ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

THE ANALYSIS OF FOOD ADDITIVES AND INORGANIC ANIONS IN BREAD BY ION CHROMATOGRAPHIC METHOD WITH CONDUCTIVITY DETECTOR

M.Sc. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

EKMEKTE BULUNAN GIDA KATKI MADDELERİ VE İNORGANİK ANYONLARIN İLETKENLİK DEDEKTÖRLÜ İYON KROMATOGRAFİK YÖNTEM İLE TAYİNİ

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Zeynep Melis Arkan, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 509181300, successfully defended the dissertation entitled "The Analysis of Food Additives and Inorganic Anions in Bread by Ion Chromatographic Method With Conductivity Detector", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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FOREWORD

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July 2020

Zeynep Melis Arkan

(Chemist)

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ABBREVIATIONS

ADI : Acceptable Daily Intake
AS : Automatic Sampler
ATO : Ankara Chamber of Commerce, Ankara Ticaret Odası
CE : Capillary Electrophoresis
CR-ATC : Continously Regenerated-Anion Trap Column
DC : Detector Chromatography
DEAE : Di-ethylamino-ethyl
DP : Double Pump
EC : European Commission
EG : Eluent generator
FAO : Food and Agricultural Organization
FDA : Food and Drug Administration
GC : Gas Chromatography
GMP : Good Manufacturing Practices
GRAS : Generally Recognized As Safe
HPIC : High Pressure Ion Chromatography
HPICE : High Performance Ion Chromatography Exclusion
HPLC : High Performance Liquid Chromatography
IC : Ion chromatography
JECFA : Joint Expert Committee for Food Additives
LD50 : Lethal Dose
LOD : Limit of Detection
LOQ : Limit of Quantification
MPIC : Mobile Phase Ion Chromatography
NaOH : Sodium Hyroxide
NOAEL : No Observed Adverse Effect Level
PES : Polyethersulfone
RFIC : Reagent-Free Ion Chromatography
RSD : Relative Standard Deviation
TMO : Soil Products Office, Toprak Mahsulleri Ofisi
UP : Ultra Pure
UV : Ultraviolet
WHO : World Health Organization

SYMBOLS

C	**:** Concentration
t	**:** Time
W	**:** Peak width
L	**:** Column length
F	**:** Flow rate
V	**:** Volume
K	**:** Partition coefficient
$\mathbf{k^{-1}}$	**:** Capacity factor
N	**:** Number of plates
$\mathbf{R_s}$	**:** Resolution

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THE ANALYSIS OF FOOD ADDITIVES AND INORGANIC ANIONS IN BREAD BY ION CHROMATOGRAPHIC METHOD WITH CONDUCTIVITY DETECTOR

SUMMARY

In this thesis, preservative food additives such as benzoate, formate, propionate, sorbate, ascorbate, and lactate used in bread and inorganic anions such as chloride, nitrite, nitrate, and sulfate transmitted by environmental factors were simultaneously determined by ion chromatographic system.

Nutrition is one of the most basic needs of human beings to sustain their lives. Bread, which is so important for human health, is the food that people consume most regardless of their income and status, ethnic and age groups. Bread is a basic foodstuff because of its simple production technology, being cheaper and easier to provide than other foodstuffs, high nutritious and satisfying properties. Bread is the leading grain consumption in our country. 400 - 450 g of bread is consumed per person per day and 50 % of daily energy is supplied from bread and cereal products. According to the report, while 1 out of every 10 bread waste produced in Turkey, economic loss is reported to amount to $ 700 million annually. The report says that the most important factor in the disposal of bread is stale, and as a reason for stale was not stored in appropriate conditions.

In the production of bread, the use of various additives is becoming more and more widespread in order to eliminate defects arising from raw materials and processes, to improve bread qualities, to delay staling and to save time. Today there are over 8000 food additives. The number of food additives approved by the FDA to date is around 2800. In the European Union, the number of permitted food additives is 297. As the consumption of these substances increases, there is also evidence of links to certain disorders. The most common diseases are eczema, asthma, headache, allergic itching, gastric disorders, diarrhea, hyperactivity, and hypersensitivity.

The number of preservatives that are allowed to be used in bread is limited and these additives are expressed in food packages with E codes. Lactic, propionic, formic, sorbic, benzoic and ascorbic acids are the most commonly used bread preservatives. And the inorganic anions such as chloride, nitrite, nitrate, sulfate are contaminated from the environment.

According to the World Health Organization (WHO) data, in 2018, 18.1 million people were diagnosed with cancer, while 9.9 million people died of cancer. In another study conducted by WHO, it was found that 15% of cancer formation was caused by genetic factors, 35% by nutrition and 50% by environmental factors. Prevention of this disease, which is so common, is only possible by eliminating the factors that cause it. Therefore, food inspections need to be made more stringent. Gas chromatography, high performance liquid chromatography, capillary electrophoresis, and ion chromatography are the most frequently used methods for these inspections.

Ion chromatography is the development of ion-exchange chromatography that arose during the Manhattan project, which was developed to separate rare earth cations that are very similar to each other with cation exchange resins. This study, which provides theoretical foundations for ion-exchange separations, It was expanded after World War II to be applied to many other items.

Nowadays, ion chromatography is widely used in many areas such as food and beverage analysis, separation and purification of charged molecules, quality assurance and control, sample purity determination, quantitative analysis of ions.

There are many studies in the literature for the determination of food additives. Analytical techniques most commonly used in determining additives in foods; gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and ion chromatography (IC). But each method has its advantages and disadvantages.

Compared to other methods, ion chromatography has many advantages such as sensitive results at mg / L level, nature friendly, high selectivity in complex matrix samples, ion species analysis, In this work, the ion chromatography method was used for the analysis of organic acid anions of these preservatives and inorganic anions. As the first stage of sample preparation, the bread samples collected from various markets were cut into small pieces (approximately 15 mm) and then, dried in the oven at 45 centigrade degrees for one day. The bread samples were weighed and then, diluted 100 times with water. The sonication process was applied to the diluted bread samples for 15 minutes. Then, this was centrifuged at 13500 rpm for 5 minutes to separate from its solid parts. To eliminate organic substances, the supernatants were passed through the C18 SPE cartridges. Finally, the solutions were filtered with PES filters prior to IC analyses.

The separation of these analytes from bread samples were accomplished by using an anion exchange column with an optimized multistep gradient eluent program that utilized 3 mM to 32 mM NaOH. Ten analytes were completely separated within 35 minutes. All the calibration curves showed good linearity ($r^2 \geq 0.999$) within the calibration ranges. Under optimized conditions, device's the limits of detection (LODs) were between 0.0087 and 0.215 μg L^{-1}. To evaluate the recovery test, three different concentrations of standard solutions were added to a bread sample and the average recoveries found between 95.34 and 101.37 %. Precision (RSD%) was evaluated by continuously performing three replicates each day within 3 days for the determination of the samples. The relative standard deviations (RSDs) were less than 5.69 %. The results indicated that this method was simple, rapid, sensitive, and accurate for the simultaneous determination of lactate, propionate, formate, sorbate, benzoate, ascorbate, chloride, nitrite, nitrate, and sulfate in various bread samples.

In 1473, Paracelsus stated the importance of the dose, saying that "All substances are poison, no non-toxic substances. It is the dose that separates the drug from poison." As Paracelsus says, it is very important to quantify the amount of food additives used. A variety of techniques have been tried in the literature for this. However, there are no studies analyzing 10 analytes simultaneously. In this project, a new method was developed for the analysis of bread additives with the IC using conductivity detector for simultaneous and short time analysis without any derivatization. Thus, it is aimed to develop a method that can be applied easily and quickly in the routine analysis of bread, which is one of the most consumed food items, and can analyze all additives at once and at once.

EKMEKTE BULUNAN GIDA KATKI MADDESİ VE İNORGANİK ANYONLARIN İLETKENLİK DEDEKTÖRLÜ İYON KROMATOGRAFİK YÖNTEM İLE TAYİNİ

ÖZET

Bu çalışmada, ekmekte kullanılan benzoat, format, sorbat, propiyonat, askorbat ve laktat gibi koruyucu gıda katkı maddelerinin ve çevre faktörlerinden bulaşan klorür, nitrit, nitrat ve sülfat gibi inorganik iyonların iyon kromatografik sistemle tayini yapılmıştır.

Beslenme, insanın hayatını idame ettirebilmesi için en temel ihtiyaçlarından biridir. İnsan sağlığı için çok önemli bir gıda olan ekmek, insanların gelir düzeyi, etnik ve yaş gruplarına bakılmaksızın en çok tükettiği besindir. Ekmek, diğer gıda maddelerine göre basit üretim teknolojisi, daha ucuz ve kolay ulaşılabilir olması ve besleyici etkileri nedeniyle temel besin maddesidir. Ekmek ülkemizde ki tahıl tüketiminin başında gelmektedir. Günlük kişi başına 400-450 g ekmek tüketilirken, enerji ihtiyacımızın %50'si ekmek ve tahıl ürünlerinden sağlanmaktadır. Yayınlanan raporlara göre, Türkiyede her 10 ekmekten 1'i israf olurken, bu durum ülke ekonomisinde yıllık 700 milyon dolar kayıp anlamına gelmektedir. Ayrıca raporda, ekmeklerin atılmasındaki en önemli faktörün ekmeğin bayatlaması olduğu ve bayatlamasının en temel nedenin ise ekmeğin uygun koşullarda saklanmadığı olarak belirtiliyor.

Ekmek üretiminde, hammaddelerin ve işlemlerin neden olduğu kusurları ortadan kaldırmak, ekmek kalitelerini iyileştirmek, bayatlamayı geciktirmek ve zaman kazanmak için çeşitli katkı maddelerinin kullanımı gittikçe yaygınlaşmaktadır. Bugün 8000'den fazla gıda katkı maddesi bulunmaktadır. FDA tarafından onaylanan gıda katkı maddesi sayısı 2800 iken, Avrupa Birliğinde kullanımına izin verilen gıda katkı sayısı 297' dir. Bu maddelerin tüketimi arttıkça, bazı sağlık sorunlarının da arttığına dair kanıtlar vardır. En sık görülen hastalıklar egzama, astım, baş ağrısı, alerjik kaşıntı, mide rahatsızlıkları, ishal, hiperaktivite ve aşırı duyarlılıktır.

Ekmekte kullanılmasına izin verilen koruyucuların sayısı sınırlıdır ve bu katkı maddeleri E kodlu gıda ambalajlarında ifade edilmektedir. Laktik, propiyonik, formik, sorbik, benzoik ve askorbik asitler en yaygın kullanılan ekmek koruyucularıdır. klorür, nitrit, nitrat, sülfat gibi inorganik anyonlar ise çevreden bulaşır.

Dünya Sağlık Örgütü'nün (WHO) paylaştığı verilere göre, 2018 yılında, 18,1 milyon insana kanser teşhisi konulurken, 9,9 milyon kişi kanserden hayatını kaybetmiştir. Yine WHO tarafından yapılan bir araştırmada kanser oluşumunun % 15'i genetik faktörlerden, %35'i beslenmeden, %50'si çevresel faktörlerden kaynaklandığı bulunmuştur. Bu kadar yaygın olan bu hastalığın önlenmesi ancak ona neden olan faktörlerin giderilmesi ile mümkündür. Bu sebeple gıda denetimlerinin daha sıkı yapılması gerekmektedir. Gaz kromatografisi, yüksek performanlı sıvı kromatografisi, kapiler elektroforez ve iyon kromatografisi bu denetimler için en sık kullanılan metodlardır.

İyon kromatografi, nadir toprak katyonlarının katyon değiştirici reçineler kullanılarak ayrılması için geliştirilen Manhattan projesi sırasında ortaya çıkmış ve zaman içerisinde gelişmiştir. Çalışma alanı, II. Dünya savaşından sonra, başka pek çok maddelere de uygulanabilecek şekilde genişletilmiştir.

Günümüzde, iyon kromatografisi, yiyecek ve içecek analizi, yüklü moleküllerin ayrılması ve saflaştırılması, kalite güvencesi ve kontrolü, örnek saflık tayini, iyonların kantitatif analizi gibi birçok alanda yaygın olarak kullanılmaktadır.

Gıdalardaki katkı maddelerinin belirlenmesinde en çok kullanılan analitik teknikler; gaz kromatografisi (GC), yüksek performanslı sıvı kromatografisi (HPLC), kapiler elektroforez (CE) ve iyon kromatografisidir (IC). Fakat her yöntemin avantajları ve dezavantajları vardır.

Diğer yöntemlerle kıyaslandığında iyon kromatografisinin mg/L seviyesinde hassas sonuçlar vermesi, doğa dostu oluşu, karmaşık matris örneklerinde yüksek seçicilik sağlaması, iyon türlerinin analizi, ayırıcı kolonların stabilitesi, hızlı sonuçlar vermesi, az miktarda örnek ile çalışılabilmesi gibi birçok avantajı vardır.

Bu tez kapsamında, gıda katkı maddelerinin ve inorganik anyonların analizi için iyon kromatografi yöntemi kullanılmıştır. Örnek hazırlamanın ilk aşaması olarak, çeşitli marketlerden toplanan ekmek örnekleri küçük parçalar halinde (yaklaşık 15 mm) kesilmiş ve 1 gün boyunca 45 santigrat derecede fırında kurumaya bırakılmıştır. Ekmek örnekleri 1:100 oranında su ile seyreltilmiştir. Sonikasyon işlemi, seyreltilmiş ekmek örneklerine 15 dakika boyunca uygulanmıştır. Daha sonra analitlerin katı kısımdan ayrılması için 13500 rpm'de 5 dakika santrifüjlenmiştir. Son olarak, numune, C18 kolonu ve PES filtresinden geçtikten sonra kolona verilmiştir. Bu analitlerin ekmekten ayrılması, 3 mM ila 32 mM NaOH arasında kullanılan optimize edilmiş çok aşamalı gradyanlı eluent programı olan bir anyon değiştirme kolonu kullanılarak gerçekleştirildi. On analit 35 dakika içinde tamamen ayrıldı. Tüm eğriler kalibrasyon aralıklarında iyi doğrusallık ($r2 \geq 0.999$) gösterdi. Optimize edilmiş koşullar altında, saptama sınırları (LOD'lar) 0,0087 ila 0,215 $\mu g\ L^{-1}$ arasında bulundu. Geri kazanım testini değerlendirmek için üç farklı standart çözelti konsantrasyonu numuneye ilave edildi ve ortalama geri kazanımlar % 95.34 ila 101.37 arasında bulundu. Hassasiyet (RSD), numunelerin tespiti için 3 gün içinde her gün sürekli üç tekrar yapılarak değerlendirildi. Göreceli standart sapmalar (RSD'ler) % 5.69' dan az bulundu. Sonuçlar, bu yöntemin, çeşitli ekmek örneklerinde laktat, propiyonat, format, sorbat, benzoat, askorbat, klorür, nitrit, nitrat ve sülfatın aynı anda belirlenmesi için basit, hızlı, duyarlı ve doğru olduğunu gösterdi.

Paracelcius 1473 yılında 'Tüm maddeler zehirdir, zehir olmayan madde yoktur. Zehir ile ilacı ayıran dozdur.' diyerek dozun önemini belirtmiştir. Paracelsus'un da dediği gibi, kullanılan gıda katkı maddelerinin miktarını ölçmek çok önemlidir. Bunun için literatürde çeşitli teknikler denenmiştir. Ancak, aynı anda 10 analiti analiz eden hiçbir çalışma yoktur. Bu projede ekmek katkı maddelerinin türevlendirme gerektirmeden, aynı anda ve kısa sürede analizleri için, iletkenlik dedektörü kullanarak IC ile analizi için yeni bir yöntem geliştirilecektir. Böylece en çok tüketilen gıda maddelerinin başında gelen ekmeğin rutin analizinde kolaylıkla ve hızlı bir şekilde uygulanabilecek ve bütün katkı maddelerinin aynı anda ve tek seferde analizleyebilecek bir yöntem geliştirmek hedeflenmiştir.

1. INTRODUCTION

Chromatography; is a method of separating and purifying the substances in a mixture by the immiscible two-phase system, one fixed and the other mobile phase. Due to its rapid, simple and effective separation method, it is frequently encountered in recent years. Chromatography is one of the most widely used analysis methods for qualitative analysis and quantitative analysis of the components.

Ion exchange chromatography is the chromatographic method in which ions and ionizing species can be analyzed by the ion-exchange mechanism. In this method; charged groups connected by chemical bonds to the ion exchanger forming the stationary phase are replaced by similar ions in the mobile phase. This displacement can be reversed at any time. Thus, the exchange of ion-exchangeable substances (acids, antibiotics, amino acids, alkaloids, etc.) is achieved.

Properties such as high sensitivity, low limit of detection for many inorganic ions, matrix problems that confuse other methods are not seen in ion chromatography, suitable for routine analysis, makes the ion chromatography method advantageous compared to other chromatographic methods. Ion chromatography is a widely used technique for the investigation of ionic components of various materials.

Ion chromatography is used in toxicology, forensic medicine, drinking water, and wastewater analysis, air pollution measurement, industrial waste analysis, determination of ionic species in biological solutions, food and beverage analysis, mass spectrometry or other spectroscopic methods in the separation of components and cations, organic acids, amines, amino acids, carbohydrates or nucleic acids.

Nutrition is one of the most basic needs of human beings to sustain their lives. Bread, which has been an important part of nutrition for centuries; it is the most common nutrient of humanity consumed all over the world. Today, bread has started to take its place in the market as differentiated products that meet changing consumer preferences. In addition to white bread, the sale of many different breads such as rye, oats, whole grain, corn, bran, and packaged products has started to increase.

Besides being a source of fiber, bread is considered as a nutrient with positive health contribution in terms of high carbohydrates, minerals, vitamins, and fat content. It also helps to protect the human body from infectious diseases by containing B group vitamins. It is an indispensable source of nutrients due to its rapid healing of wounds, prevention of arteriosclerosis and blockage, eye health, the beauty of the skin and protection against cancer thanks to vitamins E which are abundant in cereal products.

