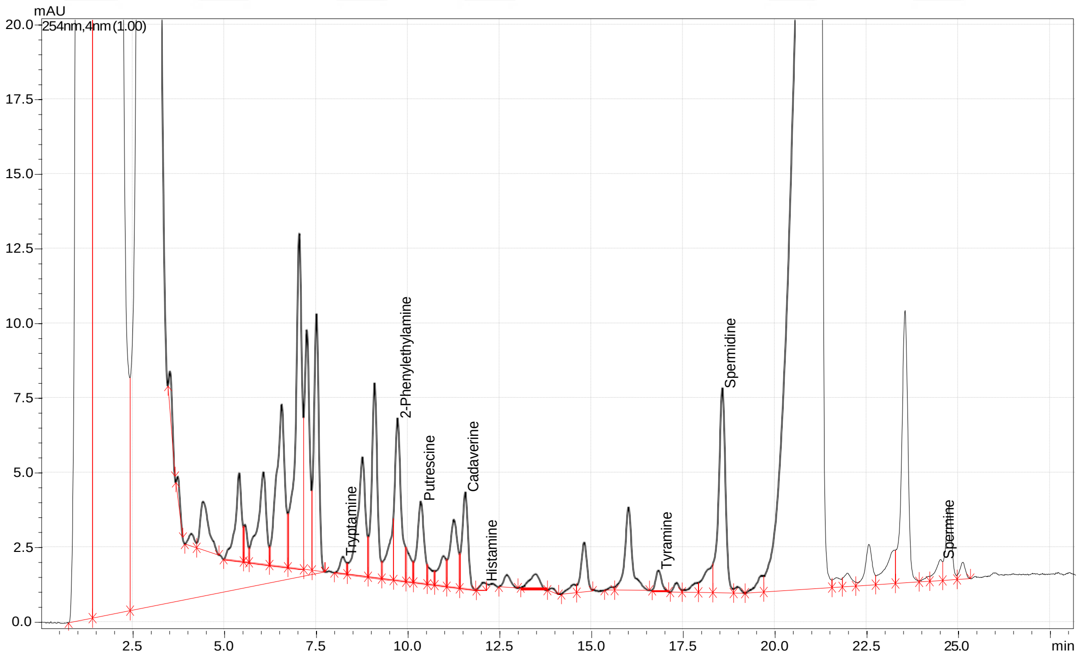

Figure 4.5. The chromatography peaks of BA content of kefir made of pasteurized milk supplemented with *L. parabrevis*

4.2.5. Sample 5: Pasteurized milk + Kefir powder + *L. plantarum*

As in other LAB strains the supplementing of pasteurized milk in kefir production with *L. parabrevis* reveals that 2-phenylethylamine is the highest BA observed. On the contrary, histamine concentration was the one that was shown to be the lowest among the Bas showed in Table 4.7.

Table 4.7. The biogenic amine content of kefir made of pasteurized milk supplemented with *L. plantarum.*

Pasteurized *L. plantarum*	**Conc. mg/L**
2-phenylethylamine	11.47
Spermidine	7.555
Cadaverine	2.803
Putrescine	2.375
Tryptamine	1.677
Tyramine	1.249
Spermine	0.397
Histamine	0.097

