

JANUARY 2023

Ph.D. in Food Engineering

AYŞE SEVGİLİ

**REPUBLIC OF TÜRKİYE
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES**

**IDENTIFICATION OF LACTIC ACID BACTERIA AND YEASTS
FROM TURKISH SOURDOUGHS AND SELECTION OF
STARTER CULTURES FOR SOURDOUGH BREAD**

**Ph.D. THESIS
IN
FOOD ENGINEERING**

**BY
AYŞE SEVGİLİ
JANUARY 2023**

**IDENTIFICATION OF LACTIC ACID BACTERIA AND YEASTS
FROM TURKISH SOURDOUGHS AND SELECTION OF
STARTER CULTURES FOR SOURDOUGH BREAD**

Ph.D. Thesis

In

Food Engineering

Gaziantep University

Supervisor

Prof. Dr. Osman ERKMEN

By

Ayşe SEVGİLİ

January 2023



©2023[Ayşe SEVGİLİ]

**IDENTIFICATION OF LACTIC ACID BACTERIA AND YEASTS FROM
TURKISH SOURDOUGHS AND SELECTION OF STARTER CULTURES
FOR SOURDOUGH BREAD**

submitted by **Ayşe SEVGİLİ** in partial fulfillment of the requirements for the degree
of Doctor of Philosophy in **Food Engineering, Gaziantep University** is approved
by,

Prof. Dr. Mehmet İshak YÜCE
Director of the Graduate School of Natural and Applied Sciences

Prof. Dr. Ali Coşkun DALGIÇ
Head of the Department of Food Engineering

Prof. Dr. Osman ERKMEN
Supervisor, Nutrition and Dietetics
Istanbul Arel University

Exam Date: 26 January 2023

Examining Committee Members:

Prof. Dr. Osman ERKMEN
Thesis Supervisor, Nutrition and Dietetics
Istanbul Arel University

Prof. Dr. Mustafa BAYRAM
Food Engineering
Gaziantep University

Asst. Prof. Dr. Gülten ŞEKEROĞLU
Food Processing
Gaziantep University

Asst. Prof. Dr. Hidayet SAĞLAM
Molecular Biology and Genetics
Kilis 7 Aralık University

Asst. Prof. Dr. Pınar GÜMÜŞ
Nutrition and Dietetics
Kilis 7 Aralık University

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ayşe SEVGİLİ

ABSTRACT

IDENTIFICATION OF LACTIC ACID BACTERIA AND YEASTS FROM TURKISH SOURDOUGHS AND SELECTION OF STARTER CULTURES FOR SOURDOUGH BREAD

SEVGİLİ, Ayşe

Ph.D. in Food Engineering

Supervisor: Prof. Dr. Osman ERKMEN

January 2023, 135 pages

In this study, 60 lactic acid bacteria (LAB) and 40 yeasts isolated from sourdoughs collected from Gaziantep, Konya and Mardin provinces were identified at the species level by MALDI-TOF MS and PCR-sequence analysis. It was determined that there were 6 different LAB from Konya sourdoughs and sent for sequence analysis. *Pediococcus acidilactici* (41.18%) and *Levilactobacillus brevis* (23.53%) were dominant among these species (most abundant). It was determined that there were 8 different LAB out of 19 LAB isolated from Gaziantep sourdoughs and sent for sequence analysis. Among these, the dominant microflora was found to be *L. brevis* (42.10%) and *Lactiplantibacillus plantarum* (21.05%). It was determined that there were 5 different LAB from Mardin sourdoughs and sent for sequence analysis. Among these, the dominant flora was found to be *L. brevis* (58.33%), *L. plantarum* (21.05%) and *P. acidilactici* (16.66%). After the identification, LAB and yeasts combinations were determined according to their technological properties and used in the production of sourdough breads. A total of 12 sourdough breads were produced from 4 different combinations of microorganisms. Physicochemical, textural and sensory analyzes of the produced breads were made and the properties of the breads were evaluated. Then, 5 combination breads were produced. Sourdough reads prepared with mixed cultures gained more appreciation as SD4, SD8, SD12 in first combination and SD17 in second combination. These breads contain more homofermentative LAB.

Keywords: Lactic Acid Bacteria, Sourdough, Yeast, PCR, MALDI-TOF

ÖZET

TÜRK EKŞİ HAMURLARINDA LAKTİK ASİT BAKTERİ VE MAYALARININ TANIMLANMASI VE EKŞİ HAMUR EKMEĞİ İÇİN STARTER KÜLTÜRLERİNİN SEÇİMİ

SEVGİLİ, Ayşe

Doktora Tezi, Gıda Mühendisliği

Danışman: Prof. Dr. Osman ERKMEN

Ocak 2023, 135 sayfa

Bu çalışmada, Gaziantep, Konya ve Mardin illerinden toplanan ekşi hamurlardan izole edilerek biyokimyasal ve morfolojik testleri sonucunda belirlenen 60 laktik asit bakterisi (LAB) ve 40 maya MALDI-TOF MS ve PCR-sekans analizleri ile tür düzeyinde tanımlanmıştır. Konya ekşi hamurlarından izole edilen ve sekans analizine gönderilen 17 LAB’da 6 farklı LAB türü olduğu belirlenmiştir. Bu türlerden baskın olanların *Pediococcus acidilactici* (%41,18) ve *Levilactobacillus brevis* (%23,53) olduğu tespit edilmiştir. Gaziantep ekşi hamurlarından izole edilen ve sekans analizine gönderilen 19 LAB’da 8 farklı LAB türü belirlenmiştir. Bunlar içerisinde *L. brevis* (%42,10) ve *Lactiplantibacillus plantarum* (%21,05) baskın flora olduğu tespit edilmiştir. Mardin ekşi hamurlarından izole edilen ve sekans analizine gönderilen 24 LAB’da 5 farklı LAB türü olduğu belirlenmiştir. Bunlar içerisinde baskın flora *L. brevis* (%58,33), *L. plantarum* (%21,05) ve *P. acidilactici* (%16,66) olduğu tespit edilmiştir. Tanımlama işlemi gerçekleştirildikten sonra, ekşi hamur ekmeği üretiminde kullanmak için LAB ve maya kombinasyonları teknolojik özelliklerine göre belirlenmiştir. Her bölgeye özgü ekmek denemeleri için 4 farklı kombinasyon yapılarak toplamda 12 ekşi hamur ekmeği üretilmiştir. Üretilen ekmeklerin fizikokimyasal, tekstür ve duyusal analizleri yapılmış ve ekmeklerin özellikleri değerlendirilmiştir. Daha sonra 5 kombinasyonlu ekmek üretimi yapılmıştır. Karışık kültürlerle hazırlanan ekmeklerden ilk kombinasyon içerisinde SD4, SD8, SD12 ve ikinci kombinasyondan SD17 daha çok beğeni kazanmıştır. Bu ekmeklerde homofermentatif LAB daha fazla bulunmaktadır.

Anahtar Kelimeler: LAB, Ekşi hamur, Maya, PCR, MALDI-TOF



'Dedicated to my mom and dad'

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof. Dr. Osman ERKMEN for his guidance and support throughout the study. I am thankful for his encouragement and motivation.

I would like to express my love and gratitude to my husband Ahmet Mert SEVGİLİ, my daughter Ayşe Hira SEVGİLİ and my son Ahmet Asaf SEVGİLİ for their endless encouragement and loving supports.

Financial support for this study was provided by 1002 - Short Term R&D Funding Program of The Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: 2180193).

TABLE OF CONTENTS

	Page
ABSTRACT.....	vi
ÖZET	vii
ACKNOWLEDGEMENTS	ix
TABLE OF CONTENTS	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvii
CHAPTER I	2
INTRODUCTION.....	2
CHAPTER II.....	2
LITERATURE REVIEW	2
2.1 History	2
2.2 Sourdough technology	4
2.2.1 Natural fermentation method (Type I)	4
2.2.2 Liquid sourdough method (Type II)	5
2.2.3 Use of starter culture (Type III)	5
2.3 Benefits of sourdough in bread production	6
2.4 Sourdough usage in bread making	7
2.5 Yeasts in sourdough	10
2.5.1 <i>Saccharomyces cerevisiae</i>	11
2.5.2 <i>Pichia kudriavzevii</i>	11
2.5.3 <i>Kluyveromyces marxianus</i>	12
2.6 LAB in sourdough	12
2.6.1 LAB.....	13

2.6.2 <i>Levilactobacillus brevis</i>	14
2.6.3 <i>Lactiplantibacillus plantarum</i>	15
2.6.4 <i>Pediococcus acidilactici</i>	16
2.7 Main pathway in LAB.....	18
2.7.1 Glycolysis.....	18
2.7.2 Lipolysis	18
2.7.3 Proteolysis	19
2.8 MALDI-TOF MS analysis	19
2.9 Polymerase chain reaction (PCR) and agarose gel.....	22
CHAPTER III	25
MATERIAL AND METHODS	25
3.1 Equipments, chemicals and media	25
3.2 Sourdough collection.....	26
3.3 Sample preparation and counting of microorganisms.....	26
3.4 Characterization and identification of isolates	27
3.4.1 Determination of morphological properties of LAB and yeasts on Petri plates	27
3.4.2 Technological properties of isolates.....	28
3.5 Identification of microorganisms	30
3.5.1 MALDI-TOF MS technique.....	30
3.5.2 PCR analysis	31
3.6 Preparation of sourdough bread culture	32
3.7 Sourdough bread production	33
3.8 Storage of breads	34
3.9 Analyzes of flour, sourdough and sourdough bread.....	34
3.9.1 Ash determination	34
3.9.2 Extensograph analysis	35
3.9.3 Farinograph analysis	35
3.9.4 Wet gluten analysis	36
3.9.5 Protein analysis in flours	36
3.9.6 Dough swelling power	36
3.9.7 Determination of moisture and total dry matter	37
3.9.8 pH and titration acidity.....	37

3.9.9 Loaf height	38
3.9.10 Volume and specific volume	38
3.9.11 Texture properties	38
3.9.12 Color properties	38
3.9.13 Determination of calorimetric (DSC) properties of breads	38
3.9.14 Microorganisms count in sourdough	39
3.9.15 Sensory analysis	39
3.10 Statistical analysis	39
CHAPTER IV.....	41
RESULTS AND DISCUSSION	41
4.1 LAB and yeasts isolations	41
4.2 Morphological characteristics of LAB and yeasts	42
4.3 Identification of LAB and yeasts with MALDI-TOF analysis.....	46
4.4 Identification of LAB and yeasts with PCR-sequence analysis	49
4.5 Technological properties of LAB and yeasts	53
4.6 Technological properties of flour	57
4.7 Sourdough bread production from first combinations of LAB and yeasts.....	62
4.7.1 Determination of LAB and yeasts	62
4.7.2 Microbiological characteristics of sourdough	66
4.7.3 pH and titratable acidity of sourdough	67
4.7.4 pH and titratable acidity of sourdough bread	70
4.7.5 Moisture, total dry matter and loaf height of sourdough breads	71
4.7.6 Crust and crumb color characteristics	74
4.7.7 Volume and specific volume of sourdough breads	76
4.7.8 Texture characteristics of sourdough breads	77
4.7.9 Determination of calorimetric properties of breads	82
4.7.10 Sensory analysis of sourdough breads	83
4.8 Sourdough bread production of second combinations	84
4.8.1 Microbiological properties of sourdough	86
4.8.2 pH and titratable acidity of sourdough in second combinations	87
4.8.3 pH and titration acidity of sourdough bread in second combinations...	89
4.8.4 Moisture, total dry matter and loaf height in second combinations	91
4.8.5 Crust and crumb color characteristics in second combinations	93

4.8.6 Volume and specific volume of sourdough breads from second combinations	94
4.8.7 Texture characteristics in second combinations.....	95
4.8.8 DSC analysis results of sourdough breads for second combinations....	99
4.8.9 Sensory analysis of second combinations	100
CHAPTER V	102
CONCLUSION	102
REFERENCES.....	105
APPENDICES	117
APPENDIX A-MEDIA.....	117
APPENDIX B- TABLES OF ANALYSIS RESULTS	121
APPENDIX C- SENSORY ANALYSIS FORM AND PICTURES	131
CURRICULUM VITAE (CV)	133

LIST OF TABLES

	Page
Table 2.1 <i>Lactobacillus</i> species as sugar fermentation	14
Table 4.1 Colony morphology, Gram staining and microscope view of LAB on MRSA.....	42
Table 4.2 Colony morphology and microscope view of yeasts on PDA.....	44
Table 4.3 Percentage of LAB with using MALDI-TOF analysis	47
Table 4.4 Percentage of yeasts with using MALDI-TOF analysis.....	48
Table 4.5 Technological properties of LAB isolated from sourdough.....	58
Table 4.6 Technological properties of yeast isolated from sourdough	59
Table 4.7 Chemical characteristics of wheat flour	60
Table 4.8 Extensograph analysis results of the flour.....	62
Table 4.9 Combinations of LAB and yeasts used in the sourdough bread production.....	64
Table 4.10 Selection characteristics of sourdough cultures	65
Table 4.11 Loaf height values of sourdough breads.....	74
Table 4.12 Results of volume and specific volume of sourdough breads	77
Table 4.13 Sensory score scale of 12 sourdough breads	84
Table 4.14 Combinations of LAB and yeasts used in the second sourdough bread production.....	85
Table 4.15 Selection characteristics of second sourdough cultures	86
Table 4.16 Loaf height of sourdough breads produced from second combinations	93
Table 4.17 Volume and specific volume results of bread produced with the second combinations	95
Table 4.18 Results of sensory analysis of sourdough breads from second combinations	101

LIST OF FIGURES

	Page
Figure 2.1 A map of ingredients and benefits of sourdough	7
Figure 2.2 Factors affecting the development of sourdough and its effect on bread quality (modified from Arendt, 2007; Ataç, 2016)	9
Figure 2.3 Image of scanning electron microscopy of <i>L. brevis</i> (Elez- Martinez et al., 2005).....	15
Figure 2.4 Micro-morphological image of <i>L. plantarum</i> (Valan et al., 2016).....	16
Figure 2.5 Scanning electron microscopy image of <i>P. acidilactici</i> (Balgir et al., 2013).....	17
Figure 2.6 MALDI-TOF MS microbial identification flowchart (Erkmen, 2022).....	21
Figure 2.7 (a) PCR workbench (Maddocks and Jenkins, 2017); (b) A solidified agarose gel (Lee et al., 2012); (c) Adding loading dye to her DNA samples (Lee et al., 2012); (d) Loading the DNA sample into a well in the gel (Lee et al., 2012).....	23
Figure 3.1 Gram-staining procedure	28
Figure 3.2 The sourdough production flowchart (Sevgili et. al., 2021).....	33
Figure 4.1 Growth of LAB (a) on MRS agar and yeast on PDA (b)	41
Figure 4.2 The purified LAB (a) on MRS agar and yeasts colonies on PDA (b)	42
Figure 4.3 Image under microscope examination; (a): Bacilli for LAB, (b) Cocci for LAB, (c) Cylindrical for yeasts and (d) Oval for yeasts	46
Figure 4.4 The image of the sequence peaks in the FinchTV program	49
Figure 4.5 Alignment created with the Bioedit Sequence Alignment Editor program.....	50
Figure 4.6 Nucleotide BLAST on NCBI website	50