Bread, which is so important for human health, is the food that people consume most regardless of their income and status, ethnic, and age groups. In the 2007 edition of the Guinness Book of Records, the country which consumes most bread has chosen Turkey. Although daily bread consumption varies according to individuals' characteristics, habits, life-working styles, and dietary composition, bread appears to be the basic nutrient.

Bread, which consists mainly of flour, water, salt, and yeast, has a short shelf life. The shelf life of bread is limited by changes such as stale and microbial deterioration. About 24 hours after leaving the oven, except for caused by microorganisms, all changes in bread are called stale. Stale bread loses its sensory properties and causes the consumer to reject bread even though it has no harmful effect on health. For this reason, tons of bread are wasted during the year and this causes a significant economic loss.

10 to 18 percent of the bread which produced in the world is wasted as surplus production and consumption surplus, and; this leads to significant losses in the world economy. As a solution to this issue, antimicrobial food additives are added to the bread in order to delay the deterioration of bread and prevent the growth of microorganisms that can reproduce. In the production of bread, the use of various food additives is becoming widespread in order to eliminate defects arising from raw materials and processes, improve bread qualities, delay staling and save time. These food additives are not harmful to health when added to very low levels of food. However, as the amount increases, the nutritional value of the food decreases and health problems such as asthma, allergy, and cancer, etc. may arise.

The use of food additives in food is limited by various laws in each country. Food additives regulations are made by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). In Turkey, the rules determined by these two

associations cooperating with the Joint Expert Committee on Food Additives (JECFA) are followed.

The preservatives which allowed for bread are limited. Acetic, benzoic, formic, propionic, sorbic, ascorbic, and lactic acids are the most widely used food preservatives. Because of their strong antimicrobial effect, as well as their lack of taste and odor, propionates are widely used as a mold inhibitor in the food industry. The highest recommended use of bread is reported to be 0.32 %. It is known to cause migraine pain when taken in large amounts. Although sorbates are more effective than propionates in preventing mold growth, they are less preferred due to their detrimental effects on dough and bread properties. It is added to food at a rate of 0.1-0.2%.

Benzoates are effective in yeast and bacteria. When sodium benzoate is mixed with very small amounts (0.5 mg/g per day), it does no harm for health. However, if this amount increases, both the nutritional value of the food decreases and health problems arise. When taken in large amounts of benzoic acid can cause various allergic reactions such as asthma or skin rashes. As an antimicrobial agent, acetic acid acts against bacteria. According to the researches, acetic acid and calcium salt are very effective for "rope" disease. The concentration of calcium acetate of 0.17 % is the most suitable amount used in the bakery. Formic acid obtained from ant secretions in nature can cause serious damage to the optic nerves such as respiratory failure and severe fatal consequences.

Ascorbate, also known as Vitamin C and abundant in vegetables and citrus fruits, is a commonly used antioxidant in foods. When used as an additive, it acts as a developing agent in the dough. Usually, it is used 25-75 mg/g in foods. Lactic acid obtained by fermenting lactose is encountered in many foods we consume daily. Although it is not limited to daily use is not recommended for young children.

Determination of anions such as chloride, nitrite, nitrate, and sulfate which are transmitted to food from various sources such as water and air, is also an important issue. The anions taken into the body are tolerated at different levels by the metabolism and in some cases, they may be highly toxic to the body.

Reliable, fast and simple analytical methods are necessary to assess the safety and quality control of food additives in the bread. Various analytical methods for the determination of these food additives have been reported in the literature. The aim of

this study is to develop a new, environmentally friendly method with ion chromatography that provides a direct, precise, cheap, fast, low limit of detection and has high reproducibility compared to the methods in the literature.

2. CHROMATOGRAPHY

2.1 General Description of Chromatography

Chromatography is a combination of Greek chroma (color) and graphein (writing) and the first time was used for the separation of colorful plant pigments by the Russian botanist Michael Tswett in 1903 [1]. Afterward, it has begun to be used in the separation and purification of components in various multicomponent samples. Today, chromatographic analysis methods are the most commonly used instrumental analysis methods for qualitative and quantitative analysis of the components of a mixture. Using these methods, it is possible to purify substances which are very difficult to separate from each other by other methods. Chromatographic methods have been developing rapidly in recent years and have been used in many years especially in terms of cheapness, simplicity, and reliability.

Chromatography systems and methods are very diverse. The common feature of all these systems and methods is that a “mobile phase” and a “stationary phase” are used for separation. In chromatography, the phase attached to a column or a flat surface is called the stationary phase; the phase including the analyte passing through or between the stationary phase is called the mobile phase. In chromatographic methods, the components in the mixture are separated from each other by passing the mobile phase through the stationary phase. During this separation, the two phases interact with each other. The interactions of the components with different physical and chemical properties to both phases are of a different extent. Thus, components driven by the mobile phase leave the stationary phase at different times since they are held to a different degree by the stationary phase. In the separation medium, the least retained component in the separation medium is first and the most retained component is last removed in the separation medium. As a result of the differences in these travel speeds and retention times, the sample components are separated from each other into different bands or regions that can be analyzed qualitatively and quantitatively.

Classification of Chromatographic Methods: Classification by Separation Mechanism [Adsorption Chromatography, Partition Chromatography, Ion chromatography, Gel permeability, Zone Electrophoresis, Affinity Chromatography, Chiral Chromatography] Classification according to mobile phase [Liquid Chromatography (LC), Gas Chromatography (GC)] Classification by Applied Technique [Column Chromatography, Layer Chromatography], Classification by Intended Use [Analytical Chromatography, Preparative Applications]

2.2 Ion Chromatography

Ion-exchange chromatography (ion chromatography) can be classified as the type of liquid chromatography applied to ions. The method is generally used for the separation of ions and ionizing species, for the qualitative distribution of oligomeric ion mixtures, and for concentration and simultaneous determination of ionic species in samples with low concentration and interfering matrices of several ions in the mixture at the same time [2]. Anion exchange resins are used to separate anions, and cation exchange resins are used to separate them with cation.

Ion-exchange chromatography, which emerged during the Manhattan project, was extended to World War II to be applied to many other substances. Modern ion chromatography was first introduced by Small et al. In this method, the conductivity detector is connected to ion chromatography to determine anions such as Cl^-, SO_4^{2-}, NO^{3-}, and PO_4^{3-} and cations such as Na^+, NH_4^+, K^+ and Ca^{2+}.

2.2.1 Types of ion chromatography

2.2.1.1 Ion-exchange chromatography (high performance ion chromatography, HPIC)

This separation method is based on an ion exchange process that occurs between the ion exchange groups bound to the column and mobile phase. The stationary phase consists of a polystyrene resin copolymerized with divinylbenzene. Ion exchange chromatography is used to separate organic, inorganic anions and cations. The separation of anions takes place with quaternary ammonium groups bound to the polymer. Sulfonate groups are used to separate cations [3].

2.2.1.2 Ion-exclusion chromatography (high performance ion chromatography exclusion, HPICE)

Ion-exclusion chromatography is used to separation of weak inorganic and organic acids from those acids which are completely dissociated at the eluent pH. All acids with high acid strengths are not retained and elute unresolved within the void volume. This separation method is also useful for determining amino acids, aldehydes and alcohols.

2.2.1.3 Ion-pair chromatography (mobile phase ion chromatography, MPIC)

Ion-pair chromatography is particularly suited for the separation of surface-active anions and cations as well as transition metal complexes. With the invention of modern grafted or hyperbranched ion exchangers, the use of ion-pair chromatography has been reduced to the analysis of surface-active ions today. However, the biggest advantage of ion pair chromatography is that the selectivity of separation can be determined by the type of mobile phase. Thus, both anionic or cationic compounds can be separated using the same stationary phase.

2.2.2 The process of ion chromatography

Ion exchange chromatography basically involves 5 steps (Figure 2.1).

Step 1: The eluent loaded on the column replaces any anion bound to the resin, so that the resin surface is saturated with the eluent anion.

Step 2: A sample is injected onto the column containing anion A and anion B.

Step 3: After the sample is injected, the Sample moves through the column with an ongoing eluent flow. Anion A and anion B interact differently on the surface of the column and move across the column at different speeds.

Step 4: As the eluent flow continues, the anion A first leaves the column.

Step 5: Then anion B leaves the column.

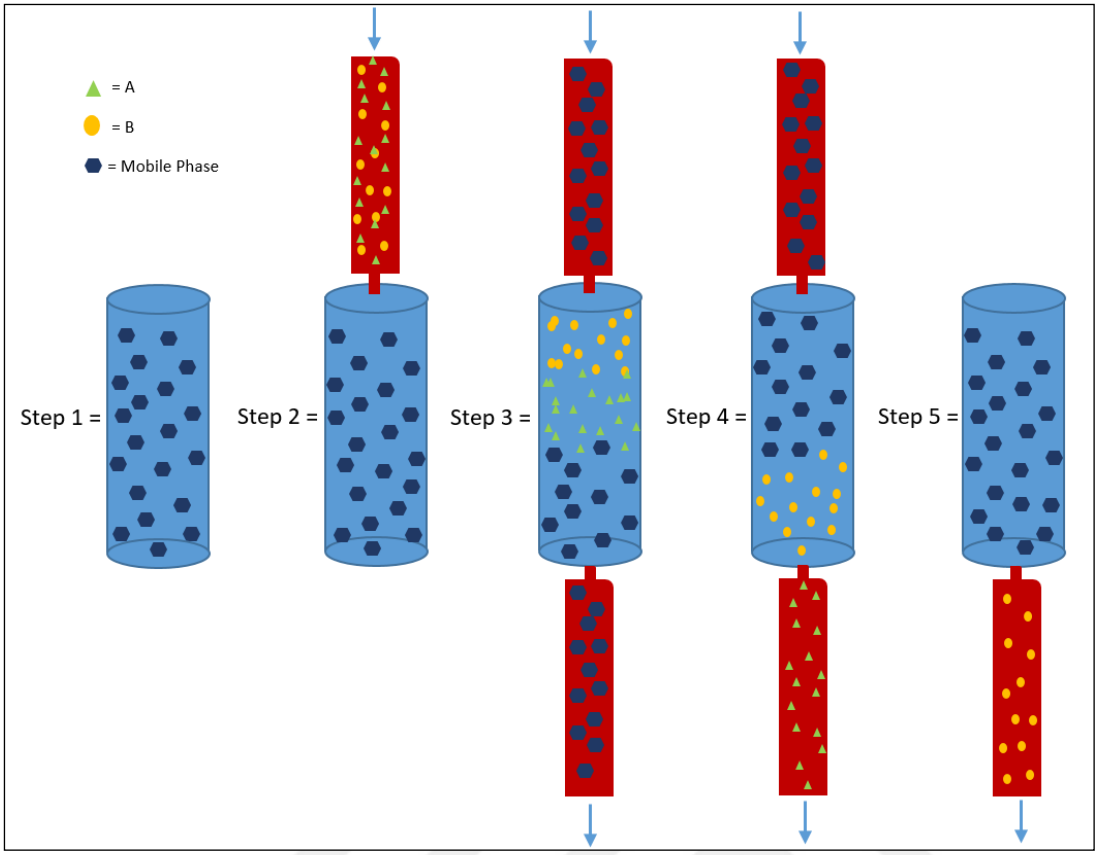

Figure 2.1 : Schematic representation of IC process.

2.2.3 The system of ion chromatography

The basic components of an ion chromatography are shown schematically in Figure 2.2 The main components of an ion chromatography device can be examined in five sections. These are pump, injector, column, suppressor, and detector [4].

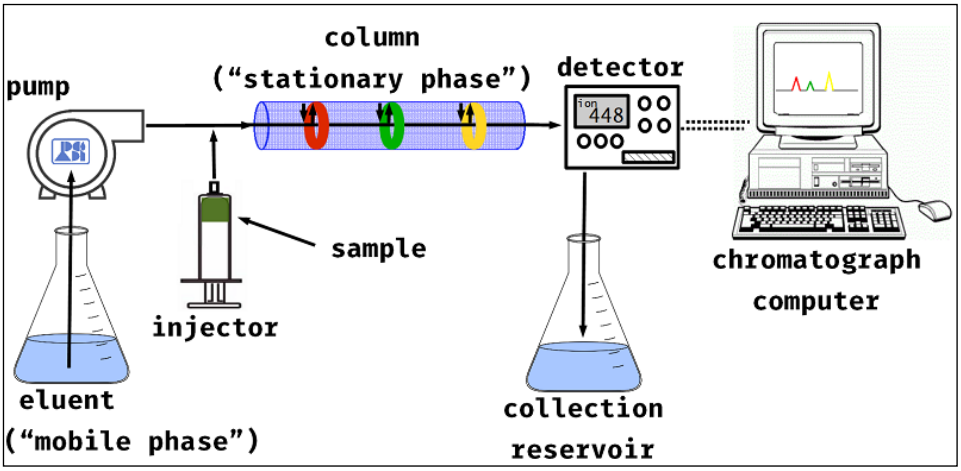

Figure 2.2 : Main components of ion chromatography apparatus.

2.2.3.1 Pump

The pump allows the solvent mixtures forming the mobile phase to pass through the injector, column, and detector at a fixed or variable speed with the help of a certain pressure. Constant flow pumps are the most commonly used pumps in ion chromatography. Constant flow systems have two basic types; reciprocating piston and dual piston pumps. Dual piston pumps are the most widely used in IC. It has advantages such as provides a constant flow, unlimited solvent reservoir allowing long-term unattended use, quick changeover, and clean out capability, wide flow rate range.

2.2.3.2 Injector

The injector is the part that is responsible for including the sample in the system. Sample entry can be done in various ways. but the simplest way is to use an injection valve. In the more advanced IC, automated systems are used in which sample promotion is made with the help of automatic sampling devices. Automatic injectors provide great convenience in unattended operation due to the flowrate and temperature optimization.

2.2.3.3 Column

The column is the fixed phase in which the analytes in the solution are captured and separated. An ion exchanger is usually a matrix (stationary phase) containing charged groups. If a group is negatively charged, a cation exchanger is used to replace positive ions. The most commonly used group in the cation exchanger is the carboxy-methyl group. However, when a group is positively charged, an anion exchanger is used to replace negative ions. Di-ethylamino-ethyl group (DEAE) is the the most commonly used group in the anion exchange chromatoraphy.

Guard column is placed in front of to the separating column. They are dependable columns designed to filter particles blocking the separation column. Thanks to this filtering process, the life of the separation column is extended.

2.2.3.4 Suppressor

Suppressors are systems used to increase the conductivity of the ion by reducing the background conductivity of the eluent. IC suppressors are membrane-based devices designed to convert ionic eluent into water for increased sensitivity.

2.2.3.5 Detector

The detector is a system that detects the signal generated by the ions separated from the separation column at different retention times. An ideal detector should have the following properties: low noise level, high sensitivity, fast response, low dead volume, wide linear dynamic range, low drift level, insensitivity to changes in type of the solvent, flow rate, and temperature, operational simplicity and reliability.

Conductivity detector is widely used in IC. The conductivity detector is used to measure the conductivity of the mobile phase. There is a suitable flow cell with two electrodes inside the conductivity detector system; electrodes are placed on one arm of a Wheatstone bridge with impedance component. Since the impedance between the electrodes changes when the ions move through the sensor, an equivalent amount of compensation signal is generated from the bridge. Ions are detected this way.

2.2.4 Factors affecting the effectiveness of ion chromatography

2.2.4.1 pH

The number and shape of ions can change with the pH of the mobile phase. pH increase in anion exchange resins increases retention time, while pH increase in cation exchange resins decreases retention time.

2.2.4.2 Ionic power

The effect of ionic power on solvent power is enormous. The increase of the ionic power increases the solvent power.

2.2.4.3 Temperature

The temperature increase lowers the mobile phase viscosity and the displacement ratio of the dissolved ions between the mobile phase and the fixed phase also increases. Reduces the retention time.

2.2.4.4 Flow rate

Unlike other HPLC systems, the low flow rate must be provided for Max separation and mass transfer kinetics.

2.2.4.5 Organic modifier

They are organic structures added to the mobile phase and increase the mass transfer kinetics by reducing the viscosity.

2.2.4.6 Salt

Solvent power will be affected by all ionic species in the environment. Adding salt has an effect that will cause pH change.

2.2.5 The applications of ion chromatography

Today, ion chromatography is used in many fields due to its strong properties such as:

Sensitive results at mg / L level, High selectivity in complex matrix samples, Ion species analysis, Stability of the separator columns, Fast results, The small amount of sample work.

It has been applied to hundreds of problems involving ionic analysis in clinical, food, pharmaceutical, industrial, plating solution, and environmental samples. Some of them are listed below.

Food and beverage analysis, Separation and purification of charged molecules, Quality assurance and control, Sample purity determination, Quantitative analysis of ions

2.2.6 Method validation

Validation is the operations performed to demonstrate that the performance of a device or system meets the specified conditions. Method validation, on the other hand, is to prove the suitability of a method or measurement procedure for the specified purposes by testing it objectively.

2.2.6.1 Sensitivity and selectivity

Indicates how much the substance analyzed according to a certain method interacts with other substances.

2.2.6.2 Specificity

The ability of a method to determine only the analyte that is searched for if there are other components than those sought in the sample matrix under specified test conditions.

2.2.6.3 Detection limit (LOD) and measurement limit (LOQ)

The detection limit (LOD); the analyte is the lowest concentration level that is statistically significantly different from that of a "blank" sample.

Measuring limit (LOQ); the lowest analyte concentration measured by acceptable precision and reality.

2.2.6.4 Linearity / measuring range

This is the analyte concentration range where the method can meet criteria such as precision and reality at acceptable levels. The lower limit of the measuring range is the measuring limit (LOQ).

2.2.6.5 Trueness

The degree of agreement between the measurement (analysis) results and the actual analyte amount in the sample. Reality studies:

With certified reference material, With the proficiency test example, With "added standard" samples (recovery tests) done.

2.2.6.6 Repeatability

It is a measure of the closeness of the results of a method, which are made by the same person in the same laboratory, with the same device, in a short time interval.