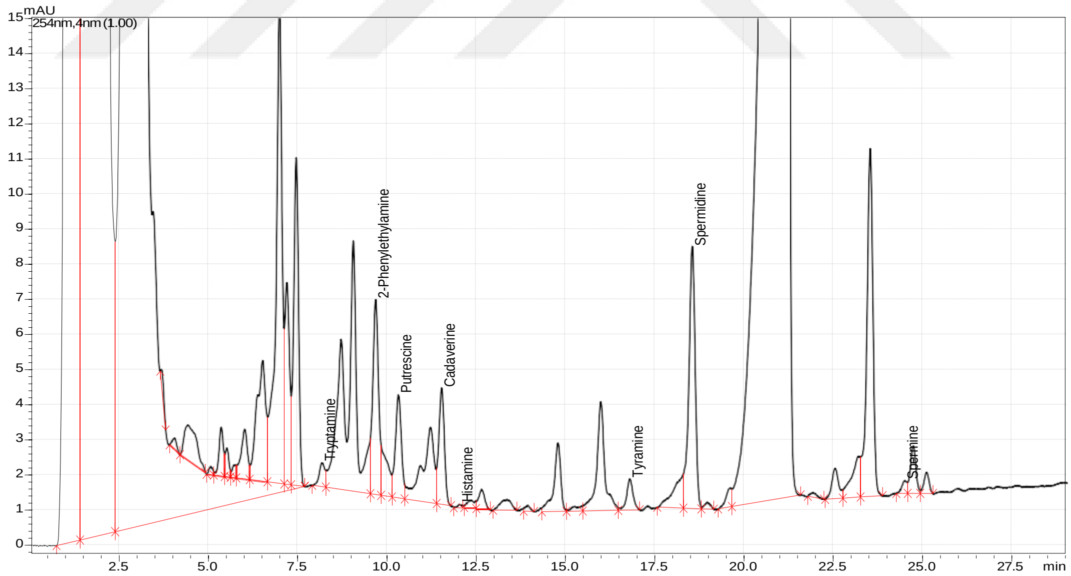

Figure 4.6. The chromatography peaks of BA content of kefir made of pasteurized milk supplemented with *L. plantarum*

4.2.6. Sample 6: Pasteurized milk + Kefir powder + *L. brevis* + *L. parabrevis* + *L. plantarum*

In the same way that it happened in the previous sample, the addition of the combination of *L. brevis*, *L. parabrevis*, and *L. plantarum* to the pasteurized milk in the production of kefir caused 2-phenylethylamine to become the highest BA found in this sample, while histamine was the lowest BA detected during this analysis as mentioned in Table 4.8.

Table 4.8. The biogenic amine content of kefir made of pasteurized milk supplemented with *L. brevis* + *L. parabrevis* + *L. plantarum.*

Pasteurized 3 MIX	**Conc. mg/L**
2-phenylethylamine	13.707
Spermidine	7.586
Cadaverine	2.894
Putrescine	2.628
Tyramine	2.107
Tryptamine	1.805
Spermine	0.389
Histamine	0.128

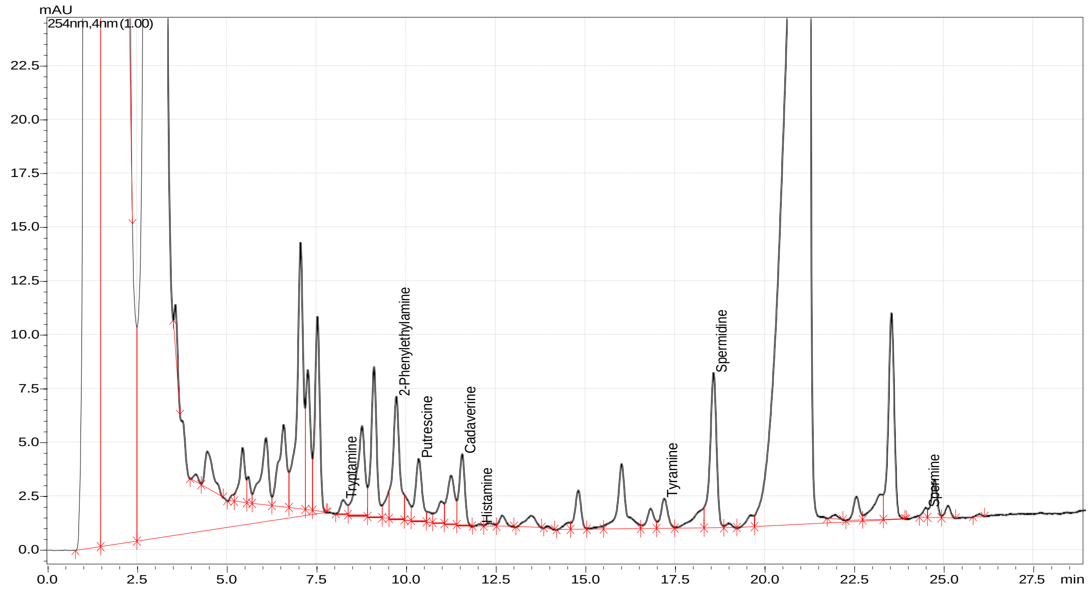

Figure 4.7. The chromatography peaks of BA content of kefir made of pasteurized milk supplemented with *L. brevis* + *L. parabrevis* + *L. plantarum*