Figure 4.7 Phylogenetic tree of LAB strains.....	52
Figure 4.8 Phylogenetic tree of yeast species	55
Figure 4.9 Farinograph analysis chart of flour	61
Figure 4.10 Extensograph analysis chart of flour	62
Figure 4.11 Microbiological analysis results of sourdoughs.....	67
Figure 4.12 pH values of sourdoughs.....	68
Figure 4.13 Titration acidity values of sourdoughs	69
Figure 4.14 Volume changes on the of sourdoughs	70
Figure 4.15 pH values of sourdough breads.....	70
Figure 4.16 Titration acidity values of sourdough breads.....	71
Figure 4.17 Moisture contents of sourdough breads.....	72
Figure 4.18 Dry matter values of sourdough breads	73
Figure 4.19 Crust color values of sourdough breads.....	75
Figure 4.20 Crumb color values of sourdough breads	76
Figure 4.21 Hardness results of sourdough breads	78
Figure 4.22 Cohesiveness results of sourdough breads	79
Figure 4.23 Springiness results of sourdough breads.....	80
Figure 4.24 Gumminess results of sourdough breads	81
Figure 4.25 Chewiness results of sourdough breads	82
Figure 4.26 Enthalphy results on sourdough breads	83
Figure 4.27 Microbiological results of sourdoughs produced in the second combinations.....	87
Figure 4.28 pH results of sourdoughs produced with second combinations.....	88
Figure 4.29 Titration acidity results of sourdoughs produced with second combinations.....	88
Figure 4.30 Volume results of sourdoughs produced with second combinations.....	89
Figure 4.31 pH of sourdough breads produced with second combinations	90
Figure 4.32 Titration acidity of sourdough breads produced with second combinations.....	90
Figure 4.33 Moisture of sourdough breads produced with second combinations.....	91
Figure 4.34 Dry matter of sourdough breads produced with second combinations.....	92

Figure 4.35 Crust color values of sourdough breads produced from second combinations.....	94
Figure 4.36 Crumb color values of sourdough breads produced from second combinations.....	94
Figure 4.37 Hardness results of sourdough breads produced with second combinations.....	96
Figure 4.38 Cohesiveness results of sourdough breads from the second combinations.....	96
Figure 4.39 Springiness results of sourdough breads produced with second combinations.....	97
Figure 4.40 Gumminess results of sourdough breads produced with second combinations.....	98
Figure 4.41 Chewiness results of sourdough breads produced with second combinations.....	99
Figure 4.42 Enthalpy results on sourdough breads produced with second combinations.....	100

LIST OF ABBREVIATIONS

μm	Micrometer
a*	Redness or Greenness
ANOVA	Analysis Of Variance
ATCC	American Type Culture Collection
b*	Yellowness or Blueness
BLAST	The basic local alignment search tool
bp	Base pair
BU	Brabender Units
cfu	Colony forming unit
cm	Centimeter
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimeter
FASTA	Fast Adaptive Shrinkage/Thresholding Algorithm
g	Gram
H₂O₂	Hydrogen peroxide
H₂SO₄	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
K₂HPO₄	Dipotassium hydrogen phosphate
kb	Kilo base
kg	Kilo gram
L	Liter
L*	Lightness
LAB	Lactic acid bacteria
MAB	Mesophilic aerobic bacteria
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass

	spectrometry
mg/g	Miligram/gram
MgSO₄.H₂O	Magnesium sulfate monohydrate
min	Minute
ml	Mililiter
MRS	De Man, Rogosa and Sharpe
NCBI	National center for biotechnology information
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pmol	picomole
RNA	Ribonucleic acid
SPSS	Statistical Package for Social Sciences
v/v	Volume/volume
w/w	Weight/weight

CHAPTER I

INTRODUCTION

Sourdough goes back to ancient times. Sourdough is produced only by mixing flour and water in a warm place. The sourdough is formed after a few replenishments (Aplevicz et al., 2014). Sourdough is used for many different production foods like pizza, snacks, bread and sweet baked products. Sourdough is significant for the preparation of bread to improve textural properties, technological properties, organoleptic properties and the development of shelf life.

Sourdough has a complex mechanism that involves changing in carbohydrate and protein components. Flour, water and microorganisms play a major role in sourdough processing. Flour is very important to produce sourdough bread and wheat flour is most preferred (Chavan and Chavan, 2011).

Sourdough microbiota has complex and varied lactic acid bacteria (LAB) and yeasts. According to Rehman et al. (2006), isolated different LAB from sourdough and most of them with in the genus *Lactobacillus*, most of the yeasts genera were *Saccharomyces* and *Candida*. *Saccharomyces cerevisiae* is the dominant yeast species in sourdough.

LAB and yeasts are identified as phenotypic and genotypic properties. LAB is coccus or bacilli, Gram-positive, non-motile, catalase-negative and non-sporulating bacteria (Thakur et al., 2017). Cellular morphology, mode of glucose fermentation, growth temperature ranges and sugar utilization patterns are still very important for the classification of LAB and molecular methods have raised the identified number of LAB (Lahtinen et al., 2011).

LAB and yeasts live symbiotic life for sourdough production. Heterofermentative LAB make the dough leaving while homofermentative LAB affects elasticity, acidity and taste of bread (Bakırcı and Köse, 2017).

This study has been included in the following purposes:

- ✓ Bread is the most consumed food. The increase in population has also increased the demand for bread. Thus, the producers aim to produce bread in a short time. This leads to the consumer away from the traditional bread.
- ✓ flavor. On account of this, there is the need for combination of lactic acid bacteria and yeasts to use sourdough bread production for improving technological, nutritional, sensory properties, leavened product and shelf life.
- ✓ There is no standard for starter culture in sourdough bread production.

Based on all these parameters;

- ✓ LAB and yeasts were isolated from traditional sourdough.
- ✓ Identification of 60 LAB and 40 yeast strains were reformed using MALDI-TOF MS and PCR-sequence analysis techniques.
- ✓ The technological properties of the identified LAB isolates (homo- or hetero-fermentation test, growth at different temperatures, oxidative and fermentative test, acid and gas formation from sugars and their ability to grow in different salt and temperature environments) were determined. Technological features of *Saccharomyces* species were determined to form acid and gas from sugars, growth at 20, 28 and 37°C.
- ✓ Firstly, 12 sourdough breads were produced with combination of LAB and yeasts.
- ✓ Considering 12 sourdough breads quality characteristics, 5 combinations with LAB and yeast species used in the production of sourdough bread.

CHAPTER II

LITERATURE REVIEW

2.1 History

Bread is among the most important food sources consumed all over the world throughout human history (Bakırcı and Köse, 2017). Bread making is one of the oldest technologies known to man. Results; it shows that the people of Babylon, Egypt, Greece and Rome used bread as part of their diet long before MS (Chavan and Chavan, 2011). In Turkey, bread firstly have from grain-based nutrition. Sixty six% of the energy consumed per person in our country is met from cereals, 56% of this is only from bread and 50% of the protein is again from bread. According to a scientific study commissioned by Turkish Grain Board in 2012; 25.295 tons of bread per day and 9.2 million tons of bread per year are produced. When this amount is converted into pieces based on the standard 250 g bread, the amount of bread produced corresponds to 101 million per day and 37 billion per year. Annual per capita bread consumption in the world is 44 kg in Australia, 180 kg in Egypt, 150 kg in Iran, 73 kg in Italy, 98 kg in Kuwait, 130 kg in Syria and 34 kg in the USA, this value is 180-210 kg in Turkey (Coşkuner et al., 1999). In the study conducted by Toprak Mahsülleri Ofisi (TMO) in Turkey, it is stated that the daily bread consumption per person is 250 g or 319 g (household and institutional population together) in 2012 (Ertürk et al., 2015). The high consumption of bread and requirements for storage have increased the use of chemical preservatives as additives in terms of increasing the shelf life of bread. This situation has led the consumer to seek quality and reliable products in bread production.

Bread is made by mixing wheat flour, yeast, salt and water in certain proportions as the main ingredient and kneading and baking the dough after fermentation. Bread is a nutritious food with rich carbohydrate content in terms of energy source. In recent

years, sourdough with rich aroma and microflora has been used to extend the shelf life of bread and increase its nutritional quality (Bakırcı and Köse, 2017).

It has been determined that breads produced with LAB and yeast strains are more volume than normal bread and the use of sourdough improves all bread characteristics. Since yeast and bacteria operate together in the previously used sourdough technique, this application is based on natural flora and sourdough bread. It is preferred due to its suitable volume, strong aroma, good crumb structure and long shelf life (Küçükçuban, 2012).

The essence of the sourdough method is based on using a piece of dough from previous dough containing wild yeasts, lactic and acetic acid-producing bacteria from the air and used ingredients and it is also used in the next dough preparation (Akgün, 2007). Proteolysis and microbial hydrolysis of starch cause some physico-chemical changes during fermentation of the sourdough bread and as a result, the stale of the bread slows down (Bakırcı and Köse, 2017).

LAB isolated from sourdough belongs to the genera *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus* and *Lactobacillus*. Yeasts isolated from sourdough are *Saccharomyces cerevisiae*, *S. exiguus*, *S. delbrueckii*, *S. uvarum*, *Candida humilis*, *C. guilliermondii*, *C. stellata*, *Issatchenkia orientalis*, *Pichia ananias*, *Pichia norvegensis*, *Pichia polymorpha*, *Pichia saitoi*, *Pichia membranifaciens* and *Debaryomyces hansenii* (Evren et al., 2011).

Ecological factors affecting the microbiota of sourdough fermentations can be divided into endogenous and exogenous factors. Endogenous factors are the composition of the grain substrate, such as fermentable carbohydrates, nitrogen sources, minerals, vitamins, oils, enzyme activities, microbial inhibitors and the original microbiology of grains. Exogenous factors are the applied process parameters such as temperature, oxygen, pulp yield, amount of recycled pulp, sodium chloride, fermentation time, spreading stages and starter cultures used. If these parameters are held constant over a longer period, their interactions induce a distinctive selection of microbiota of LAB and yeasts. These factors change the local sourdough microbiota and create a locally specific microbiota. Recently, the demand for bread made without the use of chemical additives and preservatives has increased, and ensuring the safety of the products with the bio-preservation method

comes into prominence. Thus, it is aimed to identify bacteria with natural flora for better quality bread production. In addition, the content of microorganisms in sourdough varies locally. The determination of local cultures all over the world and the use of cultures to be isolated in the production of local flavors or similar products has gained importance.

The aim of this study is to isolate LAB and yeasts from sourdough samples collected from Gaziantep, Konya and Mardin regions. First, the morphological and biochemical properties of the microorganisms were determined. Later, fermentation properties were determined. Finally, microbiota of sourdoughs were determined by using Matrix-assisted laser desorption/ionization time-of-flight and mass spectrometry (MALDI-TOF MS) and Polymerase chain reaction (PCR) methods. The isolated LAB and yeasts were used in the produce sourdough bread.

2.2 Sourdough technology

Sourdough has been used for many years and preferred in bread production in many civilizations and it is based on the gradual fermentation method. In sourdough, yeast and bacteria work together to create natural flora (Kotancilar, 2006). Sourdough fermentation has positive effect that improve texture and nutritional properties, shelf life and sensorial characteristics (Aplevicz et al., 2014), without gluten products, low glyceamic index products, improve mineral bioavailability and bioactive compounds (Katina et al., 2005). Sourdough has used the production of pizza, bread, snacks and sweet baked products. In the production of sourdough, flour and water are mixed and kept in a warm place (Aplevicz et al., 2014). Sourdoughs can be divided into 3 groups as natural fermentation method (Type I), liquid sourdoughs (Type II) and dried from of Type II (Type III) according to their differences in production technology (Yağmur et al., 2016).

2.2.1 Natural fermentation method (Type I)

Traditional sourdoughs are classified as Type I doughs. Type I sourdough production is based on back-slopping to keep of sourdough microflora active (De Vuyst et al., 2021; Yağmur et al., 2016). Type I sourdoughs are characterized by fermentation temperatures between 20 and 30°C and the pH is about 4.0 (De Vuyst and Neysens, 2005). During fermentation, LAB (especially *Lactobacillus* and *Pediococcus*

species) becomes dominant. The number of LAB in sourdough increases to 3×10^9 colony forming unit (cfu)/ml and the number of yeast to the level of 10^6 - 10^7 cfu/ml (Küçükçuban, 2012). These doughs are used for both acidification and leavening. Common LABs of Type I sourdoughs are *Lactobacillus mindensis*, *Furfurilactobacillus rossiae* (previously known as *Lactobacillus rossiae*), *Lactobacillus zymae*, *Lactobacillus acidifarinae*, *Lactobacillus hammesii*, *Lactobacillus nantensis*, *Pediococcus acidilactici*, *Enterococcus hirae*, *Enterococcus faecalis*, *L. acidophilus*, *L. alimentarius*, *L. brevis*, *L. fructivorans*, *L. pentosus*, *Companilactobacillus paralimentarius* (previously known as *Lactobacillus paralimentarius*), *L. paraplantarum*, *Lactiplantibacillus plantarum* (previously known as *Lactobacillus plantarum*), *L. pontis*, *L. spicheri*, *L. sanfranciscensis*, *Leuconostoc mesenteroides*, *L. cremoris*, *Weissella confusa* and *W. cibaria* (Arendt et al., 2007; De Vuyst and Vancanneyt, 2007; Sevgili et al., 2021). Especially isolated yeasts are *Pichia* (Sevgili et al., 2021), *Candida* and *Saccharomyces* genera and yeast microflora of sourdough occur more than 25 species (Arendt et al., 2007).

2.2.2 Liquid sourdough method (Type II)

Type II sourdough is industrial type as semi-liquid and represents long fermentations of flour and water mixture (Chavan and Chavan, 2011; De Vuyst et al., 2021). Type II sourdough is fermented at high temperatures above 30-37°C, with a long fermentation periods up to 5 days and high acid content pH<3.5 after 24 h fermentation. It is mainly used to acidify and aromatize bread dough (De Vuyst and Neysens, 2005). Isolated LABs in Type II sourdoughs are *L. panis*, *L. pontis*, *L. acidophilus*, *L. crispatus*, *L. delbrueckii*, *L. fermentum*, *L. johnsonii*, *L. amylovorus*, *L. frumenti*, *L. mucosae*, *L. paracasei*, *L. rhamnosus* and *L. reuteri*. *S. cerevisiae*, *I. orientalis* and *C. glabrata* are isolated yeasts in Type II sourdoughs (Arendt et al., 2007).