3. BREAD

3.1 Importance of Bread in Nutrition From Past to Present

Ever since humankind existed, there were substances we call nutrients. Wheat has the most important place in nutrition due to its high gluten content. For this reason, wheat is the world's most produced grain with more than 220 million planting areas. A total of 3000 varieties of wheat have been identified in the world. The most grown wheat type is triticum aestivum, also called bread wheat. Approximately 600 million tons of grain produced around 2 billion tons in the world is wheat [5]. In our country, about 20 million tons of wheat are produced every year. Although cultures are different, bread has been a passion unifying all people throughout history. This beautiful and valuable nutrient is the most important source of food produced and consumed not only in our country but also all over the world.

Prepared by mixing flour, water, salt and yeast, the bread has a unique status all over the world from past to present. Bread is a basic food item because of its simple production technology, it is cheaper and easier to provide than other foodstuffs and has high nutritional and satisfying properties. As it has a neutral taste and aroma, it acts as an ideal carrier for the consumption of other aromatic foodstuffs. It is a satisfying and energy source. Bread, which is the main source of food produced and consumed in the world, has not been easy to come from field to table. Since 10 thousand years ago, great progress has been made in agriculture and many important steps have been taken in the field of technology, from cultivation, harvesting, grinding, dough to cooking.

3.2 Nutritional Value of Bread and Its Effects on Health

Bread has different nutritional values according to the material it is made. Basically, 100 grams of wheat bread, which contains carbohydrate, protein, and fat as the main nutrients, contains 53.1 grams of carbohydrates, 9.1 grams of protein and 1.2 grams of fat and gives 246 calories. It also contains 84 mg calcium, 2.4 mg iron, 23 micrograms

B1, 14 micrograms B2 and 120 micrograms Niacin as a mineral [5]. In Table 3.1, the nutritional values of bread types can be seen more clearly.

Table 3.1 : Nutritional Values of Bread Types.

Bread Type	Moisture (g)	Protein (g)	Carbohydrate (g)	Energy (cal)	Calcium (mg)	Iron (mg)	Vitamin B1 (mg)	Vitamin B2 (mg)	Niacin (mg)
White Bread	31.8	9.1	56.4	276	7	0.7	0.09	0.06	0.8
Rye Bread	35.5	9.1	52.1	243	75	1.6	0.18	0.07	1.4
Brown bread	34	9.1	53.1	246	84	2.4	0.23	0.14	1.2

Turkey is one of the most consuming bread public on earth. In Turkey, 66 % of the energy consumed by people in their daily lives is obtained from cereals and 56 % of this energy is supplied from bread alone. Bread, which is one of the main foodstuffs, provides 50 % of the total calories in 53 % of the world countries and more than 30 % of the calories taken in 87% of the world countries. Studies show that even in western European countries, which are said to be under-consumed, it provides 30 % of protein, 50 % of carbohydrates and 50 % of B-group vitamins [6].

The yeast used in bread making is also vital for our body. Beta-Glucan, a powerful immune system promoter, is a completely natural food source found in yeast because it activates the immune system by binding to specific surfaces on macrophages (white blood cells), which are the first defense of our immune system. This substance initiates the effect that activates the cells that serve as a defense against infections, destroys foreign cells entering the body and activates the body's defense mechanism. As a result, it occurs in a strong immune system. Beta-glucan; it also contributes to the reduction of cholesterol and blood sugar levels, helps to heal damaged, damaged tissues in the body and has antioxidant properties [7].

A research in the literature shows that the supporting tissue of the plants, pulp, is of great importance for human health. The pulp, which is polysaccharides such as cellulose, hemicellulose, and pectin, and phenylpropane such as lignin; is not digested by enzymes in the digestive tract and provides movement by creating a certain volume in the intestines. This ensures that residual substances, which are composed of nutrients and the body's secretions, are removed from the body without being

converted to harmful substances. Large bowel diseases are rarely seen in populations fed with high diets, while these diseases constitute a major health problem in western societies fed with low diets. The best source of pulp is bran and dry legumes. Therefore, the consumption of whole wheat bread is especially recommended in western countries.

Whole grain bread is rich in nutrients such as pulp, vitamin E, selenium, iron, magnesium, zinc and B vitamins (B1, B6, niacin). B vitamins are important in strengthening the immune system, development of learning and cognitive functions, prevention of anemia, certain birth defects, cardiovascular diseases, and cancer [8].

Today, one of the most common disorders is tooth decay. Carbohydrates in the oral cavity cause tooth decay and it is known that bacteria in saliva produce more organic acid in the starchy environment than sugared environment. Previously, based on this information, cereal starch was thought to cause more tooth decay than sugar. However, research has revealed that white and dark bread does not cause tooth decay. On the contrary, it is estimated that brown bread has a phytatin cariostatic effect (tooth decay effect) [9]. In a study on this subject, it was claimed that high gluten, appetizing fresh bread cause less tooth decay than stale and low gluten bread.

One of the lesser-known features of bread is the effect of bread crust on human mental and physical performance. In a study conducted on school children and factory workers, it was found that workers fed with bread crust and fruit had a higher effect on performance than on the inner part of the bread and maintained their blood sugar levels for a longer period. Therefore, bread with plenty of crust is recommended for both physical and mental exertion.

3.3 Bread Consumption Statistics in the World

Bread consumption varies depending on some factors such as gender, age, occupation, income level and habits of individuals. The amount of bread consumed per person per day varies between 150 and 700 grams. An average physically working person consumes 450 grams of bread per day and heavy construction workers consume an average of 760 grams of bread per day. Bread consumption is higher in families with low socio-economic status than families with good socioeconomic status. Besides, cultural and educational differences directly affect the amount of bread consumption.

However, in general, approximately 400 - 450 g of bread is consumed per person per day in our country [10,11].

In research of the TR Ministry of Commerce, conducted to determine the relationship between gender and the amount of bread consumption, a significant relationship was found between gender and the amount of bread consumed. Daily bread consumption, which is parallel to the intensity of daily physical activity, was found to be less in women [12]. In Figure 3.1, the change in the consumption of bread depending on gender can be seen more clearly.

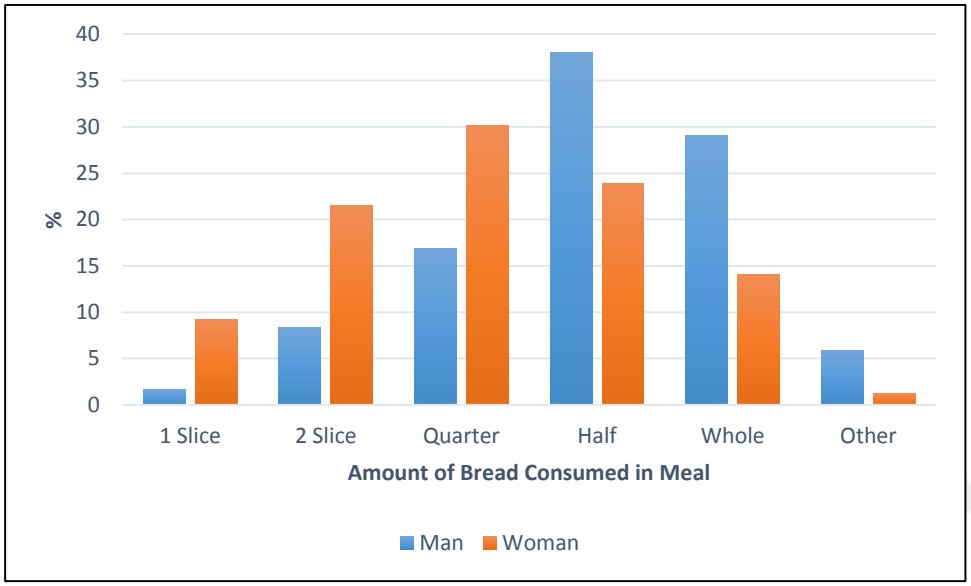

Figure 3.1 : Relationship between age and bread consumption.

It is claimed that in study, it was found that there was a significant relationship between age and the amount of bread consumed at a meal. Bread consumption shows an increasing graph between the ages of 19 and 51. This is thought to be due to the intensity of physical activity and consumption habits [12]. In Figure 3.2, the change in the age-related bread consumption rate can be seen more clearly.

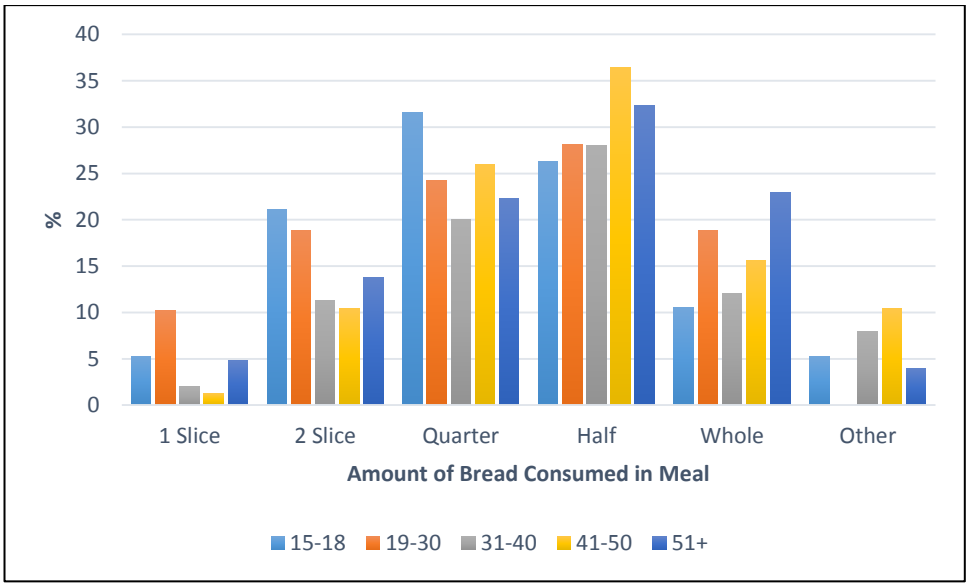

Figure 3.2 : Relationship between gender and bread consumption.

It is seen that there is a significant relationship between education level and bread consumption. While the consumption of one slice of bread is higher among the master's and undergraduate graduates, the consumption of whole and half bread at the meal is higher among the primary and secondary education graduates. This shows that there is a change in the consumption of bread in parallel with the level of education and that there is a tendency for less consumption in people with a high level of education [12]. In Figure 3.3, the change in bread consumption rate depending on the education level can be seen more clearly.

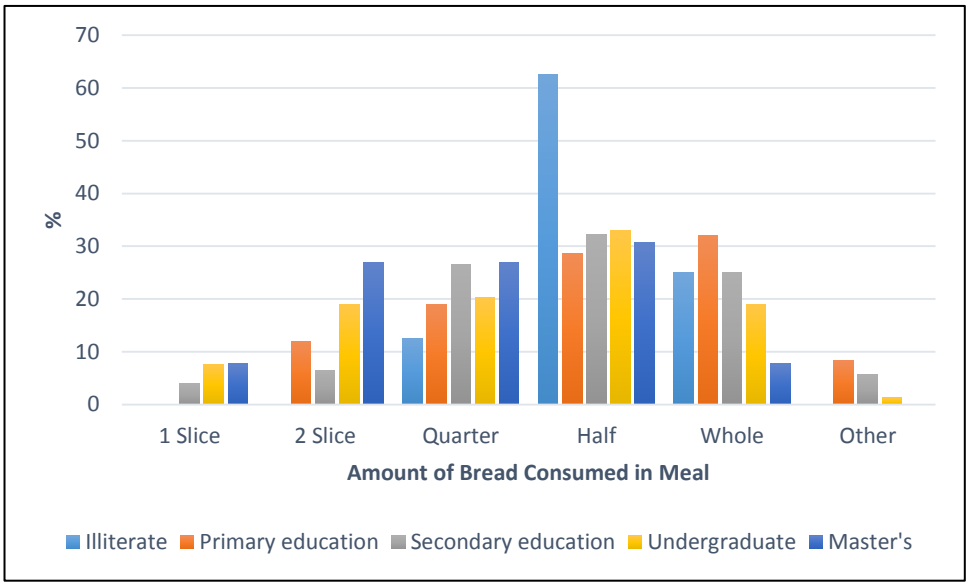

Figure 3.3 : Relationship between education and bread consumption.

It was found that there was a significant relationship between occupational status and the amount of bread consumed at a meal. While the consumption of one, two slices of bread was generally observed in the officer and student groups, the consumption of whole and half bread in the meal was seen in the worker's individuals. This shows that individuals working in occupational groups with less physical activity tend to have less consumption [12]. In Figure 3.4, the change in bread consumption rate depending on the occupation of the consumers can be seen more clearly.

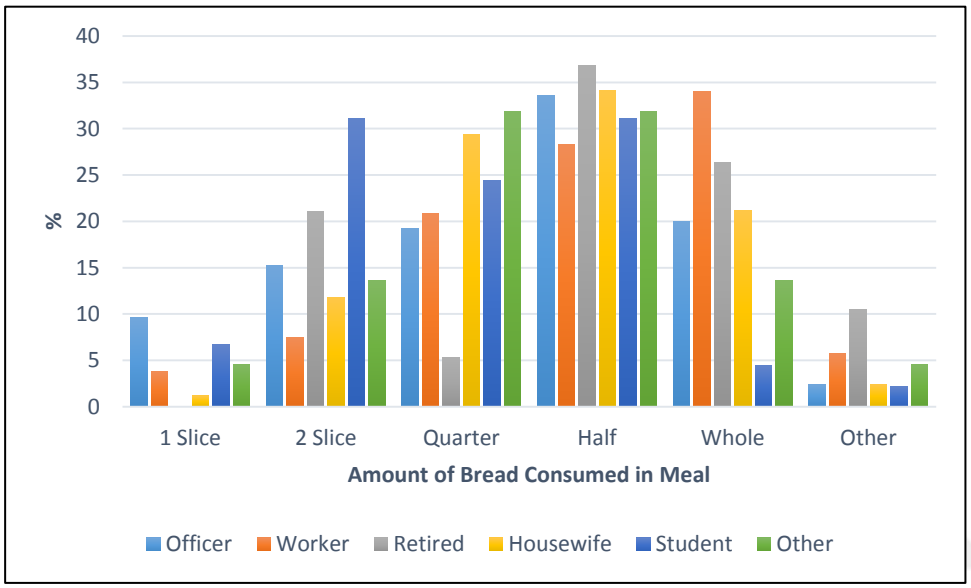

Figure 3.4 : Relationship between occupation and bread consumption.

There was a significant relationship between the income level of consumers and bread consumption. As the income level increases, all bread consumption decreases. Due to the increase in income level, it is thought that the variety of food types increases and this leads to a decrease in bread consumption [12]. In Figure 3.5, the change in bread consumption rate depending on the income level of the consumers can be seen more clearly.

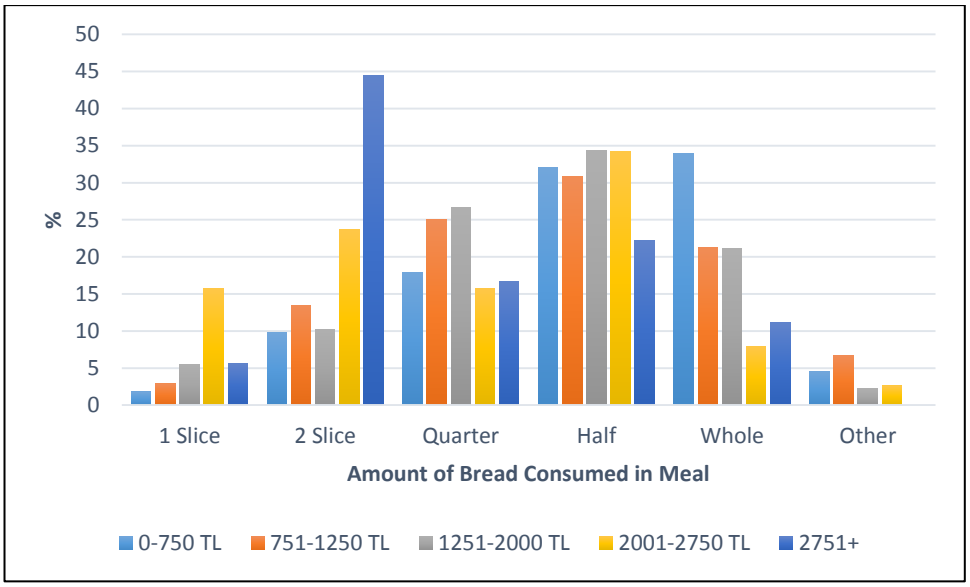

Figure 3.5 : Relationship between income level and bread consumption.

When we examined the relationship between the number of individuals in the family and the number of daily bread taken home, it was found that there is a significant relationship. As the number of individuals in the family increases, the number of bread taken daily increases. The majority of households with 7 households and overbuy more than six bread, while the majority of families with 1-2 people buy one [12]. This can be seen more clearly in Figure 3.6.