The tyrosine-decarboxylase DNA sequence was found in 44% of the lactobacilli isolates, while the histidine-decarboxylase DNA sequence was found in 16% of the isolates. Especially *E.faecium*, *E. faecalis*, *E. durans*, *E. hirae*, *E. casseliflavus*, *E. mundtii*, *L. brevis*, *L. lactis*, *L. mesenteroides*, *C. divergens* which have tyrosine-decarboxylase enzyme, converts phenylalanine aminoacids to 2-phenylethylamine, and plus *L. sakei*, *L. curvatus*, *L. plantarum*, *L. brevis*, *L. buchneri*, *L. casei*, *L. paracasei*, *L. reuteri*, *L. hilgardii*, *L. homohiochii*, *L. delbrueckii subsp. bulgaricus*, *S. thermophilus*, *S. macedonicus* converts tyrosin to tyramine (Barbieri et al., 2019)

Lactobacillus species, such as *L. helveticus*, *L. buchneri*, and *L. curvatus*, can be responsible for the accumulation of BAs in cheese and other dairy products. Although several microorganisms in cheese, including Gram-negative bacteria, are able to produce BAs, the accumulation of BAs in such products is typically caused by Lactobacillus species (Ladero et al., 2010; Burdychova et al., 2007). Several authors have been successful in isolating *L. brevis* strains that produce tyramine from cheeses (Buňková et al., 2010).

Özdestan & Üren, (2010), conducted a study on a total of ten different kefir samples. They found that tyramine had the highest concentration of any BA at 12.8 mg/L, followed by putrescine at 12.1 mg/L, spermidine at 4.5 mg/L, histamine at 4.0 mg/L, and cadeverine at 2.2 mg/L. However, the concentrations of methylamine, tryptamine, 2-phenylethylamine, and spermine were completely undetectable.

Yılmaz et al., 2017 demonstrated that an *L. plantarum* strain in yogurt has the ability to produce tyramine. They also highlighted a possible indirect effect of *L. delbrueckii subsp. bulgaricus* on accumulation of tyramine in the yoghurts, due to the synergistic interactions that this strain has with tyraminogenic LAB strains. There is evidence to suggest that the non-starter bacteria *L. brevis* and *L. curvatus* are capable of producing both tyramine and putrescine (Ladero et al., 2015).

4.3. The influence of temperature and starter culture on biogenic amines in kefir.

After conducting research on the levels of BAs found in cheeses made from raw and pasteurized milk, Schneller et al., (1997) concluded that the concentrations of BAs were caused by the high levels of enterococci and Enterobacteriaceae found in the cheeses. On the other hand, semi-soft cheeses made from pasteurized milk exhibited a much lower concentration of total BAs when compared to semi-soft cheeses made from raw milk (51–1096 mg/kg vs. 1011–3133 mg/kg).

In contrast, the effect of temperature on the amount of BAs could be clearly observed in this particular investigation due to the fact that when milk is pasteurized as part of the manufacturing process for 20 min 70 °C, the levels of some of the BAs increased, while the levels of some of the other BAs decreased, and the levels of some other BAs remain the constant. Histamine production increased by 882%, tryptamine production increased by 719%, putrescine production increased by 42%, and tyramine production increased by 2%. On the other hand, spermine production decreased by 67%, cadaverine production decreased by 44%, and phenylethylamine production decreased by 4%, but spermidine production did not change as shown in table Table 4.9.

Table 4.9. The impact of pasteurization on BAs in kefir.

Biogenic amines	***Temperature change %***
Histamine	882%
Tryptamine	719%
Putrescine	42%
Tyramine	2%
Spermidine	0%
2-phenylethylamine	-4%
Cadaverine	-44%
Spermine	-67%

Although milk pasteurization effectively reduced the quantity and quality of BA production, it did not prevent the buildup of notable BA levels in cave-ripened cheeses. Therefore, it would appear that the process of pasteurizing milk is not adequate to guarantee low BA levels in cheeses (Torracca et al., 2016).