2.2.3 Use of starter culture (Type III)

Type III sourdoughs can be considered as dried sourdough consisting of LAB resistant to drying and are mainly used as flavoring agents. Type III sourdoughs are dried dough used as dough acidifiers, flavor carriers or as a starter for sourdough in

ovens. Microbiota mainly consists of LAB resistant to drying and storage (De Vuyst and Neysens, 2005).

Pure LAB or a mixture of LAB and yeast is used to perform fermentation. In the selection of sourdough culture, cultures are selected that have the ability to acidify the dough in a short time and have the ability to create acceptable aroma when used in bread making (Küçükçuban, 2012). For this purpose, Types II and III sourdough require *S. cerevisiae* as baker's yeast (De Vuyst et al., 2005).

2.3 Benefits of sourdough in bread production

Sourdough fermentation has advantages and benefits. Gobbetti and Ganzle (2012) are reported these advantages. Grains that fermented with LAB and yeasts bring the following advantages:

- Dough rising,
- Improving sensory properties through the development of colors, aromas and textures,
- Preservation of food through the formation of lactic acid, acetic acid and alcohol,
- Increasing food safety by inhibition of pathogens,
- Improving nutritional value by removing anti-nutritional compounds (such as phytate, enzyme inhibitors, polyphenols, tannins) and enriching them in essential amino acids and vitamins,
- Detoxification by reducing mycotoxins, endogenous toxins and cyanogenic compounds, carbohydrate-producing gases,
- Reducing the cooking process and energy requirement,
- Extending the shelf life of bread through hydrolysis of organic compounds and the produce exopolysaccharides and other products.

Figure 2.1 shows ingredients and benefits of sourdough. Sourdough ingredient benefits are divided into 2 groups: improving properties of bread and increasing health benefits. Improved properties of bread are extended shelf life, improve texture and flavor and enhance aroma. Provides health benefits (such as on gastrointestinal

system), control glycemic index and rise amount of vitamins and minerals (Lau et al., 2021).

2.4 Sourdough usage in bread making

Bread is an important food item for many of the world's population. Many wheat breads and cakes are typical for Mediterranean countries while rye, wheat, barley and mixed flours are typical for Germany, Central and Eastern Europe, Scandinavia (De Vuyst and Neysens, 2005). San Francisco bread (Unites State of America), Panettone (Italy) (De Vuyst et al., 2007), Mexican “tortilla” bread, Indian and Pakistani “chappati” bread, Scottish “oatcake”, North American “fonnycake” bread, and Middle Eastern “pita” bread are consumed (Küçükçuban, 2012). In Turkey, Vakfikebir bread is significant for sourdough bread. According to a study, Vakfikebir bread is modified and produced as white pan bread and the effect of sourdough addition on bread quality was investigated. The dough supplied from Vakfikebir District of Trabzon is fermented for different times (0, 5, 10 and 15 h) and sourdough is obtained and by adding different proportions (0, 10, 20 and 30%) to 100 kg flour, white pan breads produced.

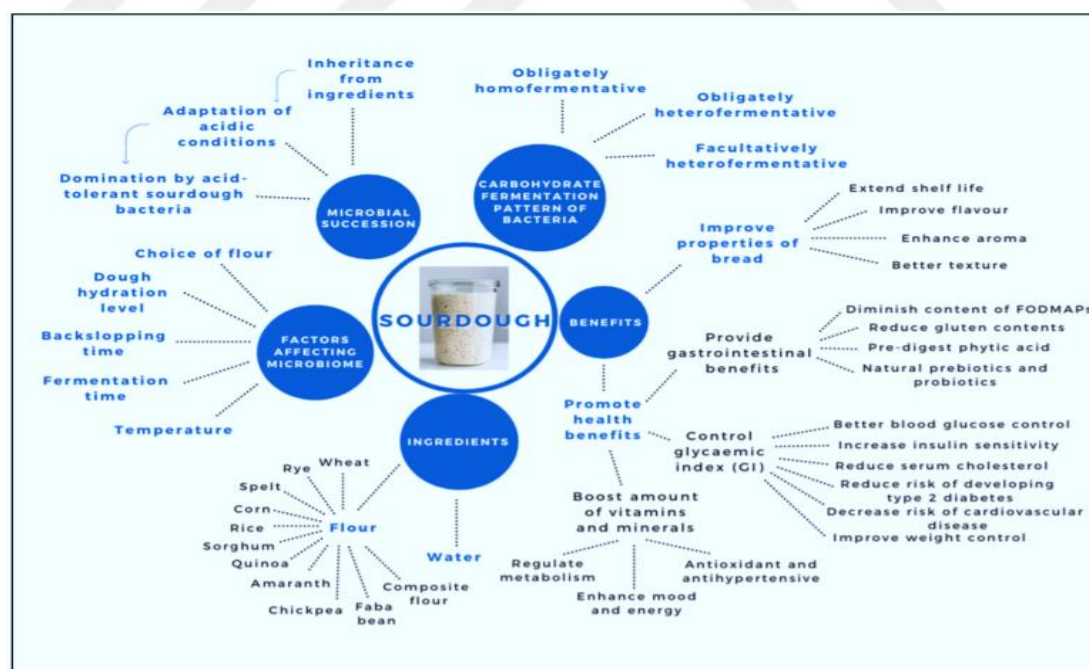


Figure 2.1 A map of ingredients and benefits of sourdough

The pH and crumb softness values of the breads are examined. As a result; it has been determined that breads made with a fermentation period of 10-15 h with 20-

30% sourdough are of good quality in terms of the softness value of the bread. As the fermentation time and sourdough addition are added; it has been reported that there is an increase in the softness of the bread and decreases in the dough and bread pH (Kotancılar et al., 2006).

In recent years, there has been an opinion about the low nutritional value of white bread. For this reason, the addition of enriching additives, the use of different starter cultures and application of specific methods increase the nutritional value of bread. One of these methods is the production of bread using sourdough. In recent years, the use of sourdough in bread making has increased due to the LAB in the consumer preference of natural products. LAB in sourdough enables the production of organic acids and flavorings, while yeasts produce metabolites such as alcohol, carbon dioxide, ester and carbonyl compounds that affect bread aroma as well as swelling of bread. While sourdough extends the shelf life of bread, it also causes significant increases in nutritional value and physical structure of bread (Plessas et al., 2011).

During fermentation, the carbohydrates and proteins in the flour undergo biochemical changes with the help of microorganisms and enzymes. These changes affect the properties of sourdough and the quality of bread. Although there are different opinions about the effect of sourdough use on bread quality, the general opinion is that dough pH affects the enzymes found in flour (Arendt et al., 2007). The effect of sourdough on bread quality the factors affecting the development of sourdough and its effect on bread quality are shown in Figure 2.2. The LAB in sourdough provide a number of technological advantages as well as functional properties. Studies have shown that LAB cause shortening of fermentation time, reduction of fermentation losses, acceleration of maturation of dough, increase of gas-forming power, improvement of dough processing properties, improvement of sensory properties of dough and increase of acidity in dough and bread. Besides, they delay the stale of the bread, bacterial spoilage and mold growth (Arendt et al., 2007).

In a study, sourdough prepared using starter culture was used in making sourdough bread. As a starter culture, *Lactobacillus acidophilus* and *Lactobacillus sakei* were used alone and mixed. Various chemical parameters such as lactic acid, total titratable acidity and pH were measured in the sourdough breads. It was stated that

the sourdough bread to which mixed culture was added kept its freshness for 12 days and this was attributed to the high lactic acid content.

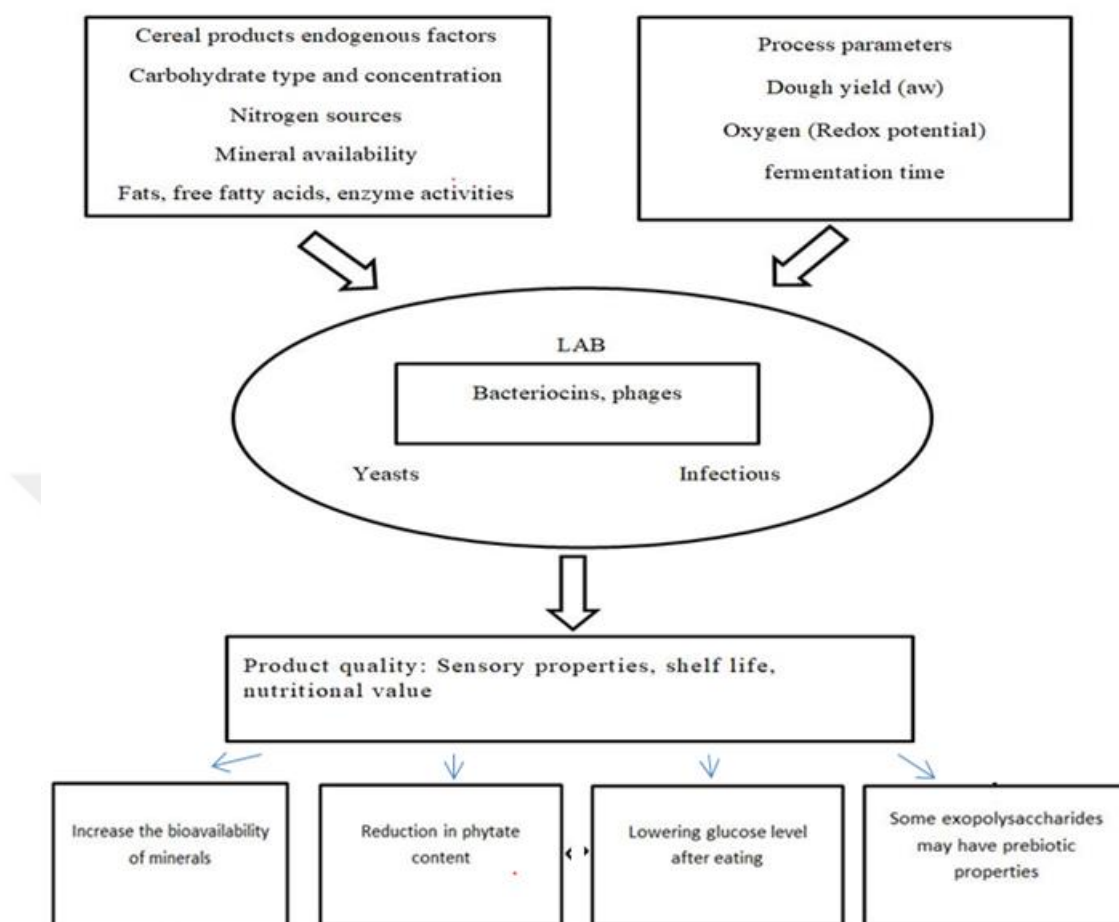


Figure 2.2 Factors affecting the development of sourdough and its effect on bread quality (modified from Arendt, 2007; Ataç, 2016)

It was determined by sensory tests and solid-phase micro-extraction gas chromatography–mass spectrometry (SPME GC-MS) analysis that the same bread showed a harder texture, good aroma, flavor and overall quality (Plessas et al., 2011).

In another study, sourdough powder was obtained from sourdough prepared using *S. cerevisiae* and *L. brevis* microorganisms and used in bread production. According to the results, the use of yeast and lactic starter was found to be effective on dough and bread properties (such as pH and acidity development). The lowest pH 3.86 and the highest total acidity of 1.71% were measured in the *L. brevis* starter cultured bread sample. The substitution of sourdough culture in bread making decreased gluten, gluten index, sedimentation and delayed sedimentation values. It was observed that

the sourdough culture did not affect the water absorption of the flour and the development time of the dough, the dough stability and kneading tolerance decreased. The softening degree of the dough increased as the rate of substitution of sourdough culture increased. Dough strength and maximum resistance were affected in bread doughs produced from sourdough powder and a decrease was observed in extensibility and energy values (Akgün, 2007).

In a different study, the possibility of using *Kluyveromyces lactis* as baker's yeast was investigated. For this purpose, the dough was enriched using lactose, sucrose and whey powder and roll-bread production was made. Textural and sensory analyzes were made by measuring protein, ash, moisture, weight loss, pH, total titration acidity, specific volume and color values in breads. Acidity, pH, protein content and color of breads prepared with whey powder added *K. lactis* are similar to breads prepared with *S. cerevisiae*. Specific volume, color change, textural analysis results, weight loss and moisture content were found to be better than *S. cerevisiae* (Akyüz, 2016).

In Plessas et al. (2011) study, sourdough made with Kefir grains developed in whey was used in bread making. The change in volatile aroma compounds and staling during bread storage was monitored by SPME GC-MS analysis for 5 days. The bread prepared with kefir sourdough added as 10 and 20% according to the amount of flour was compared with the breads prepared with the addition of 20% commercial sourdough and sourdough prepared in the laboratory. While a decrease in volatile aroma compounds was detected in all breads, volatile aroma compounds exhibited more complex profiles in kefir breads, and there was a lower loss during storage. While a significant difference was observed between the samples in sensory evaluations, it was observed that the best result was obtained in bread with 20% kefir sourdough.

2.5 Yeasts in sourdough

At the technological level, the most important features of baker's yeast: accelerate the swelling of the dough, its ability to adapt to different carbon sources and show invertase and maltase activities and stress resistance. Baker's yeast breaks down simple sugars and ferments them. With the carbon dioxide formation swelling occurs in the dough and the dough is developed with other fermentation products. The

aroma substances that occur also affect the taste and flavor of the dough (İpek, 2017). The main function of yeasts in sourdough is to make the dough swell as a result of CO₂ production and to give bread a characteristic taste and aroma with the alcohols, aldehydes, ketones and organic acids it produces (Polat, 2007).

There are many different types of yeast in sourdough composition. The most common yeasts in sourdoughs are *S. cerevisiae*, *S. exiguus*, *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala* (Rehman et al., 2006). Other yeasts isolated from sourdough are *Pichia kudriavzevii*, *Kluyveromyces marxianus*, *Geotricum candidum*, *Kazachstania unispora*, *Galactomyces candidum*, *Candida keyr*, *Wickerhamomyces anomalus*, *Kazachstania humilis*, *Candida glabrata*, *Candida tropicalis*, *Pichia guilliermondii*, *Torulaspora delbrueckii*, *Candida parapsilosis*, *Candida pararugosa*, *Candida humilis*, *Debaryomyces hanseii*, *Kazachstania barnettii* and *Kazachstania exigua* (Bakırcı and Köse, 2017; Minervini et al., 2012; Sevgili et al., 2021; Yağmur et al., 2016).