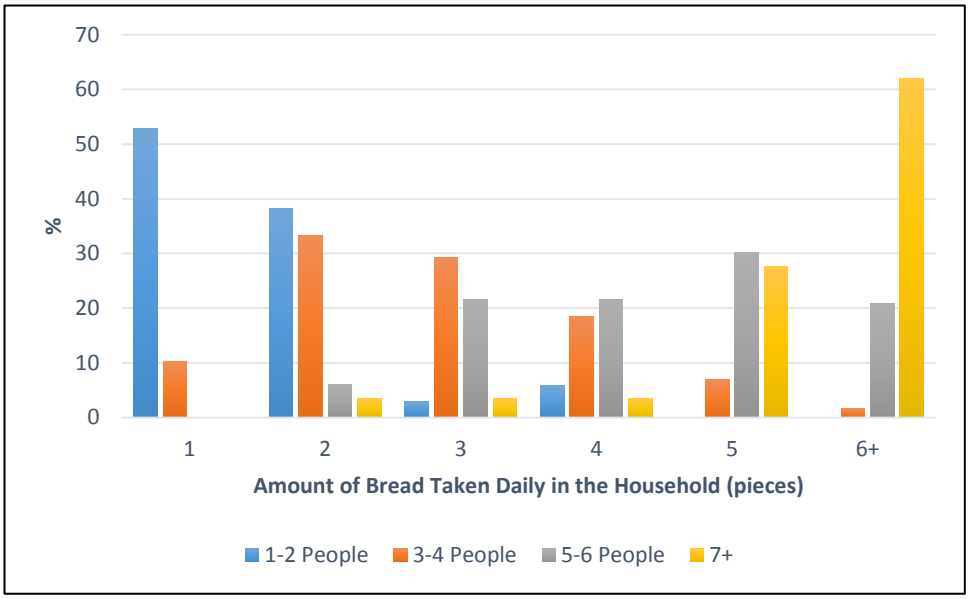

Figure 3.6 : Daily amount of bread taken to households (pieces).

3.4 Bread Waste

Some people starving in Africa, particularly in urban areas is being wasted bread in Turkey. According to the data of the Istanbul Bakers' Chamber, 18 million pieces of bread are produced daily in Istanbul, 2 million of which are thrown away. According to the data, of Soil Products Office (TMO), the total daily bread production in Turkey, 101 million; consumption is 95 million and 6 million of it is wasted [13].

According to the results of TMO's research in 2013, 4.9 million pieces of bread wasted in one day; 3 million were wasted in bakeries (62.1%), 1.4 million in households (27.7%), 0.5 million in personnel and student cafeterias, restaurants, and hotels (10.2%) (Figure 3.7).

Ankara Chamber of Commerce (ATO) official website published in 2004, "Lost Economy in Bread" report showed that bread waste in our country has reached significant dimensions. According to this report; nine out of every 10 bread are consumed and one is wasted. Approximately 44 billion bread are produced annually in our country, 40 billion of these bread are consumed and 4 billion of them are wasted. Thus, the daily loss amount exceeds 1.9 million dollars and exceeds 700 million dollars annually. In the report, the most important factor in the disposal of bread is stale [14].

According to the data collected in TMO's "Don't Waste Your Bread" campaign; 500,000 pieces of bread were wasted in Istanbul in 1991. As shown in figure 3.8, this number increased to 700,000 in 1994, to 3,750,000 in 2000, and in 2015, nearly 5.5 million bread went to waste. With the population increase over the years, the number of ovens has also increased. There has also been an increase in bread production and consumption [15].

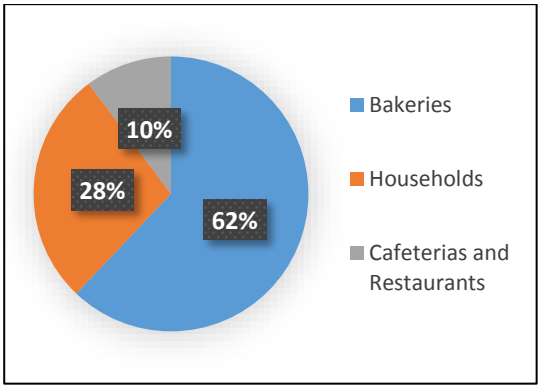

Figure 3.7 : Bread % waste rate.

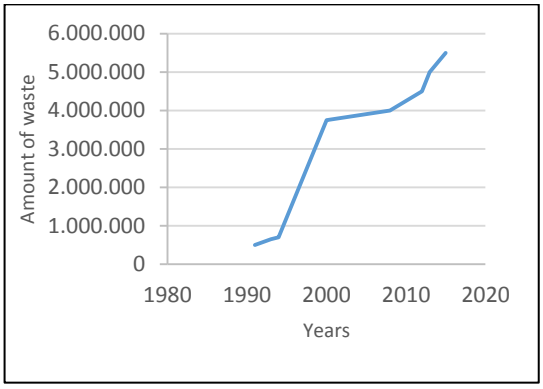

Figure 3.8 : Bread waste rate by years.

In research of the TR Ministry of Commerce, 44.3% of men and 44.2% of women make bread waste. However, there was no significant relationship between the two variables. However, a significant relationship was found between the age of consumers and bread waste. Bread waste is higher in younger age groups (Figure 3.9). This shows that older age groups are more conscious [12].

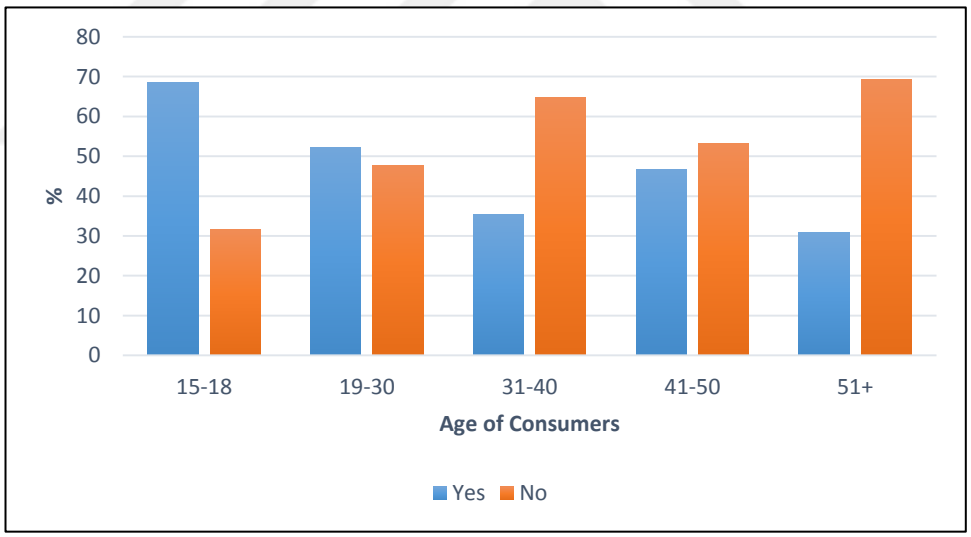

Figure 3.9 : The relationship between the age of consumers and bread waste.

It was determined that there is a significant relationship between the education level of consumers and bread waste. Bread wastage rate was lower in illiterate than in graduate education (Figure 3.10). This result shows that consumers with graduate education are not very sensitive to the waste of bread [12].

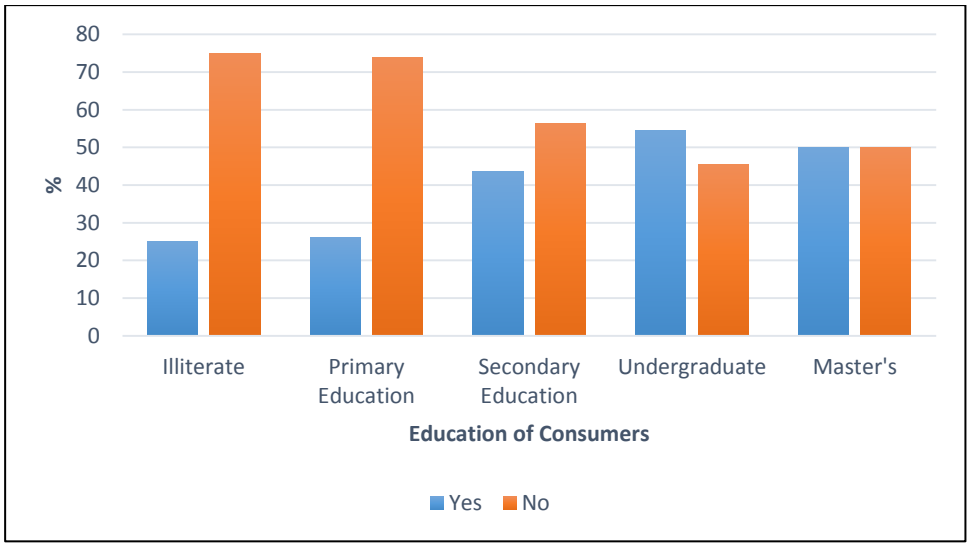

Figure 3.10 : The relationship between the education of consumers and bread waste.

It was found that there is a relationship between the professions of consumers and bread waste. Accordingly, bread waste is less by retirees and housewives, and more in students and workers (Figure 3.11). It is thought that the economic situation plays an important role in consumption habits [12].

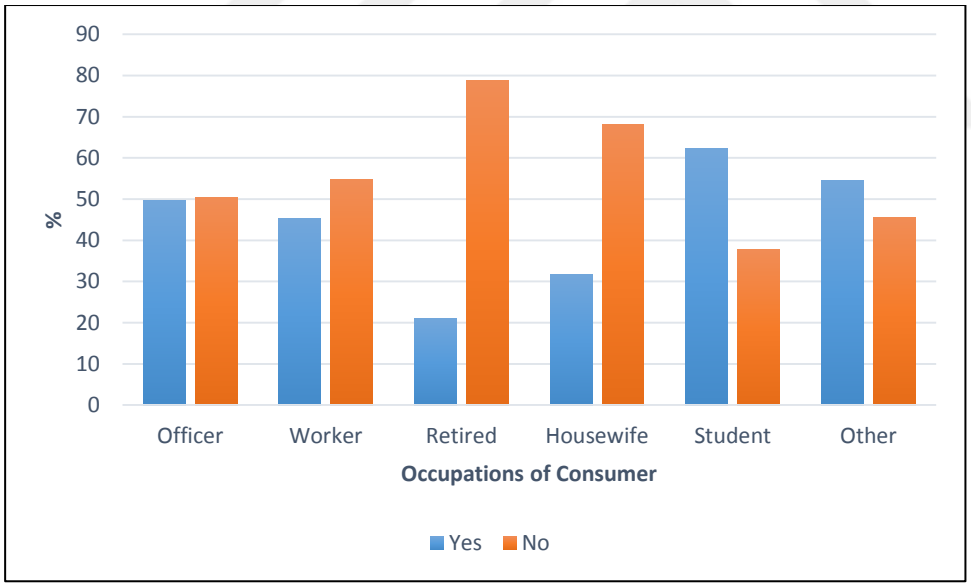

Figure 3.11 : The relationship between the occupation of consumers and bread waste.

It was found that there is no significant relationship between the income level of consumers and bread waste. This shows that despite the increase in income, the bread will continue to be consumed at a certain rate at each table [12].

It was found that the number of individuals in the family had a significant effect on bread waste. Bread wastage decreases as the number of individuals in the family increases, and increases as the number of individuals decrease [12] (Figure 3.12).

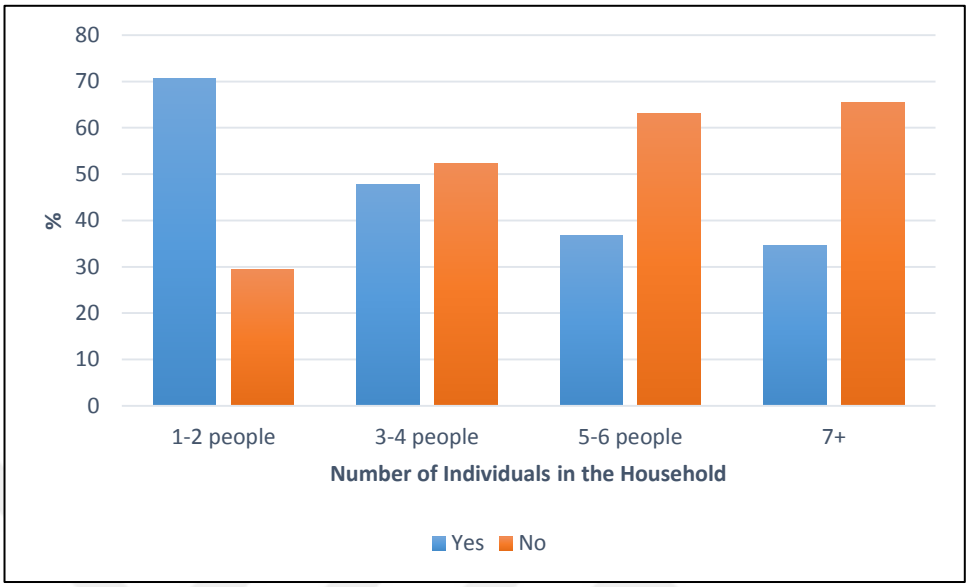

Figure 3.12 : The relationship between the number of individuals in the family and bread waste.

One of the measures taken by the participants to reduce bread waste is to purchase as much bread as needed. This is followed by evaluating the remaining bread, storing it in the refrigerator to delay stale bread, removing excess bread in the freezer, sharing it with those in need or with neighbors, and giving it to the animals.

There are two main reasons for the waste of bread. These are bread stalling and bread spoiling.

3.4.1 Bread stalling

About 24 hours after leaving the oven, except for those caused by microorganisms, all changes in bread; especially the bread crust loses its brightness, loses its aroma, softens and becomes a strap-like structure; softness and elasticity to lose hardening, tight and easily crumbling condition, the transparency and taste changes are called "stale" events.

Many changes occur in the physical properties of bread during staling:

Change of taste and odor, Increased hardness, Increased bread crumbling, Increased opacity of bread, Decreasing water-binding capacity of bread, Reduction of the amount of soluble starch extractable from bread, Decreased sensitivity of starch to amylase

enzyme, Variation of thermal properties which can be measured by "Differential thermal analysis" techniques.

There are 3 important factors affecting bread stale: water content of bread, protein quantity, and quality, retrogradation of starch.

3.4.1.1 Water content of bread

Staling occurs in bread containing water between 16-37%. Bread is not stale by preventing microbiological damages outside these limits. Staling can be prevented by drying the bread under at least the water limit or by heating with excess water. In addition, if the water holding capacity of the dough increases, the softness of the bread interior increases and its hardening is delayed.

3.4.1.2 Protein quantity and quality

Bread made of flour with strong essence hold more water and the volume is higher, but the degree and speed of hardening of the bread is less. In bread made from enriched flour, stale is delayed. Protein performs this effect by acting as a preservative around starch particles and absorbing excess water.

3.4.1.3 Retrogradation of starch

The self-agglomeration of starch material in the colloid solution state is defined as "retrogradation of starch". When heated, the retrograded starch solution returns to its former suspension state. Similarly, a stale bread containing sufficient water (< 30%) is regenerated when heated. Staling due to retrogradation of starch depends on the optimum temperature. At 60 ° C, the stale becomes difficult in closed packages.

When stale, the bread loses its sensory quality and causes the consumer to refuse bread even though it has no harmful effect on health. For this reason, tons of bread are thrown during the year and this causes an important economic loss.

3.4.2 Bread spoiling

Microorganisms that cause spoilage in foods can be found in raw materials, on equipment used, in air, in water, namely, it can be found in any environment. Microorganisms that find the appropriate environment multiply in food and cause rapid deterioration.

Diseases caused by microorganisms in bread: mold, rope disease, chalk disease, red spot disease

3.4.2.1 Mold

Mold is the most common deterioration in bread. A freshly baked bread has no mold spores, but there are intensive mold spores in the ovens and the air. A cooling product is exposed to these mold spores. During the next three days, visible mold is formed. The molds seen in the bread are Rhizophus, Aspergillus and Penicillium. Molds that develop in bakery products cause the deterioration of taste and aroma, as well as some types also cause carcinogenic substances to form. moisture and oxygen are two conditions for the growth of molds. If the moisture in the crust of the bread remains below 90 %, the mold event is greatly reduced. Another way to prevent mold; To add 0.1-0.3 % calcium propionate as a preservative.

3.4.2.2 Rope disease

Rope disease is a kind of bread disease. It is especially seen in hot summer months. Rope disease occurs as a result of the development of Bacillus subtilis (Bacillus mesentericus) bacteria due to the fact that its spores are more heat resistant in bread. However, other Bacillus genera such as Bacillus subtilis, B. licheniformis, B. megaterium, B. pumilus and B. cereus are also reported to cause rope formation.

Bacillus subtilis is a soil-borne bacterium, so its spores are usually found in flour and rarely in yeast. The spores are resistant to baking temperatures. Increases in rope spores are observed in dough in production which is not carried out under suitable conditions.

Bacillus subtilis bacteria in addition to rope formation in the product, if consumed in high amounts (108 / g), it may also cause food poisoning with symptoms such as nausea, vomiting, diarrhea, headache [16].

First, the smell of spoiled melon is spread from the bread with rope disease. Some yellowish spots appear in the bread. When the disease progresses, the inside of the bread becomes sticky (Figure 3.13). The disease does not pose a fatal danger, but the bread that are infected cannot be consumed therefore have considerable financial losses.

The additives used as a rope inhibitor and approved by the Food Codex for bread are acetic acid (vinegar), propionic acid and its salts, sorbic acid and its salts, calcium acetate and sodium diacetate.

Figure 3.13 : Bread with rope disease.

3.4.2.3 Chalk disease

In this disease, bread take a white chalky appearance. The causative agent of this disease is Endomyces fibuliger and Trichosporo variable microorganisms. This disease can be easily prevented by observing hygienic conditions.

3.4.2.4 Red spot disease

The causative bacteria is Serratia marcescens. It is a very rare type of disease. The bacterium is colorless at first, then becomes a red color and red spots appear on the bread. This disease can be easily prevented by observing hygienic conditions.

3.5 Bread Production Technology

The ingredients of the bread are flour, water, yeast, and salt. Also, emulsifiers, acidity regulators, thickeners, blowing agents and preservatives are used in bread produced for special purposes. Although the dough preparation methods vary, the processes are the same in the classical method. In the classical process (Figure 3.14), bread comes to our tables by operations such as mixing, kneading, fermentation, weighing, shaping, cooking, cooling, packaging, storage.