Cheese made from raw milk and kept in storage for 36 hours had the greatest quantity of total BAs, measuring 4817 mg/kg. This cheese was found to have been generated. The quantities of cadaverine, phenylethylamine, putrescine, and tyramine in this cheese were significantly greater than the concentrations found in any other cheese. Another raw milk cheese was found to have the highest histamine levels (573 mg/kg) of all the cheeses tested (Schneller et al., 1997)

The production of BA is only achievable under conditions that are favorable to the decarboxylation activity in the environment and under which there is availability of the free substrate amino acids (Russo et al., 2010). There are a variety of factors that are associated with such conditions, the most important of which are the bacterial density and the synergistic effect between microorganisms. These factors include the milk treatments, the use of starter cultures and enzymes, the duration and temperature of ripening, the level of proteolysis, the pH, the NaCl concentration, the presence of oxygen, the activity of water, and the relative humidity (Gardini et al., 2001).

Along with other fermented meals or drinks, fermented meat sausages are one of the food products that have the potential to accumulate greater levels of BAs over time (Suzzi & Gardini, 2003; Spano et al., 2010; Daniel Collins et al., 2011). In fermented sausages, tyramine is produced by a fermentative microbial population. This population is comprised primarily of LAB (including lactobacilli and enterococci) and more rarely coagulase negative staphylococci (Masson et al., 1999; Bover-Cid et al, 2001).

According to research conducted by Latorre-Moratalla et al. (2010), 48 percent of LAB and 13 percent of staphylococci that were isolated from sausage that underwent spontaneous fermentation were able to decarboxylate one or more amino acids.

There have been reports that certain LAB that are utilized as meat starters (i.e., *L. curvatus* and *S. carnosus*) are strong makers of BAs, primarily tyramine (Latorre-Moratalla et al., 2010; (Talon & Leroy, 2011). In contrast, microorganisms belonging to species such as *L. sakei*, *L. plantarum*, and *S. xylosus* are typically categorized as either non-aminogenic or weak (Bover-Cid et al., 2001; Aymerich et al., 2006; Latorre-Moratalla et al., 2010; Linares et al., 2011).

Several research support that a larger effectiveness in BAs reduction was achieved when *L. sakei* was utilized as the starter in contrast to other species (González-Fernández et al., 2003; Latorre-Moratalla et al., 2010; Baka et al., 2011). *L. sakei* are typically very well suited to the ecology of meat fermentation, and they are competitive at temperatures ranging from 15 to 25 degrees Celsius, which is the temperature range that is used for the production of sausages in European countries (Bover-Cid et al., 2001)

Recent research conducted by Özdestan & Üren, (2010) found that the total BA contents in kefir samples ranged from 2.4 to 35.2 mg/L, with tyramine being the most prevalent BA. Similarly, kumis, a traditional fermented cow milk drink in Colombia, had a total BA concentration of 15.31 mg/L. Despite this, these values are significantly lower than the limits that are suggested. According to Novella-Rodríguez et al., (2000) and Souci et al., (2000), other milk-based fermented products, such as buttermilk or yogurt, are not likely to contain considerable levels of BA.

In the current investigation, in order to lower the levels of BAs in the kefir samples, a total of three starter cultures were introduced at a concentration of 5 percent. According to the findings, the starter cultures were able to raise the ratio of certain BAs while simultaneously decreasing the development of some BAs.

During the production of kefir, the addition of *L. brevis* to pasteurized milk led to a significant increase in tyramine levels by 631%, followed by 2-phenylethylamine by 12% and cadaverine levels of 7%%, spermidine by 6%, while the spermine value is dropped by 72%, histamine 32%, tryptamine -14%, putrescine 11% as shown in Table 4.10

Table 4.10. The impact of *L. brevis* on biogenic amine content of kefir.

Pasteurized L. brevis	***% of BA change***
Tyramine	631%
2-phenylethylamine	12%
Cadaverine	7%
Spermidine	6%
Putrescine	-11%
Tryptamine	-14%
Histamine	-32%
Spermine	-72%

During the production of kefir, the addition of 5% *L. plantarum* in pasteurized milk led to increase in tyramine ratio by 31%, followed by putrescine 24%, spermidine 4% cadaverine 3%, 2-phenylethylamine 2%, while a significant decrease in histamine value by 95% followed by spermine 39% and tryptamine 9% as indicated in Table 4.11.

Table 4.11. The impact of *L. plantarum* on biogenic amines content of kefir.