2.5.1 *Saccharomyces cerevisiae*

S. cerevisiae is about 8 µm in diameter, each cell has a two-layered wall as seen and is selectively permeable. *S. cerevisiae* reproduce by budding and generally the optimum growth temperature is 25-30°C. Their development slows down at low temperatures. In this way, yeasts can be stored at low temperatures for a certain period of time. At the same time, this species needs moisture and sugar as well as temperature. The pH degree at which *S. cerevisiae* is most effective varies between 4.4 and 4.8. The carbon dioxide bubbles formed by fermenting the yeast sugar in the dough facilitate the kneading process by making the dough rise. *S. cerevisiae* is widely used in bread making in the world and it is the most produced yeast. Scientific studies are carried out and it is preferred because of its rapid proliferation, lack of nutritional needs for reproduction and high characteristics. It provides many opportunities for producers in economic and industrial terms (İpek, 2017).

2.5.2 *Pichia kudriavzevii*

P. kudriavzevii is widely in nature and used to produce fermented foods. US Food and Drug Administration is recognized as safe because of using to make foods products in the world. It has used as a starter culture in the production of sourdough

breads, the production of fermented cassava and cacao in Africa, fermented milk in Tibet and Sudan, and maize beverages in Colombia (Douglass et al., 2018).

P. kudriavzevii cells showed extensive pseudohyphae formation after 5 days of growth and could ferment sugars like glucose, sucrose, galactose, fructose and mannose (Oberoi et al., 2011). According to Liu et al. (2018), in dough fermentation, *P. kudriavzevii* produced alcohols, aldehydes, and acids. These are significant constituents of volatiles in bread crumb.

2.5.3 *Kluyveromyces marxianus*

K. marxianus is an aerobic yeast and use lactose as a carbon source. This yeast isolated fermented milks, yoghurt, cheese and sourdough. *K. marxianus* is available for industrial use: rapid growth, thermotolerance, secretion of the enzyme inulinase and production of ethanol (Lane et al., 2011; Lane and Morrissey, 2010; Sevgili et al., 2021).

K. marxianus has Generally Recognized as Safe (GRAS) status as *S. cerevisiae* and *K. lactis* (Fonseca et al., 2008). *K. marxianus* could use to produce bread as baker's yeast because of no difference in bread flavor produced with strains of *K. marxianus* and *S. cerevisiae* (Caballero et al., 1995).

2.6 LAB in sourdough

LABs are microorganisms that in the form of rods, cocci and coccobacilli with many species, Gram positive, immobile, not forming spore, catalase negative, microaerophilic or anaerobic, acid-resistant, strongly fermentative, not reducing nitrates. They need vitamins and amino acids (Erkmen and Bozoglu, 2016).

LAB usually grows between 5 and 33°C and the optimum pH ranges are between 5.5 and 5.8. LABs do not show pathogenic properties, they form antimicrobial substances and thus prevent the development of saprophyte and pathogenic bacteria.

Although *Leuconostoc* (generally *Leuconostoc mesenteroides*), *Weissella*, *Pediococcus* (usually *Pediococcus pentosaceus*), *Lactococcus*, *Enterococcus* and *Streptococcus* species have been isolated from sourdough in studies conducted so far, the most common bacteria observed in sourdough are *Lactobacillus sanfranciscensis*, *L. plantarum*, *L. brevis*, *L. pontis* and *L. reuteri* are the most commonly isolated

lactobacilli from sourdough (Güre, 2009). Recently identified *Lactobacillus frumenti*, *Lactobacillus mindensis*, *Lactobacillus paralimentarius*, *Lactobacillus spicheri*, *Lactobacillus rossiae*, *Lactobacillus acidifarinae*, *Lactobacillus zymae*, *Lactobacillus hamnesii*, *Lactobacillus siliginis* are isolated from sourdough (Küçükçuban, 2012).

Microorganisms in the sourdough microflora interact together and contribute positively to the technological and sensory features of bread. For example, organic acid production, proteolytic activity, synthesis of volatile components, antifungal properties of LAB are among the most important metabolic properties. The benefits of using lactic starter in bread production are as follows: (Küçükçuban, 2012).

- To obtain the desired quality and quantity of products,
- Saving production time, space and labor,
- To ensure uniformity between products realized in different batches,
- To develop new products,
- To create stronger aroma,
- Improving the texture of bread,
- Increasing the bread volume,
- Extending the shelf life,
- It is to provide production assurance by eliminating the effects of harmful microorganisms.

2.6.1 LAB

Lactobacillus species are divided into three groups according to their ability to metabolize sugar and *Lactobacillus* species isolated from sourdoughs are given Table 2.1 (Küçükçuban, 2012; Lahtinen et al., 2011). The first group consists of homofermentative *Lactobacillus* species. Almost all hexoses are fermented into lactic acid by these bacteria. Although these species cannot ferment gluconates (sodium gluconate, potassium gluconate) and pentoses (ribose), they cannot generate gas from glucose and gluconate. They develop between 15 and 45°C (Küçükçuban, 2012). Homofermentative LAB use glycolysis as known Embden-Meyerhof-Parnas pathway for hexoses fermentation (Wisselink et al., 2002).

The other group consists of facultative heterofermentative *Lactobacillus* species. This group ferments almost all hexoses to lactic acid and pentoses to lactic acid and

acetic acid. This group shows growth between 25 and 35°C. Facultative heterofermentative use Embden-Meyerhof-Parnas pathway for hexose fermentation (Chavan and Chavan, 2011). *Lactobacillus casei* is a member of this group and ferment sugars to lactic acid but do not produce NH₃ from arginine (Altıok, 2004).

The third group consists of obligatory heterofermentative *Lactobacilli* species. This group ferments hexoses to lactic acid, acetic acid, ethanol and CO₂. They form gas from glucose. *Lactobacillus brevis* and *Lactobacillus fermentum* are the predominantly LAB species found in sourdough while *Lactobacillus kefir* are the predominantly LAB species found in kefir (Yörük and Güner, 2011). This group ferment pentoses by the phosphoketolase pathway (Altıok, 2004).

Table 2.1 *Lactobacillus* species as sugar fermentation

Obligatory Heterofermentative	Facultative heterofermentative	Homofermentative
<i>L. acidifarinae</i>	<i>L. plantarum</i>	<i>L. amylovorus</i>
<i>L. brevis</i>	<i>L. pentosus</i>	<i>L. acidophilus</i>
<i>L. bunchneri</i>	<i>L. alimentarius</i>	<i>L. delbrueckii</i>
<i>L. fermentum</i>	<i>L. paralimentarius</i>	<i>L. farciminis</i>
<i>L. fructivorans</i>	<i>L. casei</i>	<i>L. mindensis</i>
<i>L. frumenti</i>	<i>L. sakei</i>	<i>L. crispatus</i>
<i>L. hilgardii</i>	<i>L. curvatus</i>	<i>L. johnsonii</i>
<i>L. panis</i>		<i>L. amylolyticus</i>
<i>L. pontis</i>		<i>L. helveticus</i>
<i>L. reuteri</i>		<i>L. salivarius</i>
<i>L. rossiae</i>		
<i>L. sanfranciscensis</i>		
<i>L. siliginis</i>		
<i>L. spicheri</i>		
<i>L. zymae</i>		

2.6.2 *Levilactobacillus brevis*

Levilactobacillus brevis (previously name *Lactobacillus brevis*) is a member of LAB that is catalase-negative, non-sporulating, non-motile, rod or coccus-shaped as seen Figure 2.3 and Gram-positive bacteria. It grows at 30°C and at a pH between 4 and 6 (Feyereisen et al., 2019). It is obligatory heterofermentative that produces acetic acid, ethanol and other aromatic compounds besides lactic acid as a result of fermentation.

Acetic acid formation also gives this culture a protective property. *L. brevis* produce gas and acid. It catabolizes glucose in an aerobic environment. It converts pyruvate into lactate, acetate and CO₂ in an anaerobic environment and into lactate, small amounts of acetate and CO₂ in an aerobic environment. It does not form acetoin. It metabolizes glucose by hexose monophosphate pathway (Küçükçuban, 2012).



Figure 2.3 Image of scanning electron microscopy of *L. brevis* (Elez-Martinez et al., 2005)

2.6.3 *Lactiplantibacillus plantarum*

As seen in the Figure 2.4, *L. plantarum* (previously name *Lactobacillus plantarum*) is usually 0.9-1.2 µm wide, 3-8 µm long, single, double or short chain a Gram positive, short-rod, micro-aerophilic, acid-tolerant, non-spore forming, non-respiring, low G+C content, heterofermentative group of lactobacilli with a range of applications in the food industry as a starter culture and preservatives and colonies growing on the surface are 3 mm in diameter, round, dull, compact, white and rarely light or dark yellow (Küçükçuban, 2012; Valan et al., 2016). It usually grows at 15°C and does not grow at 45°C (Todorov et al., 2014). Optimum growth temperature is 35-40°C (Fitzsimons and O'Connell, 1994). *L. plantarum* is dominant LAB that produce lactic acid fermented foods with pH<4. *L. plantarum* is facultative heterofermentative and ferments sugars by Embden-Meyerhof-Parnas pathway or the pentose-phosphate pathway (Matejčková et al., 2019).

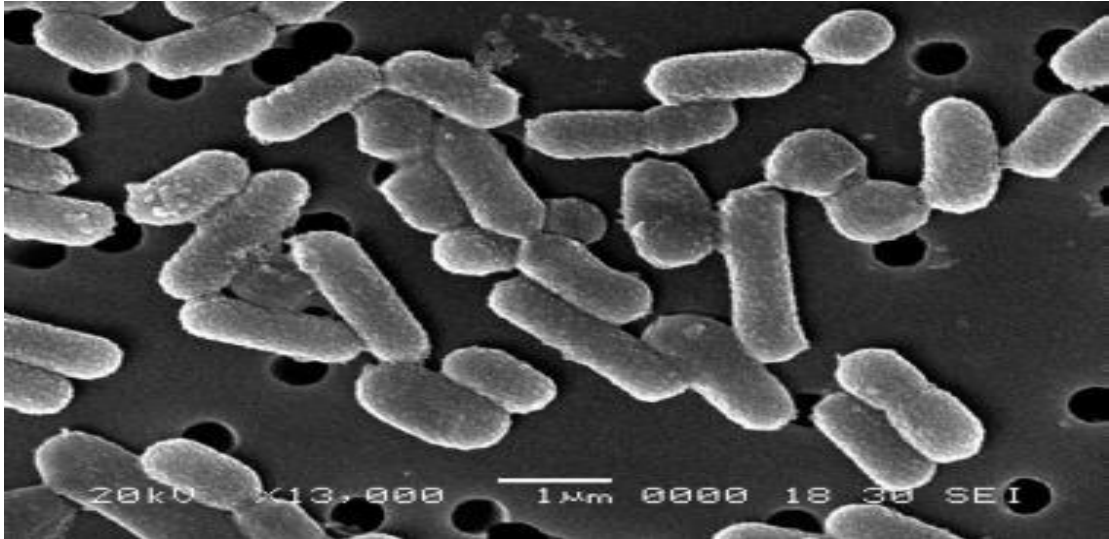


Figure 2.4 Micro-morphological image of *L. plantarum* (Valan et al., 2016)

2.6.4 *Pediococcus acidilactici*

As seen in Figure 2.5, cells are spherical and in tetrads form, but they can also be found in groups. There are no single cells or chains. They are Gram-positive, catalase negative (but some types are false positive), immobile, non-spore-forming, homofermentative, facultative anaerobes. The optimum growth temperature is 40°C and the maximum is 50-53°C. Growth occurs at pH range from 4.2 to 8.0 and sometimes at pH 8.5 (Du Toit et al., 2014).

Pediococci can lower the pH down to 3.6 by fermenting glucose into L (+) - or DL-lactic acid. Depending on the species, they can ferment sucrose, arabinose, ribose and xylose. It produces acid from galactose, arabinose, xylose, salicin and trehalose, and some strains produce weak acidity from sucrose and lactose. It does not form acid from maltose, mannitol, α-methyl glucoside or dextrin. It produces diacetyl as a flavoring agent (Küçükçuban, 2012).

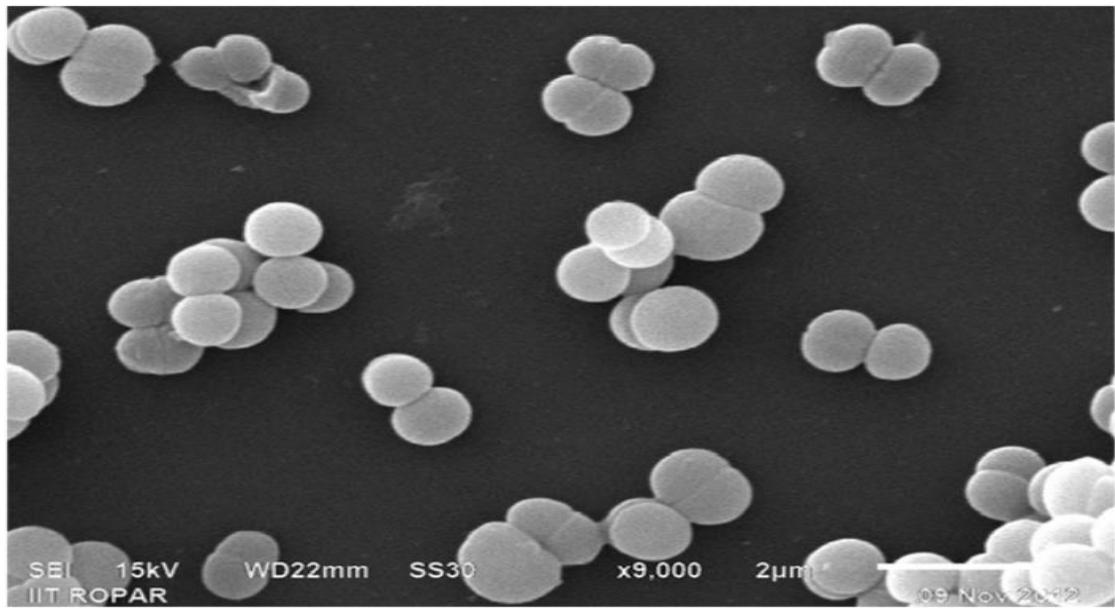


Figure 2.5 Scanning electron microscopy image of *P. acidilactici* (Balgir et al., 2013)

2.6.5 *Leuconostoc mesenteroides*

Leuconostoc genus properties are non-spore forming, catalase negative, anaerobic facultative, mesophilic (optimal growth temperature between 20 and 30°C), gram-positive, heterofermentative bacteria and occur coccus or rod-like shape after growth (Paula et al., 2015). *L. mesenteroides* and *Weissella* are able to produce exopolysaccharides (EPS) such as fructans or glucans (Bounaix et al., 2010). EPS have advantages for bread production: texture, mouth feel and taste perception. For example, *L. mesenteroides* is responsible for long storage stability in Panettone that is traditional Italian sweet bread (Manini et al., 2016).