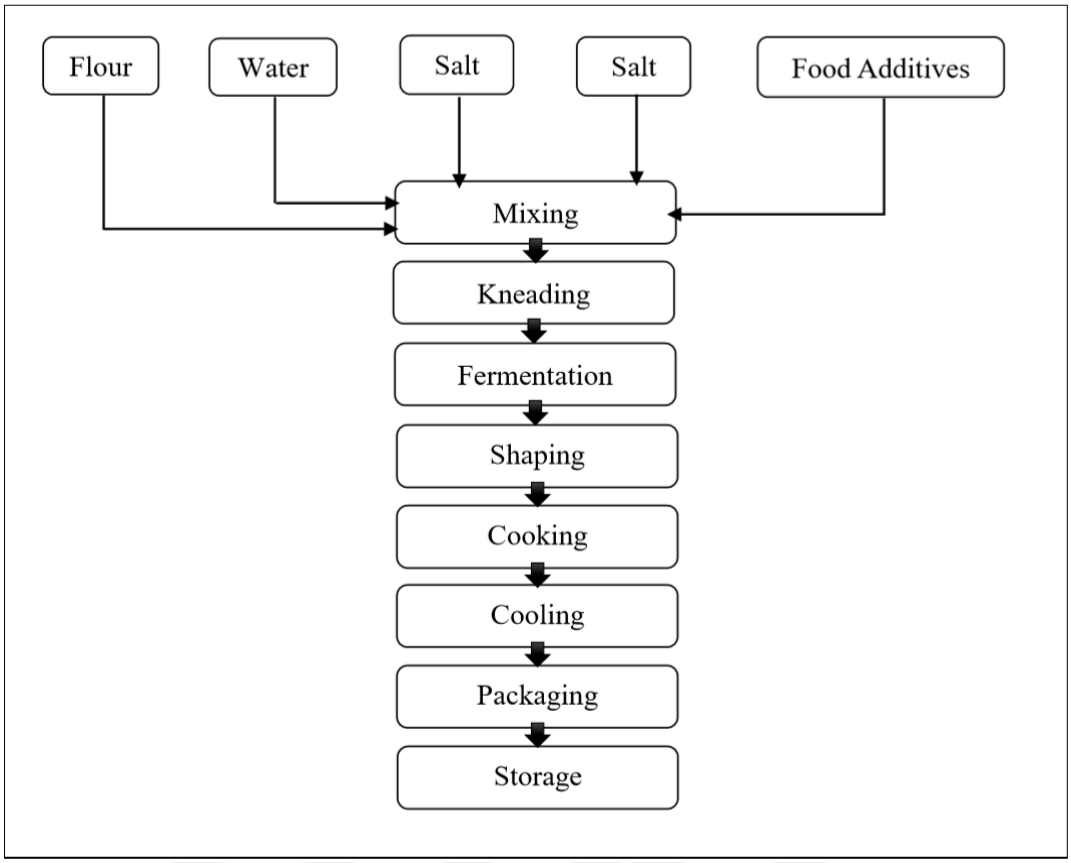

Figure 3.14 : Classical bread production process.

According to the Turkish Food Codex Communiqué on Bread and Bread Varieties, the amount of substances such as water, salt, yeast, and bread additive are calculated and added according to the flour to be processed. The classic bread formulation is expressed as a percentage over flour: 55-60 % water, 3-4 % wet yeast, 0.5-1 % bread additive, 1.5 - 1.75 % salt [17].

According to this; When 100 kg flour is used: It is calculated that 55-60 L water, 3-4 kg wet yeast, 0.5-1 kg bread additive, 1.5 kg salt should be used.

4. FOOD ADDITIVES

Food additives that derived from the Latin word “addere” is explained in the Turkish Food Codex Regulation as “Not consumed alone as food or used as food raw material or excipient; with or without nutritional value alone; residues or derivatives which may be present in the manufactured material during the process of manufacturing, used according to the selected technology; Food preservatives are substances used to preserve, correct or prevent and correct undesirable changes in the taste, smell, appearance, structure and other characteristics of food during the production, classification, processing, preparation, packaging, transportation, storage of food.”

The use of food additives in food is as old as human history. Salt, wood incense and spices are the first natural additives used by human beings. It is seen that salt and wood incense was used in the storage of meat products in B.C. 3000. In the Middle Ages, it is known that nitrate, as well as salt and wood incense, are used to protect the color of meat and to prevent spoilage. In the B.C. 50s, spices were used as flavors, and food dyes were used by the Egyptians for coloring purposes approximately 3500 years ago. In the nineteenth century, with the increase of the population and the increase in consumption, new foods were produced and parallel to this, the use of food additives became widespread. Many food additives used today, were found in the 19th century [18].

As people start to live collectively, the need for reliable methods has emerged for food preservation. Changes in agricultural practices, excessive use of nondurable foods in nutrition, increased the possibility of contamination in advanced distribution systems, and orientation to easy and practical foods have made it necessary to develop food preservation techniques. The main protection methods used in the industry are heating, freezing, drying, and irradiation. However, in cases where these cannot be applied or are insufficient, preservatives are added to the food.

Food additives are used to increase the acidity of the dough, to delay staling, to correct bread faults and diseases, to increase the water removal rate, to increase the volume,

to increase the yield of flour. Today, there are more than six thousand additives, most of them are flavors.

4.1 Benefits and Harms of Food Additives

4.1.1 Benefits

It provides the taste, flavor, and odor of foods to be much more beautiful, Corrects the shapes and colors of foods, It makes the appearance of foods more beautiful, It provides quality and durability of foods, Prevents the formation of microorganisms that cause disease in foods, It helps maintain the nutritious and biological values of foods. It provides longer food life.

4.1.2 Harms

Deterioration of the natural flavors of foods, Risk of loss of nutrients in foods, Risk of food poisoning if the food shelf life is not considered, Promotes the risk of skin rashes, asthma or even cancer if the food with additives is consumed too much.

4.2 Effects of Food Additives on Human Health

Although the daily intake values of food additives are determined, it is also disclosed that these substances can accumulate in the body and cause toxic effects over time. Risks caused by additives may occur in the long term and cause chronic diseases. For this reason, it is very important to determine the short and long term damages of the additives to be used in the food industry.

In Europe, 0.03-0.1 % of the population was found to be sensitive to food additives. Some of the colorants may cause asthma, skin rash and migraine. Allowed colorings vary from country to country. Norway and Sweden have banned all colorants used in foods. Some of the aroma enhancers may cause dizziness and palpitations [19].

According to Gülcan (2011), in England, hyperactivity was found in 75 of 277 children and allergies were found in 79 of 277 children, hyperactivity and allergy were found in 36 out of 277 children. In the study, it was determined that when artificial colorants and sodium benzoate were removed from children's diet, behavioral disorders in children improved; behavioral disorders reoccurred with the administration of foods containing these substances to children [18].

4.3 Control of Food Additives

Food additives usage considerations: It should not be harmful to human health, Technological necessity should be in use, It should be used in the allowed foods and in the allowed amounts, It should not decrease the nutritional value of the food, It should be used for the preservation of food quality and should not be used to shade poor quality.

The standardization of food production in terms of safety is a global issue. Based on this need, it has created various international structures. The first systematic treatment of additives was carried out in 1956 by the WHO (World Health Organization) and FAO (Food and Agricultural Organization), with a screening study involving 43 world countries. In 1962, JECFA was established by bringing together experts from FAO and WHO. JECFA (Joint Expert Committee for Food Additives) is an international body that undertakes the regulation, execution, and evaluation of the results of toxicological studies for each chemical substance used as an additive. In each country, there is national legislation regulating the use of food additives and official organizations that apply it [18].

Today there are over 8000 food additives. The number of food additives approved by the FDA is approximately 2800. In the European Union, the number of allowed food additives is 297 [20].

4.3.1 Duties of JECFA

Determine methodologies for toxicological assessment of additives, It applies toxicological tests and evaluates the results and determines the doses that can be taken without inconvenience (ADI), Determines the purity criteria and analysis methods for each additive, Determine and evaluate daily-annual consumption levels of food additives in various societies.

4.3.2 Control procedures

Experimental animals are given a lethal dose (LD50), the dose that causes 50% of the experimental animals to die. The dose-response relationship is investigated by slowly reducing the dose. At each dose; absorption, metabolism, and excretion of the additive.

The carcinogenic, mutagenic, teratogenic and allergic effects of experimental animals are examined. If a dose which the additive has no effect, cannot be found, it is not allowed to be added to the food.

If a dose is obtained in which shows no opposite effect to the test animal, this dose is defined as an "ineffective dose" or NOAEL (No Observed Adverse Effect Level). With the dose of NOAEL found, the daily dose amounts that do not have carcinogenic, mutagenic, teratogenic and allergic effects in various organs of the body in a period that covers 85% of the life span of the experimental animals are determined in milligrams per kilogram of the animal. Since the experiment cannot be performed on people for ethical reasons, a safety factor is used. In other words, 1/100 of the dose which shows no effect on the experimental animal is accepted for humans. ADI (Acceptable Daily Intake) = NOAEL / 100. ADI value is determined in milligrams per kilogram of human body weight. Maximum daily intake = ADI x Bodyweight.

In order to determine the maximum amount of additives to be added to the food, the following information must be known [21].

The daily amount of additive (ADI (mg/g) value), The amount required by food production technology (GMP-Good Manufacturing Practices), How many nutrients will be added to the additive, Average daily consumption of nutrients.

4.4 Classification of Food Additives

In the classifications made, food additives are sometimes grouped according to the purpose of use and sometimes according to the food used in production. We can group them into 4 groups according to their usage purpose;

4.4.1 Extend shelf life by maintaining quality

4.4.1.1 Antimicrobials (acetic acid, benzoic acid, formic acid, propionic acid, sorbic acid)

Antimicrobial additives are added to foods to destroy bacteria, molds, yeasts, and any microorganisms that can be found in food for any reason. Salt (NaCl), benzoic acid, propionic acid, acetic acid, sulfur dioxide and sulfides, nitrates and nitrites, ethylene oxide and propylene oxide are the most commonly used antimicrobial additives.

However, in this thesis, we will examine acetic acid, benzoic acid, formic acid, lactic acid, propionic acid, and sorbic acid.

Acetic acid (E 260) and its potassium (E 261), sodium (E 262), and calcium (E 263) salts

Acetic acid is one of the known preservatives since ancient times. In addition to its protective effect, it is also used as a flavoring, flavoring and sourming agent. It is in the GRAS list. Acetic acid and calcium salts (Figure 4.1) prevent rope disease in bread. It is also used as antimicrobial agent in cured meats, fish products, ketchup, mayonnaise and pickles, and has a flavor function in these products. acetic acid should contain 3.6 % to prevent the growth of lactic acid bacteria and yeasts in foods containing carbohydrates [22]. People with poor kidney function should be avoid excessive intake of this preservative.

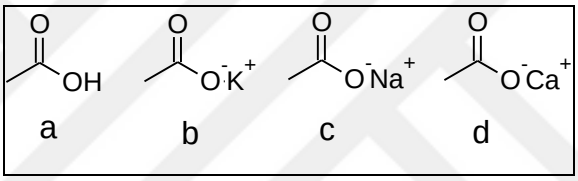

Figure 4.1 : Acetic acid (a) and its potassium (b), sodium (c), and calcium (d) salts.

Benzoic acid (E 210) and its sodium (E 211), potassium (E 212), and calcium (E 213) salts

Benzoates are effective against yeast and bacteria, but less effective against molds. As antimicrobial additives, they have the advantages of being soluble, odorless and colorless. Suitable pH limits for inhibiting microorganisms range from 2.5 to 4.0. This value is lower than the acidity of propionic and sorbic acids. Sodium benzoate is in the GRAS list. When sodium benzoate is mixed with very small amounts (0.5 mg/g per day), it does not harm for health [23]. However, if this amount increases, the nutritional value of the food decreases and health problems arise. Benzoic acid causes various allergic reactions such as asthma and skin rash. People with aspirin allergy need to be more careful.

In the United States, the use of benzoic acid and sodium salts (Figure 4.2) in the food industry is permitted within the legal limits. However, the maximum amount of use is limited and this value does not exceed 0.1 %. In other countries, this substance is allowed to be used as a food additive. Benzoates are mostly used in the food industry:

carbonated and non-carbonated drinks, fruit juices, bread, pastry, jams and jellies, margarine industry, pickles, various sauces, ketchup, table olives, marmalade, cocoa products, biscuits, wafers, and cake creams [24,25].

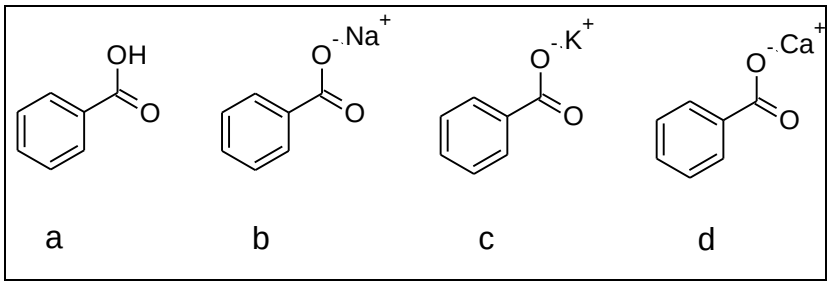

Figure 4.2 : Benzoic acid (a) and its sodium (b), potassium (c), and calcium (d) salts.

Formic acid (E 236) and its sodium (E 237), calcium (E 238) salts

Formic acid was first found in the ants' secretions in nature and was obtained by withdrawing from it. Due to the high biological activity of formic acid in metabolism, it is important to examine its sources. Since formic acid is effective with undissociated molecules, it has a protective property only for foods with a pH of 3.5 and below. Formic acid and its salts (Figure 4.3) do not have antimicrobial effects in environments with weak acid and neutral pH values. Elevated levels of formic acid in the human body can cause serious damage to the optic nerves, respiratory failure and severe fatal consequences such as liver and kidney failure. Concentrations of formic acid above 10 mmol / L (0.5 mg / mL), may induce metabolic acidosis and lead to death [26]. Formic acid can enter the body through nutrients, intestinal microflora production, by ingestion of methanol or by inhalation of methanol vapors. In some countries it is forbidden to use as a preservative.

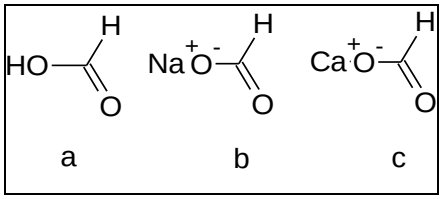

Figure 4.3 : Formic acid (a) and its sodium (b), and calcium (c) salts.

Propionic acid (E 280) and its sodium (E 281), calcium (E 282), and potassium (E 283) salts

Propionic acid, a colorless liquid, is rarely used in the food industry as it is corrosive and pungent. Therefore, the Na, Ca, K salts (Figure 4.4) are preferred. These salts form

free acids in low pH foods and are easily soluble in the solvent. The effect on mold is higher than sodium benzoate but they are ineffective against yeasts. It is also incapable of preventing bacteria. Na and Ca propioanates are mainly used as mold and rope inhibitors in bakery products. Propionates are included in the GRAS list. Propioanates are widely used in the food industry due to their strong antimicrobial effects, lack of taste and odor. In literature, it has been reported that 0.2-0.4% propioanate is added to jam, peeled apple slices, figs, black grapes, cherries, peas, and beans to delay the growth of molds. It is stated that 0.32% in white bread flour and 0.3% in cheese is the highest recommended amount [27]. When taken in large amounts, it may cause migraine pain.

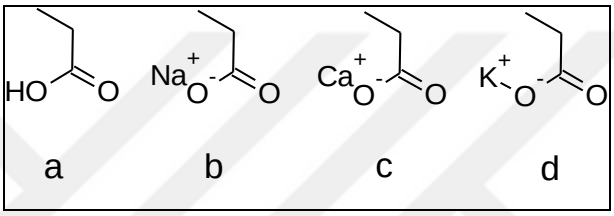

Figure 4.4 : Propionic acid (a) and its sodium (b), calcium (c), and potassium (d) salts.

Sorbic acid (E 200) and its potassium (E 202), calcium (E 203) salts

The only unsaturated organic acid that is allowed to be used as antimicrobial in food is sorbic acid. The pH at which sorbic acid is effective is higher than benzoic acid. They can be used against bacteria, yeast, and molds in foods with a pH up to 6.5 [28]. Sorbic acid and its salts (Figure 4.5) are added to food at a rate of 0.1-0.2 %. When used with NaCl or phosphates, it prevents Clostridium botulinum, which grows in acidic foods and produces toxins. Sorbates are used in various cheese products, cereals, jams, jellies, marmalades, and sauces.

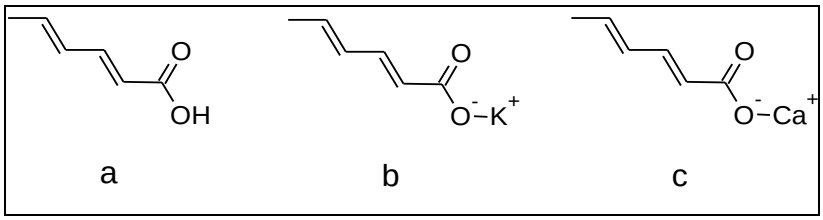

Figure 4.5 : Sorbic acid (a) and its potassium (b), calcium (c) salts.

Table 4.1 : Comparison of the effects of various antimicrobials.

Antimicrobial Agents	Bacteria	Yeast	Mold
Acetic Acid	+++	+++	+
Benzoic Acid	++	+++	+++
Formic Acid	+	++	++
Propionic Acid	+	++	++
Sorbic Acid	+	+++	+++

Determination of appropriate antimicrobials

For the application to be effective, the question of "which additive for which food" should be answered first. Then, "which dosage for which additive" question should be answered. When answering these questions, numerous factors should be considered. Factors are generally grouped under the headings of the pH of the food, the solubility and the spectrum of the substance, the labeling state of the food and the interaction of the chemical compounds (synergistic and antagonistic).

The pH value of food: The chemicals used for food preservation are usually acid or salt. These compounds are more or less ionized in the aqueous medium. There is a relationship between this phenomenon called dissociation and the antimicrobial effect of the substance. It is the non-dissociated part of the chemical substance that shows the antimicrobial effect. The antimicrobial effect is reduced as far as ionized. The lower the pH of the food, the higher the antimicrobial effect. In short, each chemical is effective only at a certain pH range. In determining the preservative, the pH value of the food must first be considered.