Pasteurized L. plantarum	***% of BA change***
Tyramine	31%
Putrescine	24%
Spermidine	4%
Cadaverine	3%
2-phenylethylamine	2%
Tryptamine	-9%
Spermine	-39%
Histamine	-95%

The addition of *L. parabrevis* by 5 to pasteurized milk duting kefir production led to increase in the ratio of spermine by 32% followed by putrescine 27%, cadaverine 6%, 2-phenylethylamine 4%, spermidine 1% while a significant decrease in the ratio of histamine which is by 84%, tryptamine by 25%, tyramine by 11% as shown in table Table 4.12.

Table 4.12. The impact of *L. parabrevis* on the biogenic amine content of kefir.

Pasteurized L. parabrevis	***% of BA change***
Spermine	32%
Putrescine	27%
Cadaverine	6%
2-phenylethylamine	4%
Spermidine	1%
Tyramine	-11%
Tryptamine	-25%
Histamine	-84%

As indicated in table Table 4.13 on 6th sample the additon of a mixture of sarter cultures *L. brevis, L. parabrevis*, *L. plantarum* by 5% (16 mL from *L. parabrevis*, 17 mL *L. brevis*, and 17 mL *L. plantarum*) led to boost the level of tyramine by 121% followed by putrescine 37%, 2-phenylethylamine 22%, cadaverine 6% spermidine 5%. At the same time, it reduced the ratio of histamine by 93%, spermine 41%, tryptamine 2%

Table 4.13. The impact of the mixture (*L. brevis, L. parabrevis, L. plantarum*) starter cultures on biogenic amine content of kefir.

Pasteurized 3 MIX	***% of BA change***
Tyramine	121%
Putrescine	37%
2-phenylethylamine	22%
Cadaverine	6%
Spermidine	5%
Tryptamine	-2%
Spermine	-41%
Histamine	-93%

Table 4.14. Biogenic amine content of all samples (mg/L)

Sample	Trp	Phe	Put	Cad	His	Tyr	Spermine	Spermidine
Raw Milk	0.225	11.709	1.355	4.885	0.183	0.932	7.202	1.979
Pasteurized	1.839	11.219	1.919	2.720	1.798	0.952	0.656	7.236
Pasteurized + *L. parabrevis*	1.379	11.646	2.432	2.877	0.292	0.845	0.864	7.284
Pasteurized + *L. brevis*	1.578	12.511	1.703	2.918	1.215	6.958	0.186	7.680
Pasteurized + *L. plantarum*	1.677	11.47	2.375	2.803	0.097	1.249	0.397	7.555
Pasteurized + 3 MİX	1.805	13.707	2.628	2.894	0.128	2.107	0.389	7.586

The association between the BAs, temperature, and starter cultures is depicted graphically in Figure 4.8 as a histogram.