2.6.6 *Enterococcus faecalis* and *Enterococcus hirae*

Enterococci is Gram-positive, facultative anaerobic cocci (Anderson et al., 2016) and member of LAB of significant in food microbiology. *E. faecalis* and *E. hirae* growth at 10 and 45°C. *E. faecalis* and *E. hirae* are typical enterococci that have beneficial role in traditional food products in Europe and may be used such as probiotics (Franz et al., 2003). Some fermented foods contain enterococci especially meat and cheeses (Giraffa, 2002).

2.7 Main pathway in LAB

LAB is a member of heterogenous group bacteria that plays an important role in the fermentation process. LAB produces very significant beneficial compounds such as organic acids, polyols, EPS and antimicrobial compounds. There are three main pathways for development of flavour in the production of fermented foods. These pathways are glycolysis, lipolysis and proteolysis. Glycolysis is about fermentation of sugars. Lipolysis is about degradation of fat while proteolysis is related to degradation of proteins (Bintsis, 2018).

2.7.1 Glycolysis

Wheat flour is significant ingredient for bread making because provides specific characteristics of bakery products. It is composed of carbohydrates, proteins, lipids, ash, fibers, water, vitamins, minerals and enzymes. There are small amounts of vitamins, minerals and enzymes (Chavan et al., 2011). LAB need carbohydrate for energy and growth (Bintsis, 2018) and assimilate carbohydrates as glucose, fructose, sucrose and maltose. For this process, homofermentative and heterofermentative LAB are significant. Homofermentative LAB use glycolysis pathway as known homolactic fermentation or EMP pathway while heterofermentative LAB use 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway (heterolactic fermentation) (Corsetti and Settanni, 2007). Obligate homofermentative and facultative heterofermentative LAB break down hexoses by EMP pathway to produce lactic acid. Homofermentative lactobacilli does not ferment pentoses and gluconate while facultative heterofermentative lactobacilli ferment pentoses and gluconate by phosphoketolase that is an enzyme pentose phosphate pathway. Obligate heterofermentative possess aldose and ferment pentoses and hexoses by phosphogluconate pathway to produce lactic acid, ethanol and CO₂ (Salvetti et al., 2012).

2.7.2 Lipolysis

Metabolism of LAB can cause lipid oxidation or produce a strong antioxidative effect during fermentation. Homofermentative lactobacilli increase lipid oxidation. This effect has been characterized as hydrogen peroxide formation in homofermentative glucose metabolism. Conversely, obligate heterofermentative

lactobacilli reduce the oxidation reduction potential of sourdough. Lactobacilli hydrate oleic, linoleic and linolenic acids into hydroxylated fatty acids (Hendek Ertop, 2014). *Enterococci* have specific biochemical properties. These are lipolytic, proteolytic and production of aromatic volatile compounds and EPS (Bartkiene et al., 2017).

2.7.3 Proteolysis

Proteolysis is the breakdown of proteins or peptides into smaller peptides or amino acids as a result of hydrolysis of peptide bonds by protease enzymes. Amino acids and peptides affect the taste of fermented products (Ganzle et al., 2008). 1/3 of the proteolytic activity is realized by yeasts originating from cereals. In other studies, it is stated that protein hydrolysates are formed from gluten proteins by proteinases produced by LAB. Recent results in sourdough also show that LAB has proteolytic activity (Hendek Ertop, 2014). Gluten protein determines the quality of bread making from wheat flours. Gluten proteins contribute to dough hydration and gas retention. Protein depolymerization during sourdough fermentation depends on the metabolic activity of microorganisms and enzymes present in the grain. The lowering of the ambient pH and the accumulation of low molecular weight thiol group-containing compounds increase the solubility of gluten proteins while reducing enzyme activities (Ertop and Hayta, 2016). The degradation of wheat proteins is of crucial importance for bread flavour, volume and texture (Ganzle et al., 2008).

2.8 MALDI-TOF MS analysis

MALDI-TOF MS has been used new technology for identification LAB and yeasts for ten years. Matrix-assisted laser desorption/ionization (MALDI), time-of-flight (TOF) and mass spectrometry (MS) has been known and has been used as one of the most powerful tools for biomolecules analysis (Jurinke et al., 2004).

Bacterial identification is generally based on phenotypic analysis that Gram-staining, biochemical properties, morphological and growth characteristics. These identification tests are complex and takes longer time (Biswas and Rolain, 2013). MALDI-TOF MS is one of the methods used in molecular identification because it is fast, reliable and inexpensive (Yılmaz et al., 2014). Today, commercial MALDI systems are available for biological research studies as well as diagnostic

applications in clinical medicine, biotechnology and industry. This device can detect ribosomal proteins mostly at protein level (Uysul et al., 2019).

First, pure yeasts and LAB cultures were taken from the De Man, Rogose and Sharpe (MRS) and Potato Dextrose (PD) Agar with a loop, inoculated to PD broth and incubated at 30°C, inoculated to MRS broth to activate cultures and then they were left for incubation at 35°C, respectively. One loop full of pure yeast colony (from 6000 rev min⁻¹ centrifugation of liquid culture for 5 min) was suspended in the sterile distilled water (or in 70% alcohol) in a small serological tube. The cell suspension was mixed with acetonitrile/formic acid/water (50:35:15, v/v) solution in the extraction process. α -Cyano-4-hydroxycinnamic acid (CHCA; 50% acetonitrile and 2.5% trifluoroacetic acid in pure water) was chosen as the matrix solution. For analysis, the extracted cell suspension in the matrix (1:1) is applied dropwise with an automatic dispenser onto the MALDI target plate and then the mixture is dried at room temperature. After matrix and sample mixture were dried on the target plate. The dried sample plates were loaded into the MALDI-TOF MS device and mass spectrum of the sample was obtained. Finally, identification of the microorganisms had done by matching the spectrum in the database with the spectrum obtained from the analysis as shown in the Figure 2.6. The results were obtained from the spectrum are given as score values depending on type of devices. Score values > 2.0 (green color) and from 1.7 to 2 (yellow color) are recognized as reliable at the species and genus level, respectively. When the score is < 1.7, the fingerprint is not reported as “reliable identification”, meaning that it cannot match sufficiently with any reference microbial species or genus in the database (Erkmen, 2022).

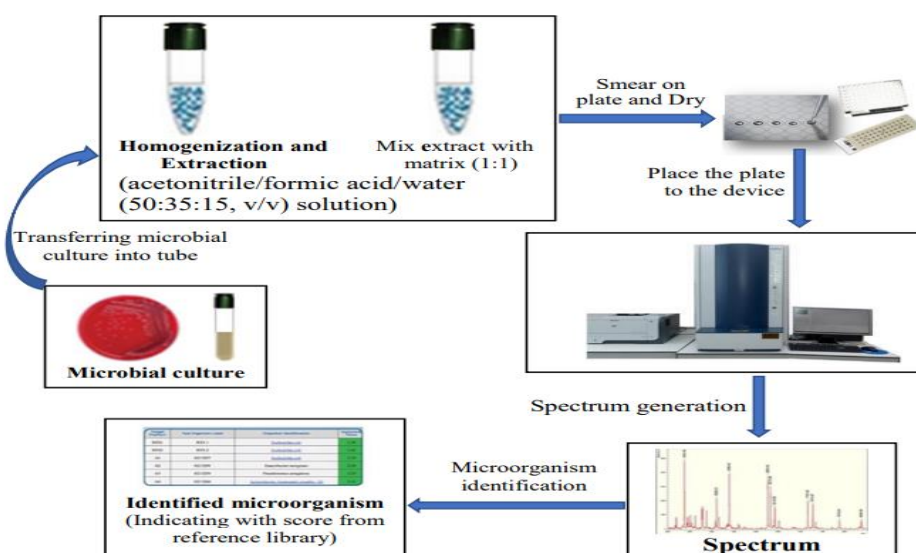


Figure 2.6 MALDI-TOF MS microbial identification flowchart (Erkmen, 2022)

Microorganisms can be isolated from food. Identification studies of microorganisms isolated from various foods were carried out in MALDI-TOF MS. Nacef et al. (2017) studied to identification of LAB that isolated from Maroilles cheeses with using MALDI-TOF MS. In Nacef et al. (2017) study, *L. plantarum*, *L. paracasei*, *L. rhamnosus*, *L. parabuchneri*, *L. brevis*, *L. curvatus*, *L. fructivorans*, *Leuconostoc pseudomesenteroides* and *Enterococcus devriesei* were identified with using MALDI-TOF MS. Soro-Yao et al. (2014) studied to isolated and identified LAB from fermented cereal foods available in Abidjan, Côte d'Ivoire. *Pediococcus* sp. and *Lactobacillus* sp. were identified with using MALDI-TOF MS.

Sogawa et al. (2011) studied to compare conventional method, MALDI-TOF MS and 16sRNA method for clinical microbial identification. The bacterial species analyzed isolates were *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The similarity of identification at the species level was 100%, 100%, 97%, 100% and 100%, respectively. *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* were identified with using MALDI-TOF MS in Sogawa et al. (2011) study. The similarity of identification at the species level was 100% in all. The MALDI-TOF MS was successful in fungi.

Angelakis et al. (2011) studied probiotic food for isolated and identified bacteria. These bacteria were identified as *L. casei*, *Lactococcus lactis*, *Bifidobacterium*

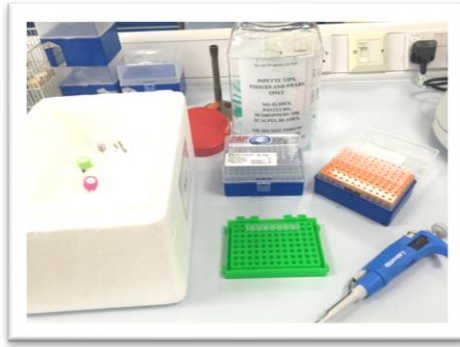
animalis, *L. delbrueckii* and *Streptococcus thermophilus*. The similarity of identification was 92% compared to the molecular method.

MALDI-TOF MS is high performance, accuracy, short time, automated analysis (Jurinke et al., 2004) and simple (Sogawa et al., 2011). DNA analysis based on MALDI-TOF MS has been used during the last years for identification microorganisms.

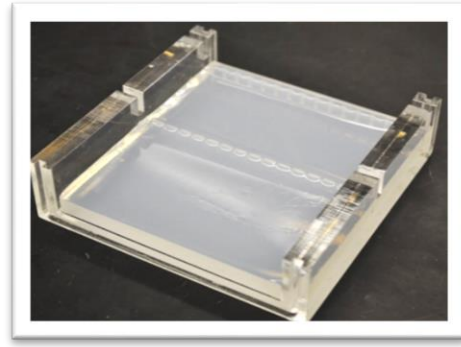
2.9 Polymerase chain reaction (PCR) and agarose gel

PCR is a technique that is used to amplify fragments of DNA. When PCR is used, the most important factor is free possible sources of contamination, clean because of affecting contamination of environment (Maddocks and Jenkins, 2017). Today, the diagnosis and epidemiological studies of infectious diseases of humans and animals have gained tremendous momentum with the introduction of PCR-based techniques. With the introduction of PCR, applications that require a long and laborious series of laboratory procedures for the isolation and identification of bacteria, viruses, fungi or parasites are no longer necessary (Kahya et al., 2014).

PCR is a simple method for analysis of DNA. In this analysis, the first stage is pure suspension of DNA (Sgaramella, 1996). The isolated DNA samples were run in agarose gel electrophoresis. Agarose gel electrophoresis is the most important and effective way to divide DNA fragments (Lee et al., 2012). Figure 2.7 consists of a bench which is arranged for PCR work and agarose gel. Agarose gel electrophoresis that DNA bands show up when electrophoresis is completed (Lee et al., 2012).



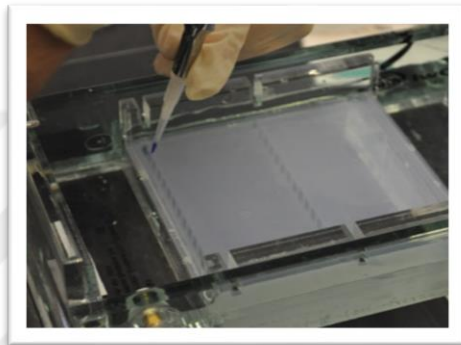
(a)



(b)



(c)



(d)

Figure 2.7 (a) PCR workbench (Maddocks and Jenkins, 2017); (b) A solidified agarose gel (Lee et al., 2012); (c) Adding loading dye to her DNA samples (Lee et al., 2012); (d) Loading the DNA sample into a well in the gel (Lee et al., 2012)

After DNA bands are seen on PCR screen, finding gene sequences using online databases are very important. A lot of online databases are used to find gene sequences. One of them is NCBI database. On the NCBI homepage;

- Select “DNA and RNA” from the blue menu on the left-hand side of the screen,
- Select “Genbank” from the list of databases that appear. Genbank is the National Institute of Health genetic sequence database, which provides an annotated collection of all DNA sequences that are currently publicly available,

- Searching Genbank using basic local alignment search tool (BLAST), depending on the information that you have regarding the sequence you wish to amplify (Maddocks and Jenkins, 2017).



CHAPTER III

MATERIAL AND METHODS

3.1 Equipments, chemicals and media

The following equipments were used:

- Autoclave (HMC Hirayama, Tokyo, Japan),
- Dry air oven (JSR-JSOF 150, Korea),
- Waring blender (Waring blender 8011ES, Waring Commercial, USA),
- Anaerobically incubator (Sanyo, CO₂ incubator),
- Aerobic incubator (Nüve, EN 500),
- Microscope (LEICA DM500, Germany),
- MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany),
- PCR (Proflex PCR Systems, Thermo Fisher Scientific),
- Hunter (Color Meter/HunterLab Color Flex, Virginia, USA) color analyzer,
- pH meter (pH-211 Microprocessor, Hanna Instruments, Sigma-Aldrich, Darmstadt, Germany),
- HPLC (Shimadzu Corp., Kyoto, Japan),
- Differential Scanning Calorimeter (DSC) Perkin Elmer Pyris-6 (PerkinElmer Inc., Istanbul),
- Heating mantle (ISOLAB, Germany),
- Oven (Tecnoeka Srl, Italy),
- Volumeter for bread (Bastak, Ankara, Türkiye),
- Magnetic stirrer (Heidolph MR Hei-Standard, Germany),
- Refrigerator (Arçelik, Türkiye) and
- Sterile petri dishes, graduated cylinder, erlenmayer flask, pipettes, drigalski spatula, sterile tubes, inoculation loop, flasks, Durham tubes and test tubes.