The solubility of chemical substance: Any chemical substance used to protect food is effective only if it is truly soluble. In general, the solubility decreases as the molecular weight of the acids increases. The distribution of preservatives in the oil and water phase in emulsion foods is also important. In these systems, microbial growth is mostly in the water phase. If the preservative passes more into the oil phase, the expected effect is not achieved. This is explained by the distribution coefficient, which is the

ratio of the concentration in the oil phase to the concentration in the water phase. For the emulsion system, the preservative must exhibit a low coefficient of distribution.

Specific antimicrobial effect: The antibacterial effect of a preservative is not the same against every microorganism. Some have a greater effect and this is called the "antibacterial effect specificity". Although microorganisms causing food spoilage are generally not uniform, it is known that some types have priority over spoilage, depending on the composition of the food and especially the pH.

Food labeling: Some preservatives have a negative effect on the composition and sensory properties of foods. Therefore, the choice of preservative according to the type of food is important. In addition, although it varies depending on the variety and concentration of the preservative, it can produce a change in the sensory properties and the taste of the food.

Interaction of antimicrobials: In order to reduce the negative properties of antimicrobial substances and increase their positive properties, there are cases where more than one compound is used together. Synergism which is a desired result and antagonism which is an undesirable result may occur in the use of preservative combinations. The synergistic effect is to increase the effect of one compound by another compound. The antagonistic effect is the reduction or elimination of the effect of one compound by another compound.

Effect of water activity: In general, the effects of preservatives increase with the decrease in water activity of food. On the other hand, toxin formation of microorganisms is closely related to the water activity of the environment, but water activity is not an effective factor in this regard.

Effect of physicochemical factors: Some physicochemical substrate factors such as the redox potential of the medium and partial pressure of oxygen may also affect the growth and development of microorganisms. For example, sulfurous acid also exhibits antimicrobial action by reducing the redox potential of the medium. Likewise, gases such as carbon dioxide and nitrogen gas affect the oxygen partial pressures of the environment and are widely used in food preservation.

Effect of food components: Food components have different effects and interactions in food preservation. For example, salt and sugar reduce the water activity of the medium and increase the effects of preservatives or prevent the growth of

microorganisms due to low water activity. Also, since salt has negative effects on enzymes, it can increase the effects of preservatives.

4.4.1.2 Antioxidants (ascorbic acid, lactic acid)

The function of oxygen in living organisms is associated with the task of respiration; however, it is possible to reduce some of the molecular oxygen during this function. In other words, oxygen, which is essential for life, also has a toxic effect. This toxicity is due to oxygen-generated free radicals, all of which are harmful to biological systems. Free radicals and other reactive oxygen derivatives cause disease in humans and food spoilage. Antioxidants are known to prevent the degradation of processed foods and provide a longer shelf life. Ascorbic acid is one of the most widely used antioxidants.

Ascorbic acid (E 300) and its sodium (E 301), calcium (E 302) salts

Ascorbic acid, also known as 'Vitamin C', is a vitamin that found in green vegetables and citrus fruits. It is also an antioxidant. When used as an additive, it prevents the discoloration of meat by reacting with oxygen in the air and helps to preserve its aroma. It also prevents the formation of nitrosamines, a carcinogenic substance. Ascorbic acid and its salts (Figure 4.6) used as an antioxidant in beer industry; It improves the shelf life of beer by preventing turbidity and is a protective agent for the maintenance of color in the meat industry, also it is used as a developing agent in the bakery industry and the prevention of discoloration in fruit pulp and juices.

In the experiments, when vitamin C was added at a rate of 2.5 g /100 kg, it was observed that the volume increased by 20 cm^3 during fermentation. In general, vitamin C is used at a rate of 25-75 mg/g [29].

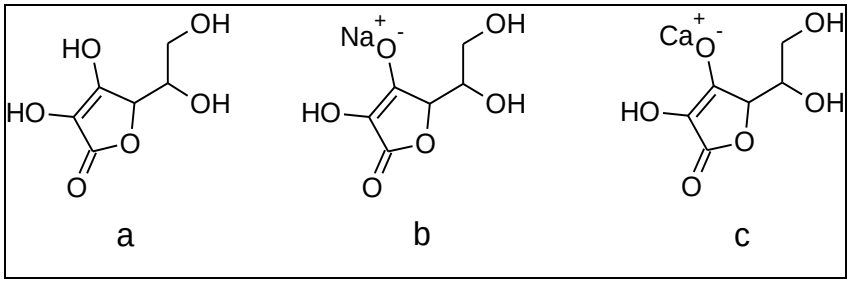

Figure 4.6 : Ascorbic acid (a) and its sodium (b), calcium (c) salts.

The following changes were observed with the addition of ascorbic acid to flour:

Development of bread structure, Short dough rest period, The dough maturation is accelerated, Increased volume of dough and bread, Fineness and homogeneity in pores.

Lactic acid (E 270) and its sodium (E 325), potassium (E 326), and calcium (E 327) salts

Lactic acid and its salts (Figure 4.7) are commonly used additive. Produced by fermentation of lactose (milk sugar). It is used as flavor, preservative and acid regulator in food industry. There is no limit to the daily intake. But it is difficult to digest for infants and young children. Therefore not recommended for children under three years.

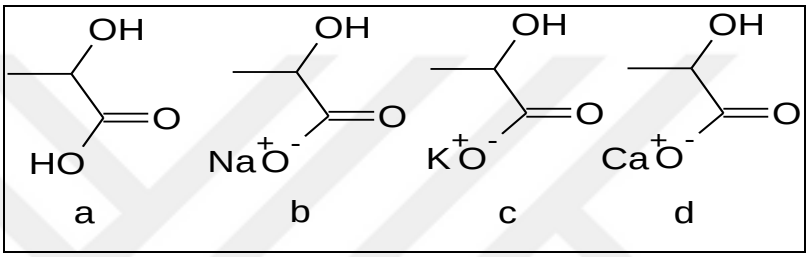

Figure 4.7 : Lactic acid (a) and its sodium (b), potassium (c), and calcium (d) salts.

4.4.2 Preparing the structure and improving the cooking properties

4.4.2.1 pH regulators

They are used to control and change the pH of nutrients and to achieve the desired level. They may also exhibit bacteriocidal and bacteriostatic effects on the nutrients by lowering the pH. Increased acidity also increases the heat sensitivity of many microorganisms. Cooking and other heat treatment destroy bacteria. Increased acidity inhibits the growth of microorganisms and prolongs the shelf life of some foods. They prevent enzymatic blackening in fruits and vegetables. They delay the formation of the bitter taste of oil by binding with iron and copper. In addition, they affect the properties of nutrients such as sweetness, sourness and provide the desired flavor.

4.4.2.2 Anti-caking

Anti-caking agents are used to preserving the flowability of powdered mixtures such as salt, powdered sugar, spices, instant soups, milk powder, and to prevent caking and aggregation.

4.4.2.3 Emulsifiers

They are used for mixing water and oil in food and providing homogeneous distribution by reducing surface tension.

4.4.2.4 Stabilizers, gelling agents, sweeteners

Stabilizers; They are used to prevent re-separation of oil and water.

Gelling agents; Gives texture to food with gel formation.

Artificial sweeteners; It is used to make the taste more attractive and to give the sweet taste (without calories).

4.4.3 Flavor and color enhancers

4.4.3.1 Flavor enhancers

Flavor enhancers are used to increase the flavor and odor present in the food, to make the aroma more attractive, to preserve and improve the original aroma.

4.4.3.2 Colorants

They participate to regain the natural color lost during processing and storage, to strengthen the weak color, and to give color to the colorless food.

4.4.4 Nutritional preservatives

They can be grouped under two headings as replacing nutrients lost during processing (B1, B2, niacin) and adding nutrients that may be missing in the diet (vitamins A, D)

Additives generally used in bread products: E 300 Ascorbic Acid, E 471-E477 Mono- and diglycerides and their modified forms, E 282 calcium propionate, E 281 sodium propionate, E 262 Sodium diacetate, E 260 acetic acid, E 280 propionic acid, E 202 potassium sorbate, E 200 sorbic acid, E 202 potassium sorbate E 203 calcium sorbate, E 283 potassium sorbate, E 170 calcium carbonate, E 332 Potassium citrate, E 481 Sodium stearol-2-lactylate, E422 Glycerol (glycerin), E 322 Lecithin / Soy lecithin, E 341 Calcium phosphate, and E 412 Guar gamut.

Also, carcinogenic and allergic substances such as E928 benzoyl peroxide and E924 potassium bromate are used as whiteners to produce whiter appearing flour, and additives such as E920 Cystain is also used as bulking agents produced from human and porcine hair.

4.5 E Codes

Two formats can be used for labeling food additives. The first is “the function and name of the contribution” and the other is “the function and E code of the contribution”. Since the second application is more common, it is very important for the consumer to read the food label and know the E code. The E code is an international symbol given to food additives whose toxicological investigations have been completed and the dose of harmlessness has been determined. The E-codes used in our country also show the codes given to the food additives that have been passed to the required safety tests of the relevant health and food authorities of the European Union and all specifications have been determined. It is an expression of security.

E codes are codes consisting of the letter E and the three-digit number as the symbol of the European Commission (EC), which is used to identify food additives and to avoid confusion. The E code comes from the initial letter of the word “European”. These codes are determined by the “Food Science Committee” for each additive. Each food additive has an internationally recognized number. The Turkish Food Codex Regulation contains about three hundred food additives used for various purposes. These food additives are grouped as follows:

Colorants; E100 - E180, Preservatives; E200 - E285, E330, Antioxidants; E300 - E321, Thickener, gelling agents; E400 - E495, Acid-base providers; E500 – 578, Fragrances; E620 – 637, Sweeteners; E950 - E959.

5. INORGANIC ANIONS

Anions are negatively charged ions formed by ionization of acids and salts. They can be monatomic (such as F^-, Cl^-) or polyatomic (such as NO_3^-, PO_4^{3-}). Some anions are necessary for the body, while others are highly toxic to the body. The amount of anions taken into the body is tolerated at different levels by the metabolism. Therefore, a reliable determination of anions is an extremely important issue.

Chloride (Cl^-) ; is one of the most common anions in foods. It plays an important role in acid-base balance in metabolism. However, intake at high concentrations may cause too many side effects. Disinfectants such as chlorine used in water treatment, carry carcinogenic risks in the long term. When taken at high concentrations, irritates the liver, causes respiratory distress, causes throat constriction and pulmonary edema [30,31]. The most common foods containing chloride ion are table salt, mineral waters, celery, lettuce, olives, rye sea water, sea grass and tomatoes.

Nitrate (NO_3^-) and nitrite (NO_2^-) are found in many vegetables naturally or as a source of irrigation water. Due to the widespread use of nitrogenous pesticides, nitrite and nitrate levels in foods have increased. Nitrite and nitrate, which are generally used as color preservatives in meat products, cause methemoglobinemia in case of excessive intake. When combined with hemoglobin in the blood, oxygen delivery of the blood is prevented or reduced. This condition is known as “blue baby syndrome” in children [32]. Although nitrate is more stable and less toxic than nitrite, it can easily be converted to nitrite by a microbial reduction in food products.

$$2\ NO_2^- + O_2 \rightarrow 2NO_3^- \qquad (5.1)$$

Nitrite can be converted to carcinogenic nitrosamines by combining with secondary amines in food products and the human digestive system. Numerous investigators have reported carcinogenic effects of nitrosamines in both humans and animals. The current acceptable daily intakes (ADIs) for nitrite and nitrate, set by JECFA in 2002, is 0.07 mg/kg and 3.7 mg/kg, respectively [33].

Sulfate (SO_4^{2-}) can be found in almost all natural water. The origin of most sulfate compounds is the oxidation of sulfite ores. And also, it is a natural and necessary component found in the bodies of humans and animals. The sulfate level in human serum is between 0.25 and 0.38 mmol / L. The sulfate is involved in biochemical activities, including the production of chondroitin sulfate and the sulphation of exogenous chemicals. Sulfate, which is absorbed in small amounts in the intestines and rapidly discharged from the kidneys, cleans the intestines by showing a laxative effect in humans. It is not recommended to take more than 25 mg / L due to the laxative effect in case of exposure to high sulfate concentrations [34].

Due to the side effects described above, the determination of anions in bread is important. There are so many studies in the literature for this. Faster, more reliable and more environmentally friendly method is required for the detection of these anions.

6. EXPERIMENTAL

In this work, a novel ion chromatography method was developed for the analysis of organic acid anions used for preservatives and inorganic anions.

6.1 Chemicals and Tools

Eluent (NaOH) was created on-line in a multi-gradient format on the EG (DIONEX, USA) electrolytic eluent generator module.

1000 mg / L certified standard solutions of chloride, nitrate, nitrite, sulfate were purchased from Dionex (Sunnyvale, California, USA).

Weighing of solid chemicals was made in Precisa brand electronic scale (Precisa XB 220A, Swiss), which can weigh up to the fourth digit after the comma.

Wisemix brand vortex device was used to mix the solutions homogeneously.

Ultra-pure water, used for stock solutions, dilutions, and eluent; was supplied from the New Human Power I Scholar UV (Human Corporation, Seoul, Korea).

6.2 Chromatographic System

In this study, DIONEX ICS-3000 (Dionex Corporation, USA) Ion Chromatography System was used. This system consists of DP double pump (isocratic pump used in the study), EG eluent generator suitable for single and double pump systems, DC Detector-Chromatography Module and AS automatic sampler parts.

The sampling volume of the autosampler is 40 µL, and the sample loop volume is 10 µL. The EG eluent generator, which includes the Eluent generator cartridge, RFIC-Eluent degassing and continuously regenerated anion trap column (CR-ATC) parts, prepares NaOH at the desired concentration at the desired time and supplies the degassed eluent into the column.

DC module has RFIC TM IonPac® AS20 Analytical (2 × 250 mm) anion exchanger column and pre-guard column RFIC TM IonPac® AG20 Guard (2 × 50 mm) in the

chromatographic separation part, and CD conductivity detector with ASRS ULTRA II mm suppressor in the detector part.

The resin of the AS20 column is a cross-linked supermacroporous polyvinylbenzyl ammonium polymer with a particle diameter of 7.5 µm, the functional group of which is the quaternary ammonium group and divinylbenzene.

AS20 is a hydroxide selective anion exchanger column that can separate polarizable anions in a short time. AG20 is a guard column with a particle diameter of 11 µm, resin composition, microporerous polyvinylbenzene and functional group, quaternary ammonium group, attached to the prior to analytical column and preventing contamination of the column. Since AG20 is micropore, it shows optimum performance for a long time.

6.3 Preparation of Solutions

6.3.1 Preparation of standard solutions

To prepare stock solutions of lactate, propionate, formate, sorbate, benzoate, and ascorbate with 1000 mg / L concentration for each one, appropriate amount of their salts were weighed as exhibited in Table 6.1 and each solid was transffered into different 100 mL of volumetric flasks and then UP water was added up to the marked line.

Table 6.1 : Weighed amount of organic acid anions for preparation of stock solutions with 1000 mg / L concentration

Analyte	Prepare From	Amount (g)
Lactate	Lactic Acid	0.09
Propionate	Calcium Propionate	0.26
Formate	Formic Acid	0.08
Sorbate	Potassium Sorbate	0.14
Benzoate	Benzoic Acid	0.1
Ascorbate	Ascorbic Acid	0.1

1000 mg / L of certified chloride, nitrite, nitrate, sulfate standards were commercially obtained (vice supra).

Intermediate stock solutions were prepared from the main stock solutions. The standard solutions were seperated on the analytical column by utilizing NaOH eluent concentration of 3 mM between 0-8 minutes; 8 mM between 8.1-13 minutes; 14 mM between 13.1-18 minutes; 32 mM between 18.1-30 minutes; 3 mM between 30.1-35

minutes during analysis. The other chromatography conditions; suppressor current was 20 mA, detector cell temperature was 35°C, mobile phase flow rate was 0.250 mL/min and sample loop volume was 10 µL.

6.3.2 Preparation of the bread samples

As the first stage of sample preparation, the bread samples collected from various markets were cut into small pieces (approximately 15 mm) and then, dried in the oven at 45 centigrade degrees for one day. The bread samples were weighed and then, diluted 100 times with water. The sonication process was applied to the diluted bread samples for 15 minutes. Then, this was centrifuged at 13500 rpm for 5 minutes to separate from its solid parts. To eliminate organic substances, the supernatants were passed through the C18 SPE cartridges. Finally, the solutions were filtered with PES filters prior to IC analyses.

6.3 Optimization of Chromatographic Conditions

In this study, various attempts were conducted to achieve optimal chromatography conditions for the best separation. Firstly, the separation of 10 analytes with 15mM isocratic elution was tried. As seen in figure 6.1 (a), all of the analytes leaved from the IC column, but an effective separation was not achieved.

Secondly, the 10mM isocratic elution has been tried. As seen in figure 6.1 (b), peak seperation was better than the previous one. But again, sufficient separation was not achieved. Analysis time was less than 15 minutes.

Thirdly, the 5mM isocratic elution tried. As seen in figure 6.1 (c), peak seperation better than the previous one. But again, sufficient separation has not been achieved. Retention time is less than 25 minutes.

And then, the gradient elution from 4mM to 40mM has been tried. As seen in figure 6.1 (d), peak seperation is better than the previous one. But again, sufficient separation has not been achieved.

Finally, the gradient elution from 3mM to 32mM has been tried. As seen in figure 6.1 (e), the best results were obtained under this conditions.