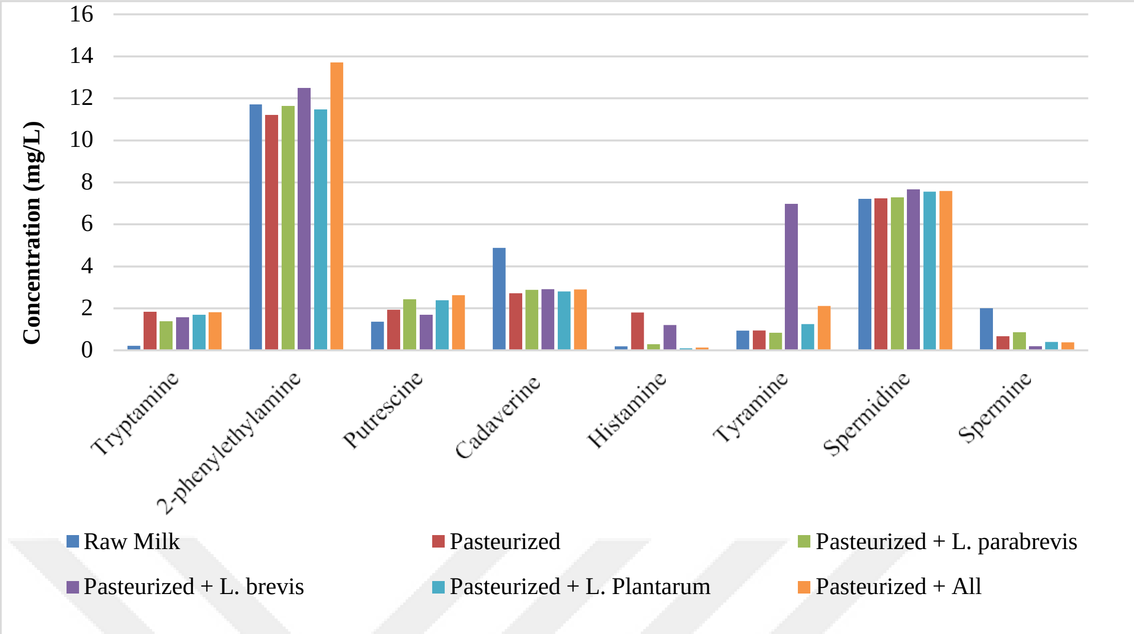

Figure 4.8 Statistical histigogram of BAs in Kefir samples

It suggests that the starter cultures, if not selected in a particularly careful manner, have the potential to boost the levels of some of the BAs. At the same time, though, they are able to decrease a number of them. Furthermore, it indicates a direct connection between the amount of BA present and the degree of temperature at which the milk was heated in the process of making kefir. Because of this, there needs to be a sufficient amount of research done to determine the temperature that would produce the best results during the pasteurization step of kefir production.

5. CONCLUSION

The growing population of the world has resulted in an increase in the amount of food that is required. At the same time, one of the most severe worries that people have is the safety of the food they eat. Because access to nutritious food is one of the most fundamental human rights, national governments are obligated to pass laws governing food production, storage, and distribution.

Food manufacturers are obligated to give thought to the ingredients that are used in the production of their products. In order to manufacture items of a high-grade standard, they are required to work with materials that are both fresh and of a high quality. In addition to this, they are required to apply sanitary practices across the food production chain in order to prevent any losses in terms of both material and economic value.

Because it has a wide variety of nutrients in its composition, milk is an excellent medium for the growth of microorganisms. As a consequence of this, milk is regarded as a food that has a short shelf life. Milk and dairy product manufacturers have a responsibility to handle milk straight from the farm in a sanitary manner and to prevent any kind of contamination at every stage of the food manufacturing process. In order to bring the number of microorganisms found in the milk down to an acceptable level, they must use a variety of thermal application procedures, which change depending on the kind of product being processed.

The fermentation process results in the production of nitrogenous organic chemicals known as biologic amines, which can be found in certain foods. They are produced when free amino acids are subjected to decarboxylation by bacteria. The presence of these materials in low concentrations is unlikely to have a significant impact on human health because certain enzymes can remove them from the body, however, the presence of these compounds in high concentrations can cause serious health problems, beginning with food poisoning. The levels of BAs in food products are often used as a benchmark for overall quality. Foods that are high in the amount of BAs typically have a low quality, whereas foods that are low in the amount of BAs generally have a high quality.

In the dairy industry, the process of pasteurization of milk is essential; however, the process can take varying amounts of time and require varying temperatures depending on the product. The purpose of pasteurization is to limit the amount of potentially hazardous germs that present naturally, through the handling of the milk, or during the process of milking the animal. On the other hand, pasteurization has the ability to change milk proteins in certain circumstances. It does this by breaking the peptide connections that hold casein and whey protein together and releasing their separate free amino acids. Because of this, it requires to be a significant amount of research done so that the optimal amount of time and temperature can be selected for the process of pasteurizing milk in order to prevent the milk proteins from being deformed.

In the present investigation, six different kefir samples were made and supplemented with starter cultures so that the effect of these cultures on the BAs could be analyzed. While some of the cultures were successful in lowering the ratio of BAs, others caused a considerable increase in the amount of BAs produced.

The fermentation industry makes extensive use of starter cultures for a variety of applications due to its extensive application. One of the aims is to employ them as food preservatives in order to avoid the production of certain compounds, such BAs. This will help achieve one of the goals. The LAB that was utilized in this study was able to reduce some of the BAs. At the same time, they were able to decarboxylate some of the amino acids that were present in the milk, which caused a large increase in some of the BA ratios. For this reason, LAB strains should be chosen with caution in order to prevent the creation of BAs. This is because some of the LABs have the ability to enhance the formation of BAs, particularly when paired with other FBPs.

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