The following chemicals and media were used;

- de Man Rogosa and Sharpe (MRS) agar/broth (Merck, Darmstadt, Germany)
- Potato Dextrose (PD) agar/broth (Merck, Darmstadt, Germany)
- HHD medium (Merck, Darmstadt, Germany)
- All Purpose Tween (APT) agar (Merck, Darmstadt, Germany)
- Sugars (glucose, fructose, maltose, lactose) (Merck, Darmstadt, Germany)

Crystal violet, iodine, ethyl alcohol (95%) and safranin were used for Gram staining procedure. H_2O_2 (3%) were used for catalase tests. The preparation and ingredients of chemicals and media is given in Appendix A.

3.2 Sourdough collection

Thirty six sourdoughs (about 600 g) were obtained from Gaziantep, Konya and Mardin regions (without commercial cultures) and brought to the laboratory. Sourdoughs from Konya were obtained from Özkan bakery located in the center of Konya, Alsancak patisserie, Derbent district (domestic sourdough), Ilgın (Çiğil district) and Kadınhanı district. Sourdoughs from Mardin were obtained from central Araplar district, Kızıltepe district and Midyat district. Sourdoughs from Gaziantep were obtained from Metro bakery oven, Cevizler district, Morcalı village and Nizip district.

3.3 Sample preparation and counting of microorganisms

Twenty five grams sourdough sample was weighed and added to 225 ml of sterile peptone water (0.1%) in the sterile Waring blender (Waring blender 8011ES, Waring Commercial, USA) and homogenized by blending for 1 min. Spread plates were performed on the Petri plate for LAB and yeasts with de Man Rogosa and Sharpe (MRS) and Potato Dextrose Agar (PDA) agar (Erkmen, 2022), respectively. Then inverted MRS agar plates were incubated anaerobically (Sanyo, CO₂ incubator) at 28°C for 48 and 72 hrs, and inverted PDA plates are incubated in an aerobic incubator (Nüve, EN 500) at 28°C for 48 and 72 hrs.

After incubation of plates, colonies formed on Petri plates were counted based on colony morphology from plates containing 25 to 250 colonies (Erkmen, 2022). After counting different colony types (at least 5 colonies from each plates) according to the colony morphology were selected from the Petri plates and each colonies were

purified by streak plating on MRS and PDA for LAB and yeasts, respectively and morphological analysis using Gram staining. Single colonies of purified isolates were stored in 20% (v/v) glycerol and kept at -20°C until experiments. Pure cultures were used in morphological and technological identification tests.

3.4 Characterization and identification of isolates

3.4.1 Determination of morphological properties of LAB and yeasts on Petri plates

The morphological characteristics of the isolates were determined by microscopic analysis. Morphological analyzes were performed on LAB after Gram staining (Gram reaction, cell shape and appearance of cells). Before the analysis, pure cultures were growth in MRS and PD broth (Erkmen, 2021).

Morphological analyzes were applied for yeasts (Erkmen, 2021): Direct microscopic examination: cell size, cell shape and appearance of cells.

Gram staining: Gram-staining procedure is given in Figure 3.1. According to this method, liquid or solid culture was taken from the medium with a loop and spread on the slide, allowing the culture to dry in the air, and physical fixation was made by passing it through the flame 3 times. Crystal violet was dropped on the slide and stained for 1-2 min and washed with distilled water. Afterward, iodine waited for 1-2 min and washed with distilled water and 95% ethyl alcohol were added for 5 to 30 sec and washed with distilled water until the stain did not flow, and then safranin was poured on the slide and waited for 1 min. The safranin was poured and the slide was washed with distilled water and dried in air. Finally, a drop of immersion oil was dropped and preparations were examined according to the microbial shapes, appearance and colors under microscope (Erkmen, 2021).

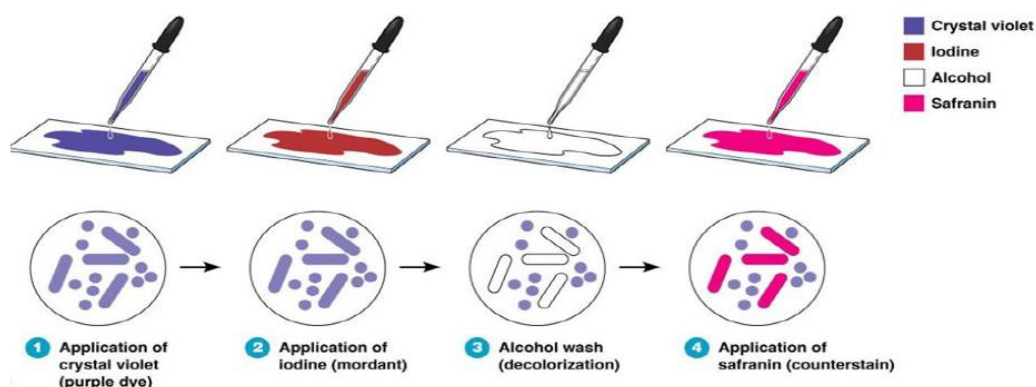


Figure 3.1 Gram-staining procedure

3.4.2 Technological properties of isolates

a) Following technological properties of LAB isolates were identified: Catalase test, oxidative-fermentative test (OF), homo fermentation test, hetero fermentation test, growth at 15, 28 and 45°C and at 1.5, 4, 6.5, 7 and 10% salt concentrations. The growth, acid and gas formation ability from sugars (glucose, sucrose, lactose and maltose) were also determined.

b) Technological properties of yeast isolates were identified for *Saccharomyces* and non-*Saccharomyces* species: Acid and gas production from sugars (glucose, sucrose, lactose and maltose) and growth at 25, 28 and 37°C.

3.4.2.1 Catalase test

The colony to be tested was transferred to the slide, then a drop of 3% H_2O_2 was added on the colony. The development of air bubbles was recorded as test is positive (Erkmen, 2021).

3.4.2.2 Oxidative-fermentation (OF) test

Carbohydrate solution containing 0.5% sugar in 10 ml distilled water was added to test tubes. A sterile carbohydrate solution was added aseptically into the sterile molten base medium to give a final concentration of 0.5%. After the microorganism was inoculated, the surface of one of the media was covered with sterile liquid paraffin. It was incubated at 35°C up to 5 days. The decomposition of glucose in oxidative character was seen only in the paraffin-free tube (open tube). Oxidation

was considered positive when the color of medium turned yellow. If both covered and non-covered tubes turn yellow, it indicates positive fermentation test. If both covered and non-covered tubes including the absence of color change indicates glucose is not metabolized (Erkmen, 2021).

3.4.2.3 Homofermentative and heterofermentative tests

Homofermentative and heterofermentative LAB tests were performed using Homofermentative-Heterofermentative Differential (HHD) medium. Pure 48h LAB cultures were inoculated to MRS broth and incubated at 35°C to obtain activate pure culture. Then isolated LAB cultures inoculated to HHD agar and incubated at 35°C for 10 days. On this medium, heterofermentative LAB reduce fructose to mannitol (white colony), but homofermentatives do not (blue green colony), and heterofermentative bacteria were confirmed with All Purpose Tween (APT) agar (Erkmen, 2021).

3.4.2.4 Temperature test

Inoculated pure 48h yeast cultures were taken from the PD agar with a loop and they were inoculated to PDB broth and pure 48h LAB culture were inoculated to MRS broth. MRS broth was used for LAB and their growth at 15, 28 and 45°C was observed in the anaerobic incubator (5% CO₂) up to 10 days. PD broth was used for yeasts and their growth at 25, 28 and 37°C was observed in the aerobic incubator. The results were evaluated turbidity of LAB and yeasts in medium (Erkmen, 2021).

3.4.2.5 Growth at 1.5, 4, 6.5, 7 and 10 % salt concentrations test

Salt was added by 1.5, 4, 6.5, 7 and 10 % into MRS broth for LAB and into PD broth for yeasts. One loop full of LAB was inoculated into MRS broth containing salts and they were incubated in 35°C at 48-72 h. One loop full of yeasts inoculated into PDB containing salt and they were incubated at 28°C at 48-72 h. The control: (i) the liquid media without salt were inoculated with respective isolates. (ii) the broth containing salt without inoculation. The results were evaluated according to the presence of turbidity in the medium. The results were determined as positive for turbidity and negative for without turbidity (Erkmen, 2021).

3.4.2.6 Carbohydrate fermentation test

Carbohydrate fermentation test detect the ability of microorganisms to ferment a specific carbohydrate. Phenol red carbohydrate broth was prepared. One percent carbohydrate was added into fermentation medium. LAB and yeast pure cultures from the MRS and PD broth with a loop were inoculated into respective 10 ml fermentation medium with Durham tube. Glucose, lactose, maltose and sucrose were used separately as test carbohydrates. Inoculated tubes were incubated at 35 and 30°C for LAB in an anaerobic incubator and yeast in an aerobic incubator, respectively, up to 10 days. Gas and acid formations were controlled every morning. Positive test was indicated with yellow color through medium to indicate acid formation and bubble (space) in the Durham tube to indicate gas formation. Negative test was determined purple color (no color change) or no bubble (non space) in the Durham tube or no acid production on the medium (Reiner, 2012; Erkmen, 2022).

3.5 Identification of microorganisms

LAB and yeasts were isolated and identified from 36 sourdoughs obtained from Gaziantep, Konya and Mardin regions according to their morphological and technological characteristics. These isolates further identified by Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis (Figure 2.6). Finally, all isolates (60 LAB and 40 yeasts) were identified by polymerase chain reaction (PCR). Service procurement from MALDI-TOF MS analysis was obtained from Centre for Implementation and Research of Plant Health Clinic of Mustafa Kemal University in Hatay, Türkiye. DNA amplification was also performed by mixing PCR (Proflex PCR Systems, Thermo Fisher Scientific) in Gaziantep University Biology Department. A total of 60 LAB and 40 yeasts were identified in this way. These isolates were used in the sourdough bread production.

3.5.1 MALDI-TOF MS technique

MALDI-TOF MS has emerged as one of the most reliable tools with promising applications in the field of rapid identification, typing and classification of microorganisms based on protein fingerprinting based on whole cell analysis. This device can detect ribosomal proteins, mostly at the protein level. Diagnosis of the bacteria and yeast isolates were performed using the MALDI-TOF MS (Bruker

Daltonics GmbH, Bremen, Germany) from microorganism identification device in Hatay Mustafa Kemal University, Centre for Implementation and Research of Plant Health Clinic. At this stage, pre-extraction processes were carried out using the ethanol-formic acid method for protein isolation from pure bacterial and mold cultures. Then, the spectra obtained with the device's flex control software program (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) were compared with the Maldi Biotyper Real-Time Classification (RTC) software and the diagnosis process was continued. As a result of the analysis, high probability species identification; 2.000-3.000 (green color), genus level and probable species level diagnosis; It was evaluated using scale values between 1.700-1.999 (yellow color) (Aysun et al., 2019; Erkmen, 2022).

3.5.2 PCR analysis

Sequence analysis was performed with service procurement and its types were made. Isolated LAB and yeasts DNA were extracted using PureLink™ genomic DNA kits (Thermo Fisher, Bleiswijk, Netherlands), according to the manufacturers' protocols. Isolated LAB 23S rRNA region were amplified through polymerase chain reaction (PCR) by ISR forward (5'GCTGGATCACCTCCTTTC-3') and ISR reverse (5'CCTTTCCTCACGGTACTG-3'). The PCR reactions were conducted in 30 µl reaction volume that contained 3 µl 10xBuffer, 50 mmol/L MgCl₂, 10 mmol/L deoxynucleotide triphosphate (dNTP), 10 pmol of each primer, 0.4 µl Taq DNA polymerase and 3 µl DNA. The PCR conditions were 3 min at 94°C for initial denaturation, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 1.5 min at 72°C and a final extension for 10 min at 72°C. Yeasts were identified from ITS region through polymerase chain reaction by ITS1 forward (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 reverse (5'-GGAAGTAAAAGTCGTAACAAGG-3'). The PCR conditions were 2 min at 94°C for initial denaturation, followed by 35 cycles of 30 s at 94°C, 30 s at 53-59°C, 1 min at 72°C and a final extension for 10 min at 72°C. Yeasts were identified from the ITS region with bands ~450 bp. Both strands of DNA sequences were FASTA formatted and homology was determined using BLASTn within the National Center of Biotechnology Information (NCBI) database. Service procurement for PCR analysis was carried out by Biohybrid Laboratory Products in Gaziantep.

3.6 Preparation of sourdough bread culture

The sourdough culture was prepared with wheat (Golia type wheat) flour. Wheat flour was supplied from Özmen Un in Gaziantep, Türkiye. Orbital mixer (HY-10M, Mateka, Tekirdağ) is used in sourdough production. The water to be added in the preparation of the sourdough was taken into consideration in the water coming from the culture addition. In the study, each LAB and yeast species were propagated separately using 100 ml MRS broth and PDB, respectively, and centrifuged. The cultures centrifuged ($6000 \text{ rev min}^{-1}$ for 15 min) under aseptic conditions were washed twice with saline, then diluted with saline to obtain LAB 10^9 colony forming unit (cfu)/ml and yeast 10^7 cfu/ml. LAB and yeasts were counted by MRS agar (Difco, Detroit) and PDA (Difco, Detroit) respectively (Erkmen, 2021).

Ingredients were added into the orbital mixer (HY-10M, Mateka, Tekirdağ) except culture, mixed for a while and kneaded at 400 rpm for 5 min. Then the microbial cultures were added into mixture and the kneading process was continued for 10-15 min. After the kneading was completed, it was left to fermentation at 28°C for 24 h. This was mother sourdough culture (MSC). MSC was used in the preparation of bulk sourdough culture (sourdough) by three times enrichment (refreshing) through intermediate culture (IMC) with the following ingredients base.

The culture mixture was obtained by mixing equal amounts (1 ml) of each species. The following ingredients based on percent composition were used in the preparation of MSC: 100 g wheat flour (WF), 200 mL top water, 1.5 g of NaCl, 1.5 mL LAB stock culture and 1.5 mL yeast stock culture. Enrichment of sourdough was performed (Sevgili et. al., 2021):

1. Enrichment: 100 g WF + 205 mL water + 60 g MSC was mixed in the mixer and left to fermentation at 28°C for 24 h that was used as IMC-1.

2. Enrichment: 135 g WF + 200 mL water + 65 g IMC-1 was mixed in the mixer and left to fermentation at 28°C for 24 h that was used as IMC-2.