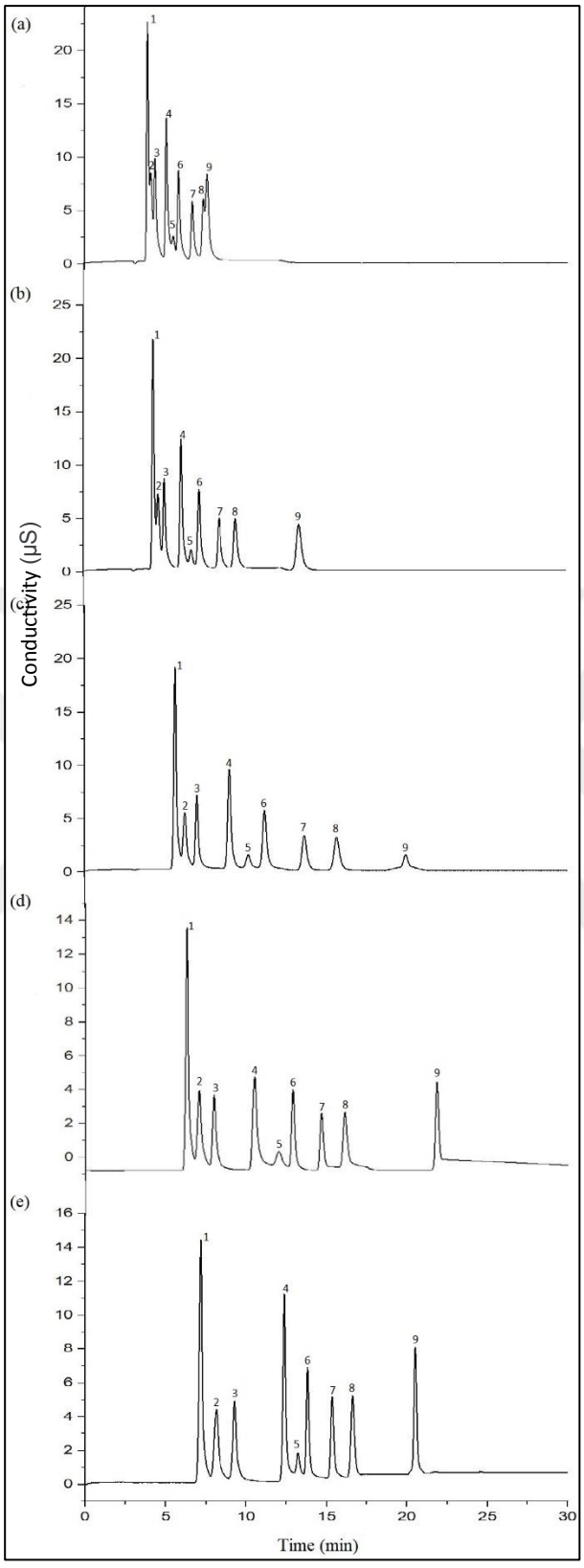

Figure 6.1 : (a) 15mM isocratic elution, (b) 10mM isocratic elution, (c) 5mM isocratic elution, (d) Gradient elution from 4mM to 40mM, (e) Gradient elution from 3mM to 32mM.

6.4 Method Validation

6.4.1 Linearity

Concentrations in standard calibration solutions prepared for 10 analytes are given in Table 6.2.

Table 6.2 : Solution concentrations prepared for the calibration chart.

Lactate	0.05	0.1	0.5	1	2	3	4	5
Propionate	0.05	0.1	0.5	1	2	3	4	5
Formate	0.05	0.1	0.5	1	2	3	4	5
Chloride	0.05	0.1	0.5	1	2	3	4	5
Sorbate	0.05	0.1	0.5	1	2	3	4	5
Nitrite	0.05	0.1	0.5	1	2	3	4	5
Benzoate	0.05	0.1	0.5	1	2	3	4	5
Nitrate	0.05	0.1	0.5	1	2	3	4	5
Sulfate	0.05	0.1	0.5	1	2	3	4	5
Ascorbate	0.05	0.1	0.5	1	2	3	4	5

Standard calibration solutions analyzed with the below conditions:

NaOH eluent concentration 3 mM between 0-8 minutes; 8 mM between 8.1-13 minutes; 14 mM between 13.1-18 minutes; 32 mM between 18.1-30 minutes; 3 mM between 30.1-35 minutes.

The suppressor current is 31 mA,

The detector cell temperature is 40 °C,

The flow rate is 0.250 mL / min,

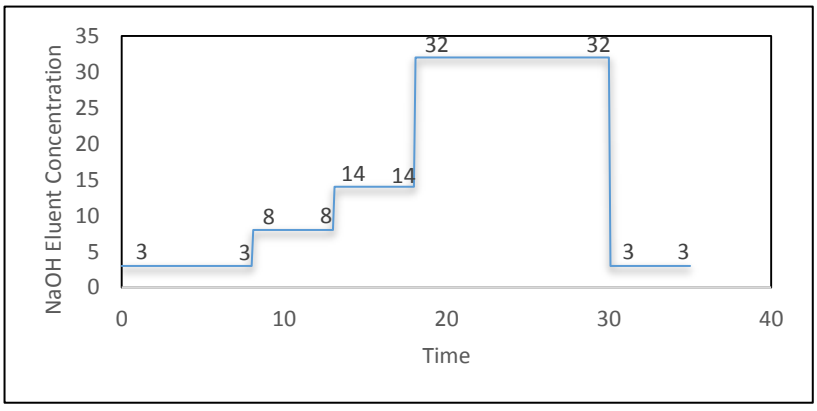

Figure 6.2 : NaOH eluent concentration change graph over time.

The overlaid chromatograms of the standard solutions with concentrations between 0.05-5.0 mg / L of lactate, propionate, format, chloride, sorbate, nitrite, benzoate, nitrate, sulfate, and ascorbate are shown in Figure 6.7.

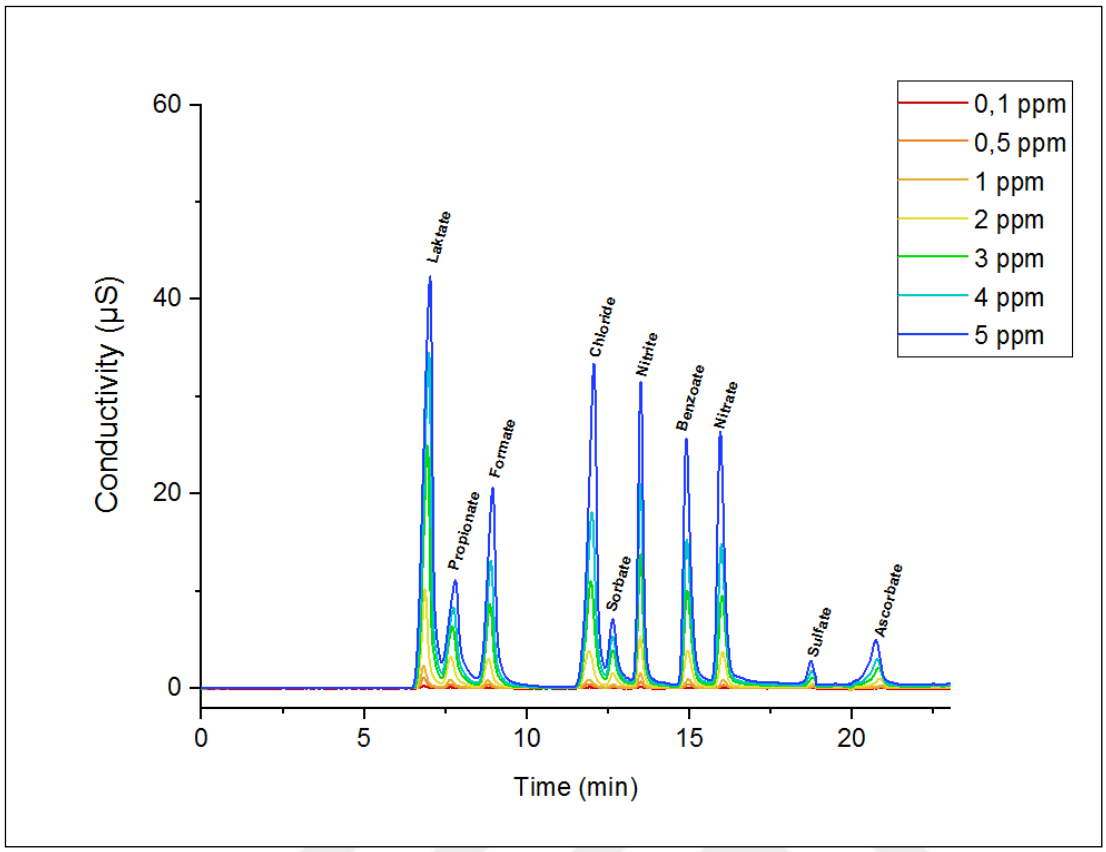

Figure 6.3 : Calibration Graphics.

Calibration curves drawn by Excel of standard solutions of 10 analytes are given in Figure 6.4.

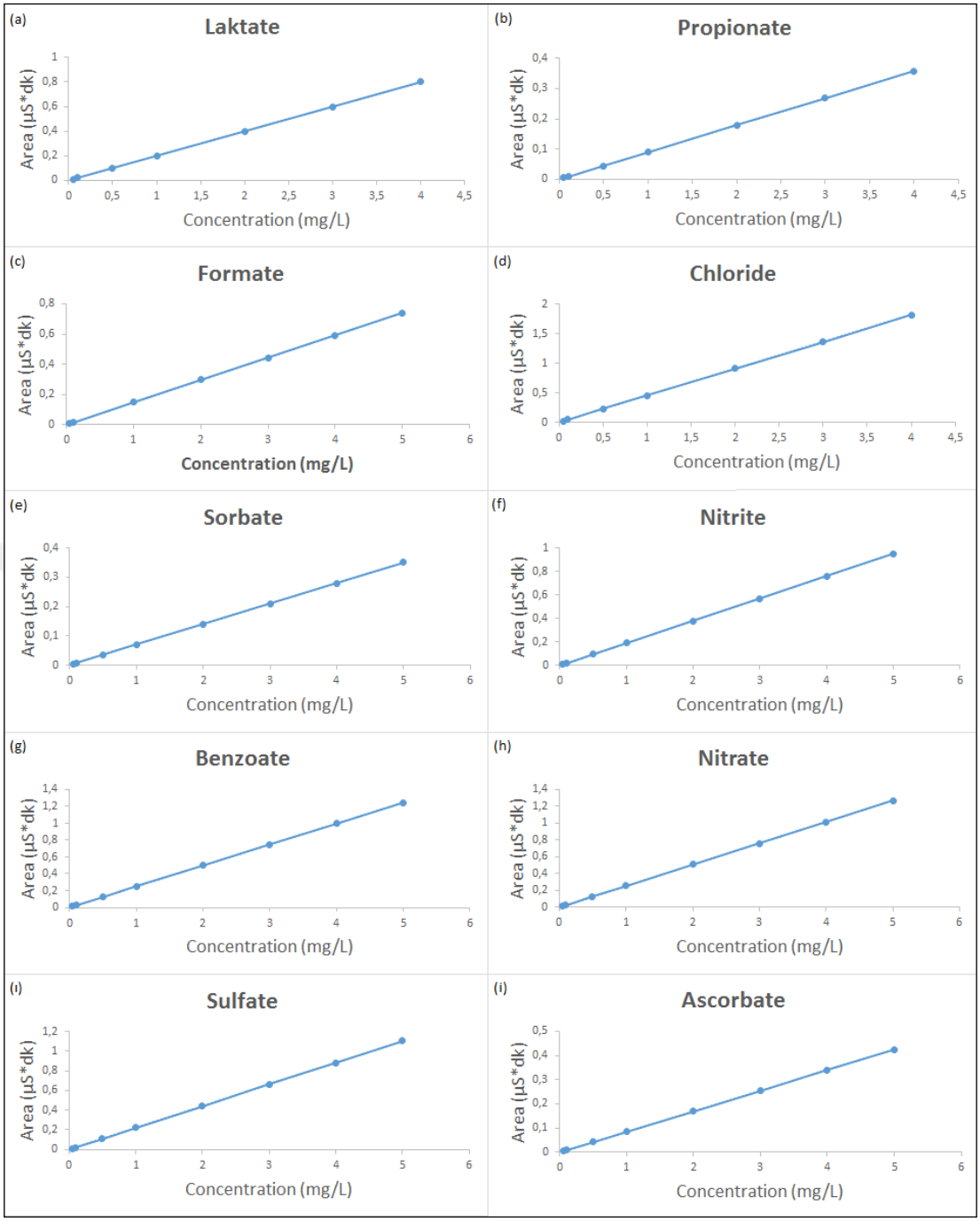

Figure 6.4 : (a) The calibration curve of 0.06-4.0 mg / L lactate standard solutions, (b) the calibration curve of 0.04-4.0 mg / L propionate standard solutions, (c) the calibration curve of 0.07-5.0 mg / L formate standard solutions, (d) the calibration curve of 0.03-4.0 mg / L chloride standard solutions, (e) the calibration curve of 0.08-5.0 mg / L sorbate standard solutions, (f) thecalibration curve of 0.06-5.0 mg / L nitrite standard solutions, (g) the calibration curve of 0.2-5.0 mg / L benzoate standard solutions, (h) the calibration curve of 0.2-5.0 mg / L nitrate standard solutions, (ı) the calibration curve of 0.7-5.0 mg / L sulfate standard solutions, (i) the calibration curve of 0.3-5.0 mg / L ascorbate standard solutions.

Separation success was determined by calculating the resolution (Rs). In general, resolution is the ability to separate two signals and it is given by below formula;

$$R = \frac{(t_{r2} - t_{r1})}{1/2(w_1 + w_2)}, \tag{6.2}$$

The resolution results obtained are listed in Table 6.3. the results indicates that the seperation is highly successful because the peaks showed resolutions is bigger than 1.5 for all analytes [35].

Table 6.3 : Resolution (Rs) of anions.

	0.05 (mg / L)	0.1 (mg / L)	0.5 (mg / L)	1 (mg / L)	2 (mg / L)	4 (mg / L)	5 (mg / L)
Lactate	1.53	1.62	1.54	1.52	1.53	1.51	1.53
Propionate	3.75	3.93	3.67	3.41	3.28	3.24	3.21
Formate	10.79	10.77	10.31	9.17	9.25	8.24	8.42
Chloride	2.36	2.36	2.26	2.04	2.00	2.07	2.01
Sorbate	3.04	3.21	3.17	2.97	2.98	2.95	2.95
Nitrite	5.41	5.65	5.54	5.30	5.16	5.17	5.08
Benzoate	2.52	2.68	2.69	2.72	2.60	2.64	2.66
Nitrate	9.09	9.52	10.61	11.96	11.59	12.08	12.03
Sulfate	4.38	4.39	4.57	5.01	4.81	4.99	5.18

In Table 6.4, method performance parameters of 10 analyte are given.

Table 6.4 : Method performance characteristics.

Analyte	Retention Time (min)	Calibration Range (mg / L)	Regression Equation (n = 6)	R^2	LOD (µg /L)	LOQ (µg/L)
Lactate	7.07	0.06-4.0	y = 0.2005x - 1E-05	0.999	18.50	61.00
Propionate	7.57	0.04-4.0	y = 0.1998x + 6E-06	0.999	10.90	36.30
Formate	8.61	0.07-5.0	y = 0.21x + 4E-06	0.999	22.00	72.30
Chloride	11.50	0.03-4.0	y = 0.3036x - 2E-06	0.999	8.70	29.30
Sorbate	12.50	0.08-5.0	y = 0.07x + 1E-05	0.999	22.00	75.30
Nitrite	13.17	0.06-5.0	y = 0.1898x - 2E-05	0.999	16.50	55.00
Benzoate	14.66	0.2-5.0	y = 0.0632x + 1E-05	0.999	68.00	233.00
Nitrate	15.71	0.2-5.0	y = 0.1493x - 8E-06	0.999	45.00	153.00
Sulfate	19.22	0.7-5.0	y = 0.221x - 1E-05	0.999	215.00	710.00
Ascorbate	20.64	0.3-5.0	y = 0.2604x + 5E-06	0.999	96.00	323.00

6.4.2 Effect of the incubation time in ultrasonic wave bath on the extraction yield

Time optimization studies for ultrasonic bath were carried out to obtain more accurate and reliable results in the experiment. The results obtained are listed in Table 6.5. As a result of the trials, 15 minutes were chosen as the optimal ultrasonic bath time.

Table 6.5 : Time optimization studies for ultrasonic bath.

Analyte (mg/L)	5 min	10 min	15 min	20 min
Lactate	20.12	26.24	28.66	28.62
Propionate	3.92	5.24	5.44	5.44
Formate	1.58	1.61	1.85	1.84
Sorbate	5.70	6.79	7.35	7.34
Nitrite	0.15	0.19	0.21	0.21
Benzoate	0.81	1.23	1.42	1.42
Nitrate	0.44	0.59	0.75	0.75
Sulfate	14.48	17.92	21.14	22.03
Ascorbate	1.85	2.18	3.69	3.71

6.4.3 Recovery and repeatability studies

A bread sample was prepared by the optimized procedure described in the bread preparation section and then it was analyzed. Considering the anion amounts in the analyzed non-spiked bread sample, three-level spikes were applied at appropriate rates. The sample was spiked with standard solutions at three concentration levels depending on the each analyte result.

For the first stage, 200 µL of 1000 mg / L standard lactate solution; 60 µL of 1000 mg / L standard propionate solution; 40 µL of 1000 mg / L standard formate solution; 80 µL of 1000 mg / L standard sorbate solution; 10 µL of 1000 mg / L standard nitrite solution; 10 µL of 1000 mg / L standard benzoate solution; 10 µL of 1000 mg / L standard nitrate solution; 200 µL of 1000 mg / L standard sulfate solution; 15 µL of 1000 mg / L standard lactate solution was taken and 0.2 g bread sample was added and the total volume was completed to 20 mL and shaken for 3 minutes.

For the second stage, 400 µL of 1000 mg / L standard lactate solution; 120 µL of 1000 mg / L standard propionate solution; 80 µL of 1000 mg / L standard formate solution; 160 µL of 1000 mg / L standard sorbate solution; 20 µL of 1000 mg / L standard nitrite solution; 20 µL of 1000 mg / L standard benzoate solution; 20 µL of 1000 mg / L standard nitrate solution; 400 µL of 1000 mg / L standard sulfate solution; 30 µL of

1000 mg / L standard lactate solution was taken and 0.2 g bread sample was added and the total volume was completed to 20 mL and shaken for 3 minutes.