3. Enrichment: 135 g flour + 200 mL water + 65 g IMC-2 was mixed in the mixer and left to fermentation at 28°C for 24 h. This bulk sourdough culture (sourdough) was used in the determination of sourdough physicochemical and microbiological properties. Bread sourdough was prepared as indicated in Figure 3.2.

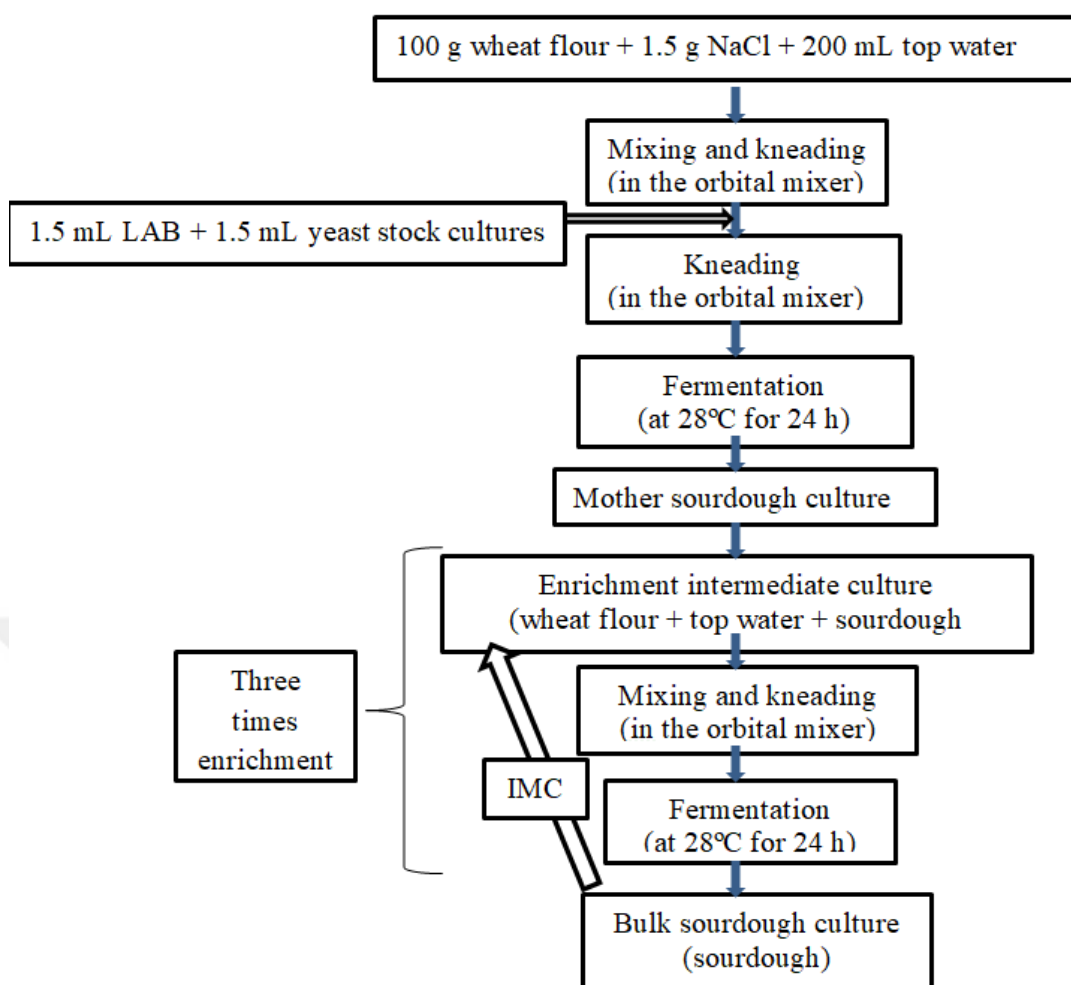


Figure 3.2 The sourdough production flowchart (Sevgili et. al., 2021)

3.7 Sourdough bread production

Twelve types of sourdough bread were produced from each of sourdough. Sourdough for sourdough bread was prepared using 900 g wheat flour, 1.5% salt (w/w) and 20% (w/w) bulk sourdough culture and water that was the total amount of water in the sourdough will be 58%, taking into account the amount of water coming from ingredients and remaining water was added. The mixture was mixed in the orbital mixer for 6 min and kneaded. The fermentation was carried out in two stages: aeration and cold holding: Aeration was done by folding the dough 6 times. The first folding was done once in 30 min; 4 times folding in 2 h. Salt was added after the 1st fold. One hour later after 4th folding, the 5th folding was done, the sixth was made one hour after the fifth fold. After the 6th folding of fermentation, the dough in basket made of bamboo was kept in the refrigerator for 12 h and the fermentation

process was completed. The fermented dough is divided into 350 g pieces and rolled. The rolled dough was left to rest for 10 min. After resting, the dough was shaped and put into a bread mold. The bread was baked in a baking oven at 240°C for 25 min. In the preparation of control bread, commercial yeast was used instead of sourdough culture. After the sourdough breads were baked and cooled to 25°C, they were wrapped in a bread cloth and stored in the cold room (4°C) in the bread basket. Analyzes on breads were made on 1, 4 and 24 h, 3 and 7 days. Samples were taken from the breads to be analyzed within 1 min and placed back in the refrigerator.

3.8 Storage of breads

After the breads were baked and cooled to 25°C, they were wrapped in a bread cloth (made cotton) in the bread basket made of bamboo and stored in the refrigerator (4°C). Analyzes on breads were performed on 0 (4 h after production), 3 and 7 days. Samples were taken from the breads to be analyzed, then put back in the refrigerator. In the research, texture and color analysis of bread were made after 4 and 24 h.

3.9 Analyzes of flour, sourdough and sourdough bread

a) Analyzes of flour: Ash, extensograph, farinograph, wet gluten and protein analyzes were made on the flour.

b) Analyzes of sourdough: Microbiological counts (LAB, mesophilic aerobic bacteria and yeasts), dough swelling, pH and titratable acidity analyzes were made on the sourdough.

c) Analyzes of sourdough bread: pH, titratable acidity, moisture, total dry matter, loaf height, volume, specific volume, crust and crumb color, texture, thermal calorimetric (DSC) and sensory analysis were done on the sourdough bread.

3.9.1 Ash determination

Ash refers to the inorganic residue left after the complete combustion of organic matter. Ash determination crucibles were dried in an ashing furnace at 550-600°C. The crucibles were cooled in the desiccator and weighed. Two grams sample was placed in the crucible and weighed. It was burned at 550-600°C until white ash was formed in the ash furnace for 12-18 h. After cooling in the desiccator, the weighing

process was done. Ash content % was calculated by the formula below (Park and Bell, 2004):

$$\text{Ash \%} = \frac{\text{weight of residue} \times 100}{\text{sample weight}} \quad (3.1)$$

3.9.2 Extensograph analysis

Extensograph analysis was determined as the elongation ability and resistance to elongation of pieces of 150 g weight cut from the dough prepared with flour, water and salt after being kept at 28°C for a certain period in the fermentation cabinet of the tool. The dough was shaped into a standard form in the rounder of the extensograph and the cylinder shaper. After waiting in the fermentation cupboard for a certain time, it was extended in the special part of the tool and the applied force was recorded. Extensograph analyzes were performed according to AACC 54-10 using an extensograph device (Aydoğan et al., 2012).

3.9.3 Farinograph analysis

Farinograph analysis was performed according to AACC 54-21 using a fanograph device. It measures the water absorption of the flour and the resistance of a certain density of dough prepared from this flour against kneading (Aydoğan et al., 2012). Firstly, moisture content of the flour was determined the as directed in any oven method for flour. 50±0.1 g flour was weighed. The small burette was filled with water at room temperature. The first titration attempt rarely produces a curve that has maximum resistance centered on the 500 BU line; therefore, in the subsequent titration, adjust absorption either up or down until this is achieved to within 20 BU. It was calculated the absorption on a 14% moisture basis determined with the large bowl using the following equation (AACC, 2011):

$$\text{Absorption \%} = 3(x + y - 300) \quad (3.2)$$

x = mL of water needed to produce a curve with maximum consistency centered on the 500 BU line,

y = g of flour used, equivalent to 300 g, 14% moisture basis.

3.9.4 Wet gluten analysis

Ten g of flour sample was weighed into a bowl. A hard dough was prepared by adding 5 mL of water onto this flour. The dough was taken in the palm and washed gently under running salty water in drops. During washing, the starch portion was removed from the pulp. Washing was continued until the water flowing from the dough became clear. The gluten index value was calculated with the following formula by weighing the gluten obtained (Akgün, 2007).

$$\text{Wet gluten amount (dry matter \%)} = \frac{\text{wet gluten content}}{(100 - \text{flour moisture \%})} \times 100 \quad (3.3)$$

3.9.5 Protein analysis in flours

One gram of flour was weighed in a filter paper. It was folded and placed in a 250 ml dry Kjeldahl flask with filter paper. It was mixed thoroughly by adding 5 g of catalyst mixture and 10 ml of concentrated sulfuric acid. The balloon was attached to the smoke-free digestion device and heated moderately until the mass was charred. During this time, the gases that came out were removed using a water tramp. After that, the flame was raised and the mixture was boiled until dissolved. The solution was clear and greenish-blue (within 1-2 h) then heated for another half an hour. It was left to cool. With stirring, 100 ml of water was added by carefully cooling it gradually under tap water. A piece of zinc and a small litmus paper was added. Without mixing, 20 g of potassium hydroxide (KOH) was poured from the wall into the flask in 20 ml of water and immediately attached to the distillation apparatus. Distilled ammonia into 25 ml of 0.1N HCl or H₂SO₄. The results multiplied by a conversion factor of 5.70.

3.9.6 Dough swelling power

To determine the swelling strength values of the dough prepared with sourdough cultures, the sourdough was prepared from 100 g wheat flour, 1.5 g table salt (w/w), water and 20 % liquid sourdough culture. Soft consistency dough was prepared by mixing with the help of a glass stirring rod. The dough samples in the cylinder were

left to incubate for 4 h in the incubator set at 30°C. The initial volume values (V_i) of the dough samples and the volume values at the end of each hour (V_s) were recorded. The change in the volumes of the dough samples was calculated in terms of ml and % volume (İpek, 2017; Bircan et al., 2017).

$$V \text{ (mL)} = V_s - V_i$$

$$\text{volume \%} = [(V_s - V_i) / V_i] \times 100$$
(3.4)

V_i = initial volume (ml); V_s = final volume (ml)

3.9.7 Determination of moisture and total dry matter

Five grams sample was weighed into the tared drying vessels which were dried at 135°C and brought to constant weight and cooled in a desiccator. The samples were placed in drying ovens openly and dried at 135°C for about 2 h until they reached constant weight. At the end of the period, they were placed in the desiccator with the caps closed, cooled, and weighed. Then, the amount of moisture and dry matter was calculated from the formula below (Akgün, 2007).

$$\text{Moisture \%} = [(m_2 - m_3) / (m_2 - m_1)] \times 100$$
(3.5)

m_1 : Dry empty drying container weight (g)

m_2 : Analysis sample + drying vessel weight (g)

m_3 : weight of the drying vessel containing the analysis sample after drying (g)

% total dry matter (g/100 g) = 100 - moisture content (%)

3.9.8 pH and titration acidity

In the analyzes, after adding 90 ml of distilled water to 10 g of sample in beaker and it was homogenized for 1 min, the pH value was measured by pH-meter (Erkmen, 2021).

3-4 drops of 3 % phenolphthalein indicator were dropped onto 10 ml of homogenized sample and titrated with 0.1 N NaOH until the light red (pink) color remained constant for 1 min. The amount of 0.1 N NaOH consumed was calculated as the amount of lactic acid to be consumed per 100 g sample.

3.9.9 Loaf height

The maximum height and highest point of each loaf of bread were measured in cm with a standard ruler (Park et al., 2017).

3.9.10 Volume and specific volume

The weight and volume of the breads were measured 1 h after the oven exit. The sourdough bread weight was determined by using weight balance. Bread volume was determined based on rapeseed displacement method at a volumeter (Bastak, Ankara, Türkiye). Specific volume was obtained from volume and weight and calculated by dividing the volume value by weight (Huang et al., 2008; Elgün et al., 2002; Aplevicz et al., 2013).

3.9.11 Texture properties

Texture profile analysis (TPA) of breads was performed 4th and 24th h after bread production by using a texture analyzer (TA-XT2i). The hardness, cohesiveness, springiness, gumminess and chewiness properties of breads were determined. In the study using an aluminum cylindrical probe with a diameter of 36 mm, the bread pieces were compressed twice from their midpoints. (Elgün et al., 2002; Bircan et al., 2017).

3.9.12 Color properties

Color analysis of bread crust and crumb was made on slices of bread production. L*, a*, and b* values were measured using the Hunter (Color Meter/HunterLab Color Fleex, Virginia, USA) color analyzer (Elgün et al., 2002). Colorimeter was standardized by white and black disks. "L*" shows lightness (0 = black, 100 = white), "b*" shows chromaticity on a blue (-) to yellow (+) axis and "a*" shows chromaticity on a green (-) to red (+) axis.

3.9.13 Determination of calorimetric (DSC) properties of breads

Calorimetric properties of breads were determined using Differential Scanning Calorimetric (DSC) Perkin Elmer Pyris-6 (PerkinElmer Inc., Istanbul) device. To determine the effect of sourdough cultures on staling, enthalpy values were measured

by DSC analysis. DSC analysis was performed on 0, 3 and 7 days of the bread samples stored at 4°C. The calibration was made using an indium standard. The samples were hermetically and precisely weighed about 3 mg in aluminum pans. The container temperature was exposed to heat with an increase of 5°C/min from 20°C to 130°C (Hendek Ertop, 2014; Parniakov et al., 2018).

3.9.14 Microorganisms count in sourdough

Twentyfive g of the samples were weighed and added into 225 ml of peptone water in the sterile Warring blender. A homogeneous mixture was obtained by mixing with Waring blender (100 rpm, steel, 1 L) for 1 min. Inoculation was performed using Brain Heart Infusion Agar for mesophilic aerobic bacteria count (MAB), MRS agar and M17 agar for LAB counts. Petri plates were incubated at 30°C for 1-3 days. After the incubation, the number of LAB was counted from plates containing 25 to 250 colonies (Erkmen, 2021).

For yeast count from the homogenized mixture, it was inoculated with spreading to PDA and incubated at 30°C for 3-5 days. Then, the yeast colonies were counted from plates (Erkmen, 2021).