For the third stage, 800 µL of 1000 mg / L standard lactate solution; 240 µL of 1000 mg / L standard propionate solution; 160 µL of 1000 mg / L standard formate solution; 320 µL of 1000 mg / L standard sorbate solution; 40 µL of 1000 mg / L standard nitrite solution; 40 µL of 1000 mg / L standard benzoate solution; 40 µL of 1000 mg / L standard nitrate solution; 800 µL of 1000 mg / L standard sulfate solution; 60 µL of 1000 mg / L standard lactate solution was taken and 0.2 g bread sample was added and the total volume was completed to 20 mL and shaken for 3 minutes.

The spiked solutions were injected to the IC system. The results of the analysis were evaluated and the necessary calculations were made, and recovery data were obtained.

The overlaid chromatograms of the spiked and unspiked bread samples can be seen in Figure 6.9.

The recovery values of 10 anions with a three-stage level spike applied on the bread sample are given in Table 6.6.

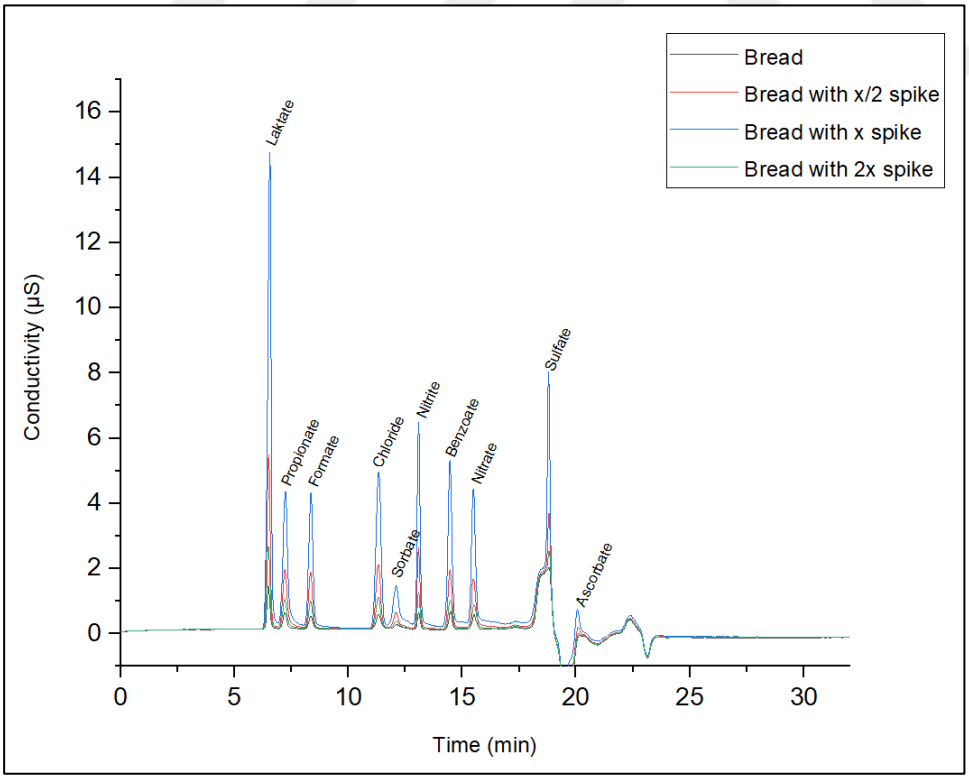

Figure 6.5 : The overlaid chromatograms of the spiked and unspiked bread samples.

Table 6.6 : Recovery % values of 9 anions. (N=3)

Analyte	Found (mg/g)	Spike No	Added (mg/g)	Total (mg/g)	Average Recovery	SD	RSD%	t_{cal}
		Spike 1	10.00	37.30	101.30	3.00	2.99	0.75
Laktate	27.17	Spike 2	20.00	47.18	100.07	2.32	2.31	0.05
		Spike 3	40.00	67.56	100.97	1.30	1.29	1.29
		Spike 1	3.00	8.55	97.85	2.98	3.05	1.25
Propionate	5.62	Spike 2	6.00	11.52	98.35	3.74	3.81	0.76
		Spike 3	12.00	17.67	100.64	3.08	3.06	0.36
		Spike 1	2.00	3.83	98.51	3.90	3.96	0.66
Formate	1.87	Spike 2	4.00	5.75	97.68	3.54	3.62	1.13
		Spike 3	8.00	9.66	97.35	2.98	3.06	1.54
		Spike 1	4.00	11.33	100.51	3.32	3.31	0.27
Sorbate	7.31	Spike 2	8.00	15.41	101.01	2.67	2.65	0.65
		Spike 3	16.00	23.55	101.30	2.61	2.57	0.86
		Spike 1	1.00	1.21	100.35	1.68	1.67	0.36
Nitrite	0.21	Spike 2	2.00	2.19	99.40	1.59	1.60	0.65
		Spike 3	4.00	4.26	101.64	1.74	1.71	1.63
		Spike 1	1.00	2.42	101.07	2.10	2.08	0.88
Benzoate	1.42	Spike 2	2.00	3.43	100.96	1.86	1.84	0.89
		Spike 3	4.00	5.43	100.30	1.58	1.57	0.33
		Spike 1	1.00	1.72	98.96	2.49	2.52	0.72
Nitrate	0.74	Spike 2	2.00	2.74	99.97	2.12	2.12	0.02
		Spike 3	4.00	4.76	100.40	0.97	0.96	0.71
		Spike 1	10.00	30.56	96.40	2.83	2.93	2.20
Sulfate	21.03	Spike 2	20.00	40.60	97.74	2.09	2.14	1.87
		Spike 3	40.00	61.51	101.30	1.48	1.46	1.52
		Spike 1	1.50	5.11	97.30	2.22	2.29	2.10
Ascorbate	3.65	Spike 2	3.00	6.63	99.07	3.37	3.41	0.48
		Spike 3	6.00	9.62	99.64	4.15	4.16	0.15

According to the results, the calculated t values were smaller than the theoritical, critical t value for 2 degrees of freedom equal to 4,30 at a level of significance $\alpha = 0.05$ (see Table 6.6).

Repeatability data was obtained with multiple measurements (N=3) taken with spiked and unspiked bread samples. Repeatability was examined with concentration, retention time, peak areas, and peak heights parameters.

Repeatability results of the concentrations of the analytes in bread 3 are given in Table 6.7.

Table 6.7 : Repeatability results of concentrations of lactate, propionate, formate, sorbate, nitrite, benzoate, nitrate, sulfate, and ascorbate. (N=3)

	Lactate (mg/g)	Propionate (mg/g)	Formate (mg/g)	Sorbate (mg/g)	Nitrite (mg/g)	Benzoate (mg/g)	Nitrate (mg/g)	Sulfate (mg/g)	Ascorbate (mg/g)
1st Day									
Average	26.04	5.82	1.76	7.35	0.21	1.42	0.76	21.58	3.59
Stand. Deviation	0.37	0.2	0.06	0.3	0.03	0.01	0.02	0.49	0.12
RSD %	1.44	2.51	3.04	1.46	1.35	1.03	2.71	2.27	3.34
2nd Day									
Average	27.06	5.8	1.84	7.5	0.2	1.48	0.77	20.49	3.82
Stand. Deviation	1.49	0.13	0.01	0.11	0.01	0.01	0.01	0.41	0.06
RSD %	3.52	2.32	0.48	2.19	1.93	1.05	0.88	2.19	1.62
3rd Day									
Average	24.49	5.96	1.89	7.27	0.21	1.49	0.73	21.78	3.33
Stand. Deviation	0.84	0.04	0.08	0.09	0.01	0.01	0.03	0.84	0.05
RSD %	3.44	0.74	3.96	1.21	3.02	0.93	2.22	3.87	1.45
Inter-Days									
Average	25.86	5.86	1.83	7.37	0.21	1.46	0.75	21.29	3.58
Stand. Deviation	1.48	0.21	0.12	0.24	0.03	0.04	0.03	1.47	0.17
RSD %	5.7	3.6	6.76	4.05	5.15	2.77	4.27	6.9	4.98

Repeatability results of the retention times of the analytes in bread 3 are given in Table 6.8.

Table 6.8 : Repeatability results of retention times of lactate, propionate, formate, sorbate, nitrite, benzoate, nitrate, sulfate, and ascorbate. (N=3)

	Lactate (Min)	Propionate (Min)	Formate (Min)	Chloride (Min)	Sorbate (Min)	Nitrite (Min)	Benzoate (Min)	Nitrate (Min)	Sulfate (Min)
1st Day									
Average	7.00	7.45	8.50	12.11	12.46	13.15	13.83	15.62	19.03
Stand. Deviation	0.02	0.01	0.01	0.09	0.01	0.02	0.07	0.00	0.03
RSD %	0.30	0.13	0.11	0.72	0.11	0.18	0.48	0.01	0.17
2nd Day									
Average	6.96	7.40	8.47	12.06	12.42	13.16	13.77	15.61	19.01
Stand. Deviation	0.03	0.04	0.02	0.06	0.03	0.00	0.01	0.01	0.00
RSD %	0.37	0.48	0.19	0.49	0.23	0.02	0.07	0.06	0.03
3rd Day									
Average	6.92	7.37	8.50	12.17	12.56	13.36	13.96	15.68	18.96
Stand. Deviation	0.00	0.01	0.03	0.12	0.13	0.16	0.15	0.06	0.01
RSD %	0.03	0.10	0.25	1.02	1.04	1.21	1.09	0.37	0.04
Inter-Days									
Average	6.96	7.4	8.49	12.11	12.48	13.22	13.85	15.64	18.9
Stand. Deviation	0.04	0.04	0.02	0.23	0.25	0.27	0.27	0.10	0.04
RSD %	0.58	0.56	0.33	1.89	1.97	2.02	1.92	0.66	0.2

Repeatability results of the peak areas of the analytes in bread 3 are repeatabilities are given in Table 6.9.

Table 6.9 : Repeatability results of peak areas of lactate, propionate, formate, sorbate, nitrite, benzoate, nitrate, sulfate, and ascorbate. (N=3)

	Lactate (µS*min)	Propionate (µS*min)	Formate (µS*min)	Sorbate (µS*min)	Nitrite (µS*min)	Benzoate (µS*min)	Nitrate (µS*min)	Sulfate (µS*min)	Ascorbate (µS*min)
1st Day									
Average	1.64	1.13	0.39	0.50	0.02	0.09	0.13	4.64	0.40
Stand. Dev.	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.00
RSD %	0.78	0.92	3.77	2.00	5.00	3.33	6.69	0.14	1.19
2nd Day									
Average	1.66	1.13	0.35	0.53	0.02	0.09	0.16	4.65	0.42
Stand. Dev.	0.01	0.01	0.02	0.02	0.00	0.00	0.01	0.01	0.00
RSD %	0.36	0.54	2.79	2.21	7.49	1.65	6.25	0.11	1.17
3rd Day									
Average	1.64	1.13	0.40	0.51	0.02	0.09	0.13	4.64	0.40
Stand. Dev.	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.01
RSD %	0.79	0.54	2.75	1.28	6.05	0.86	4.44	0.08	1.43
Inter-Days									
Average	1.65	1.13	0.41	0.52	0.02	0.10	0.14	4.66	0.41
Stand. Dev.	0.02	0.02	0.01	0.01	0.00	0.00	0.01	0.03	0.00
RSD %	0.92	1.84	4.32	2.90	10.09	4.04	8.24	0.62	1.98

Repeatability results of the peak heights of the analytes in bread 3 are repeatabilities are given in Table 6.10.

Table 6.10 : Repeatability results of peak heights of lactate, propionate, formate, sorbate, nitrite, benzoate, nitrate, sulfate, and ascorbate. (N=3)

	Lactate (µS)	Propionate (µS)	Formate (µS)	Sorbate (µS)	Nitrite (µS)	Benzoate (µS)	Nitrate (µS)	Sulfate (µS)	Ascorbate (µS)
1st Day									
Average	7.39	4.86	1.67	2.71	0.18	0.65	0.76	31.44	4.75
Stand. Dev.	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.37	0.13
RSD %	0.20	0.18	1.24	0.74	4.51	2.36	2.00	1.16	2.67
2nd Day									
Average	7.42	4.86	1.62	2.73	0.18	0.66	0.78	31.11	4.87
Stand. Dev.	0.01	0.00	0.02	0.02	0.01	0.02	0.01	0.25	0.12
RSD %	0.15	0.06	0.93	0.66	6.53	3.07	1.73	0.81	2.48
3rd Day									
Average	7.41	4.85	1.65	2.72	0.18	0.66	0.77	31.21	4.83
Stand. Dev.	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.11	0.17
RSD %	0.13	0.28	1.30	0.33	6.53	1.65	0.72	0.35	3.04
Inter-Days									
Average	7.41	4.85	1.66	2.71	0.18	0.66	0.78	31.36	4.83
Stand. Dev.	0.02	0.02	0.03	0.02	0.01	0.01	0.02	0.49	0.18
RSD %	0.24	0.32	1.78	0.91	7.29	3.14	2.46	1.55	3.74

6.4.4 Analysis of the real samples

Different bread samples (homade bread, 4 different bread taken from the market, and lavash) were used to examine the amounts of additives and inorganic anions in bread. These examples were examined with the developed method. The analytical results of the analytes are given in Table 6.11.

Table 6.11 : Results of the food additives and the inorganic anions in the bread samples.

	Lactate (mg/g)	Propionate (mg/g)	Formate (mg/g)	Sorbate (mg/g)	Nitrite (mg/g)	Benzoate (mg/g)	Nitrate (mg/g)	Sulfate (mg/g)	Ascorbate (mg/g)
Homemade Bread	-	1.06	0.15	-	0.08	0.77	0.13	7.12	0.68
Bread 1	4.61	4.79	-	1.80	-	1.92	-	6.95	1.53
Bread 2	0.08	2.67	-	-	0.60	0.13	0.05	8.18	1.07
Bread 3	27.26	4.81	2.88	10.81	0.07	-	0.38	1.02	1.44
Bread 4	2.44	5.01	0.75	10.68	0.17	1.16	0.73	5.61	0.66
Lavash	-	5.65	-	7.03	0.40	-	-	6.39	0.78

7. RESULT AND DISCUSSION

The number of preservatives that are allowed to be used in bread is limited and these additives are expressed in food packages with E codes. Lactic, propionic, formic, sorbic, benzoic and ascorbic acids are the most commonly used bread preservatives, and the inorganic anions such as chloride, nitrite, nitrate, sulfate are contaminated from the environment. Reliable, fast and simple analytical methods are required to evaluate the safety and quality control of the addition of these food additives and anions in the bread.

Various analytical methods have been reported in the literature for the individual determination of these analytes [36-39]. Analytical techniques most commonly used in determining additives in foods; gas chromatography (GC) [40], high performance liquid chromatography (HPLC) [41], capillary electrophoresis (CE) [42,43], and ion chromatography (IC) [44,45]. But each method has its advantages and disadvantages.

GC method is a sensitive, specific and fast method. However, analytes need to be prepared and derivatized at long extraction times. In the study conducted with HPLC, it was seen that the effects of the matrix, long analysis time and excessive mobile phase consumption negatively affect the efficiency. In the study conducted with CE method, instrumental detection limits (LODi) and quantification (LOQi) values are found high for reliable analysis. Also, the method's repeatability is low.

The most important advantages of ion chromatography are a wide range of applications, reliability with accuracy and precision, high selectivity, high speed, high separation efficiency, good tolerance to sample matrices and low consumable cost.

In this work, the ion chromatography method was used for the analysis of organic acid anions of these preservatives and inorganic anions. Five different programs have been tried for the separation of these analytes from bread. The best separation was accomplished by using an anion exchange column with an optimized multistep gradient eluent program that utilized 3mM to 32 mM NaOH. Ten analytes were completely separated within 35 minutes. All the curves showed good linearity ($r^2 \geq$

0.999) within the calibration ranges. Under optimized conditions, device's the limits of detection (LODs) were between 0.0087 and 0.215 μg L^{-1} and the limits of quantitation (LOQs) were between 0,029 and 0,71 μg L^{-1}. To evaluate the recovery test, three different concentrations of standard solutions were added to the sample and the average recoveries were between 95.34 and 101.37 %. Precision (RSD) was evaluated by continuously performing three replicates each day within 3 days for the determination of the samples. The relative standard deviations (RSDs) were less than 6.9 %. In the thesis, experiments were carried out with 5 different bread were collected from different markets and a home made bread. And we found that the highest level of additives is in the bread 3.

In our country, approximately 400 - 450 g bread is consumed per day and a thin slice of bread is 25 grams [10,11]. According to this results, someone who consumes an average of 17 slices of bread 3, 11.59 g lactate, 2.04 g propionate, 1.22 g formate, 4.59 g sorbate, 0.03 g nitrite, 0.16 g nitrate, 0.43 g sulfate, and 0.61 g ascorbate. These values are within the daily intake limits as I explained in the food additives section. In other words, all of the bread examined is suitable the Turkish Food Codex Food Additives Regulation.

The results indicated that this method was simple, rapid, sensitive, and accurate for the simultaneous determination of lactate, propionate, formate, sorbate, benzoate, ascorbate, chloride, nitrite, nitrate, and sulfate in various bread samples.

In the literature, there are methods to analyze one or more of the food additives together, but there is not any study that has been found in which all of 10 analytes are analyzed simultaneously.

In this thesis, a new ion chromatographic method has been developed for the simultaneous analysis of bread additives and inorganic anions. This new method has been proved to be direct, sensitive, fast, and environmentally friendly compared to other methods in the literature. Also, it is an inexpensive and easy method in the preparation of samples.

It is aimed to apply this method successfully in the routine determination of food additives and inorganic anions in bread samples in food analysis and quality control laboratories.

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