3.9.15 Sensory analysis

To determine sensory characteristics of bread samples, a group of 10 panelists from Gaziantep University was determined. Sensory analysis form was given in Appendix C. External features were determined as volume, crust color, shape and crust features. Internal features was determined as color, taste, wetness, dryness, swallowability, acidity, tissue softness, pore size and chewability. As a control, bread produced with commercial yeast was used. Panelist analyzes were conducted 4, 24 h and 7 days after the bread was baked (Hendek Ertop, 2014).

3.10 Statistical analysis

Bread production was repeated three times in a separate day, each repetition was run in parallel and a parallel sample was used in each analysis. The results of chemical, physical, microbiological and sensory analyzes were evaluated by analysis of variance. One-way analysis of variance and ANOVA tests were used in statistical analysis. Between differences, $p < 0.05$ was considered significant. Duncan multiple

comparison tests was applied to the important differences. "IBM SPSS Statistic v.22" program was used for this.



CHAPTER IV

RESULTS AND DISCUSSION

4.1 LAB and yeasts isolations

In this study, isolated LAB and yeasts from sourdough collected from Gaziantep, Mardin and Konya regions were used in the production of sourdough bread and the physicochemical, textural and thermal analysis and sensory properties of sourdough bread from different combinations were determined.

The purification process was carried out in the selected colonies. Appearance of LAB and yeast colonies obtained from sourdoughs are given in Figure 4.1. The purified colonies for LAB and yeasts are given in Figure 4.2.

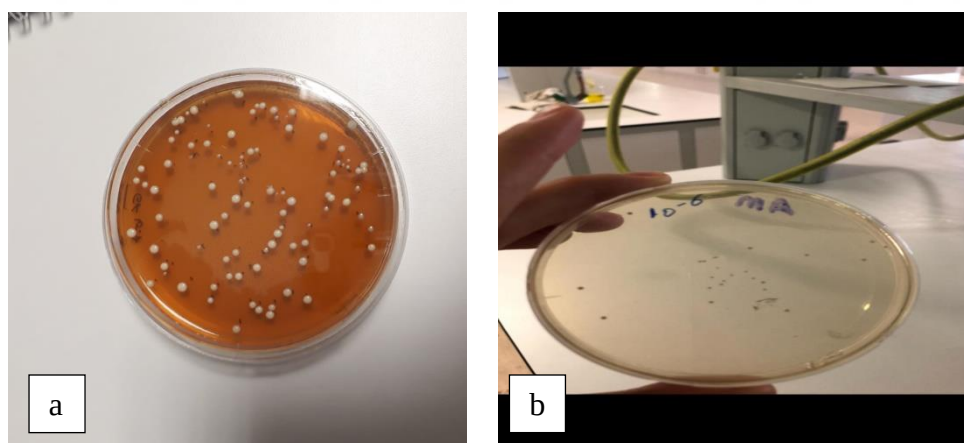


Figure 4.1 Growth of LAB (a) on MRS agar and yeast on PDA (b)

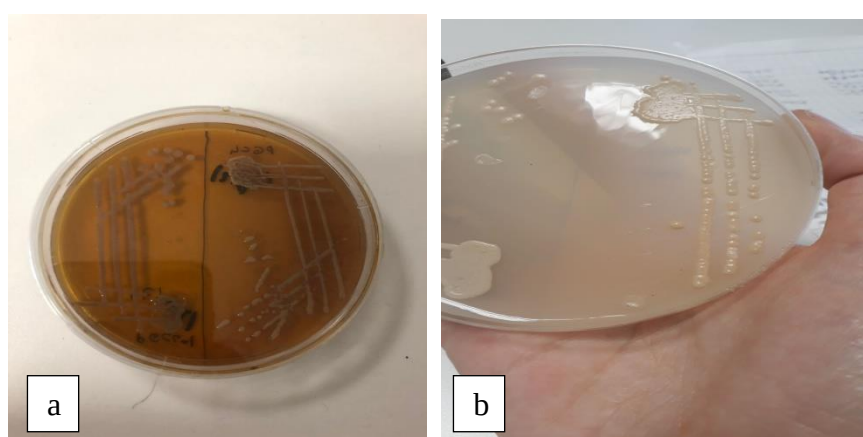


Figure 4.2 The purified LAB (a) on MRS agar and yeasts colonies on PDA (b)

4.2 Morphological characteristics of LAB and yeasts

LAB and yeasts were isolated from twelve sourdough samples that collected from each province. Colony characteristics, microscopic characteristics and Gram-staining of LAB and yeasts were determined. LAB was evaluated according to the shape, size, opacity, color, Gram reaction and microscopic view as given in Table 4.1. Microorganisms can show different characteristics in their proliferation in Petri plates as seen in the table, in general, LAB colonies were observed circular, big, matte and white. Purple-blue bacteria were evaluated as Gram-positive under microscope view after Gram staining as seen in Figure 4.3. All of LAB colonies were determined as Gram-positive. 70% of LAB colonies were found in bacilli while 30% of LAB colonies were found in cocci.

Table 4.1 Colony morphology, Gram staining and microscope view of LAB on MRSA

Sample No	Shape	Size	Opacit y	Color	Gram Staining	Microscope view
PMC3	Circular	Big	Matte	Cream	Gr (+)	Cocci
MMA2	Unround	Small	Matte	Cream	Gr (+)	Cocci
D5g	Circular	Big	Matte	White	Gr (+)	Bacilli
O50	Circular	Small	Matte	Cream	Gr (+)	Cocci
MGN1	Circular	Big	Matte	White	Gr (+)	Bacilli
MGC5-1	Circular	Big	Matte	White	Gr (+)	Bacilli
MMB1	Circular	Big	Matte	White	Gr (+)	Bacilli
MMC2-1	Circular	Big	Matte	Cream	Gr (+)	Cocci
PMB5	Unround	Small	Matte	White	Gr (+)	Bacilli

Table 4.1 (Continued)

MMC8-1	Circular	Big	Matte	White	Gr (+)	Bacilli
MME3	Circular	Big	Matte	Cream	Gr (+)	Cocci
O24	Circular	Big	Matte	Cream	Gr (+)	Cocci
MGE4	Circular	Big	Matte	White	Gr (+)	Bacilli
MGE1-1	Circular	Big	Matte	White	Gr (+)	Bacilli
MGM1	Circular	Big	Matte	Cream	Gr (+)	Cocci
K13	Circular	Big	Matte	Cream	Gr (+)	Cocci
MMF9	Circular	Big	Matte	White	Gr (+)	Bacilli
PMB2	Unround	Small	Matte	White	Gr (+)	Bacilli
MGC6	Unround	Small	Matte	White	Gr (+)	Bacilli
F1b	Unround	Small	Matte	White	Gr (+)	Cocci
PMC1	Circular	Big	Matte	White	Gr (+)	Bacilli
MGA3-1	Circular	Small	Matte	Cream	Gr (+)	Cocci
PMB3	Circular	Big	Matte	White	Gr (+)	Bacilli
O14	Unround	Small	Matte	White	Gr (+)	Bacilli
O11	Unround	Small	Matte	White	Gr (+)	Bacilli
MGE2	Unround	Small	Matte	White	Gr (+)	Bacilli
PMB5-1	Unround	Small	Matte	White	Gr (+)	Bacilli
MME5	Circular	Big	Matte	White	Gr (+)	Bacilli
MME5-1	Circular	Big	Matte	White	Gr (+)	Bacilli
MME4	Unround	Small	Matte	Cream	Gr (+)	Cocci
O55	Circular	Big	Matte	White	Gr (+)	Bacilli
A20	Unround	Small	Matte	White	Gr (+)	Bacilli
A4	Circular	Big	Matte	Cream	Gr (+)	Cocci
MMB3	Circular	Big	Matte	White	Gr (+)	Bacilli
PMB4-1	Circular	Big	Matte	White	Gr (+)	Bacilli
PMB4	Unround	Small	Matte	White	Gr (+)	Bacilli
PMD3	Circular	Big	Matte	White	Gr (+)	Bacilli
MGB6	Circular	Big	Matte	White	Gr (+)	Bacilli
MGD2	Unround	Small	Matte	White	Gr (+)	Bacilli
MGF5	Unround	Small	Matte	White	Gr (+)	Cocci
MGF4	Circular	Big	Matte	White	Gr (+)	Bacilli
MGF5-1	Circular	Big	Matte	White	Gr (+)	Bacilli
MGH4	Circular	Big	Matte	White	Gr (+)	Bacilli
MGH2	Unround	Small	Matte	White	Gr (+)	Bacilli
K2	Circular	Big	Matte	Cream	Gr (+)	Cocci
K42	Circular	Big	Matte	Cream	Gr (+)	Cocci
A14	Circular	Big	Matte	Cream	Gr (+)	Cocci
MGN5	Circular	Big	Matte	White	Gr (+)	Bacilli
MGL3	Unround	Small	Matte	White	Gr (+)	Bacilli
PGC4	Unround	Small	Matte	White	Gr (+)	Bacilli
O26	Circular	Big	Matte	Cream	Gr (+)	Cocci

Table 4.1 (Continued)

PMD1-1	Circular	Big	Matte	White	Gr (+)	Bacilli
PME5	Circular	Big	Matte	White	Gr (+)	Bacilli
PMC3-1	Circular	Big	Matte	White	Gr (+)	Bacilli
O53	Circular	Big	Matte	White	Gr (+)	Bacilli
O37	Unround	Small	Matte	White	Gr (+)	Bacilli
MGD3	Circular	Big	Matte	White	Gr (+)	Bacilli
C3	Circular	Big	Matte	Cream	Gr (+)	Cocci
D5h	Circular	Big	Matte	White	Gr (+)	Bacilli
MMB2	Circular	Big	Matte	White	Gr (+)	Bacilli

MG, PG: Represents LAB sample type which were obtained from Gaziantep sourdough

MM, PM, K13: Represents LAB sample type which were obtained from Mardin sourdough

A, C, D, O, F, K2, K42: Represents LAB sample type which were obtained from Konya sourdough

The yeasts was evaluated according to the shape, size, opacity, color, Gram reaction and microscope view as seen in Table 4.2. In general, yeast colonies were observed circular, brilliant and cream. The result of Gram staining is evaluated according to Figure 4.3. All of yeasts were found Gram-positive. According to examination of microscope, oval shape was found more than cylindrical shape.

Table 4.2 Colony morphology and microscope view of yeasts on PDA

Sample No	Shape	Size	Opacity	Color	Microscope View
KD8a-1	Circular	Big	Matte	White	Cylindrical
KDS2a-1	Circular	Big	Matte	White	Cylindrical
KDS2c	Circular	Big	Matte	White	Cylindrical
KDS2c-1	Circular	Big	Matte	White	Cylindrical
KD7a-1	Circular	Small	Brilliant	Cream	Cylindrical
KD1b-1	Circular	Small	Brilliant	Cream	Cylindrical
KD3b	Circular	Small	Brilliant	Cream	Cylindrical
KD3b-1	Circular	Small	Brilliant	Cream	Cylindrical
KD9c-1	Circular	Small	Brilliant	Cream	Cylindrical
KD11a-1	Circular	Small	Brilliant	Cream	Cylindrical
KDS4a	Circular	Small	Brilliant	Cream	Cylindrical
PGC3	Circular	Small	Brilliant	Cream	Cylindrical
PGC3-1	Circular	Small	Brilliant	Cream	Cylindrical
PGD1	Circular	Big	Brilliant	Cream	Oval
PGE2	Circular	Small	Brilliant	Cream	Oval
PGL1	Circular	Big	Brilliant	Cream	Oval
PGL2	Circular	Big	Brilliant	Cream	Oval

Table 4.2 (Continued)

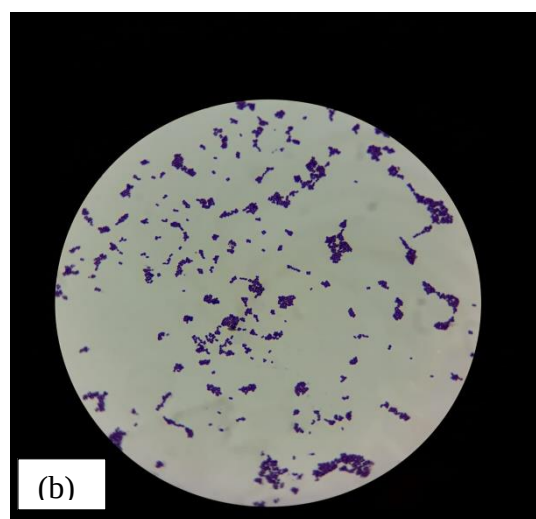
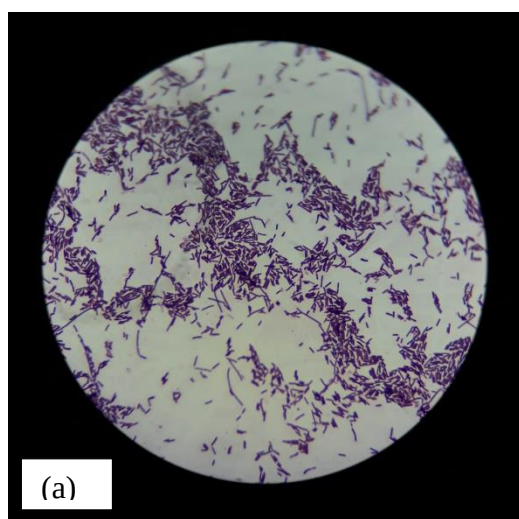
KD6a	Circular	Small	Matte	Cream	Oval
KD6b	Circular	Small	Matte	Cream	Oval
KDS2a	Circular	Small	Matte	Cream	Oval
KDS2a-2	Circular	Small	Matte	Cream	Oval
F16	Unround	Big	Matte	White	Cylindrical
MGL1	Circular	Small	Brilliant	Cream	Oval
MGL2	Circular	Small	Brilliant	Cream	Oval
PMB1-1	Circular	Small	Matte	White	Oval
KD9d	Circular	Small	Matte	White	Oval
KD1a-1	Circular	Small	Matte	White	Oval
PGB4	Circular	Big	Brilliant	Cream	Oval
PGH2	Circular	Big	Brilliant	Cream	Oval
1B	Circular	Big	Brilliant	Cream	Oval
3F	Circular	Big	Brilliant	Cream	Oval
MB	Circular	Big	Brilliant	Cream	Oval
ME	Circular	Big	Brilliant	Cream	Oval
MGB1	Circular	Big	Brilliant	Cream	Oval
PMA9	Circular	Big	Brilliant	Cream	Oval
PMA10	Circular	Big	Brilliant	Cream	Oval
PMF5	Circular	Big	Brilliant	Cream	Oval
PGM1	Circular	Small	Matte	Cream	Oval
PGH1	Circular	Small	Matte	Cream	Cylindrical
MA	Circular	Big	Brilliant	Cream	Oval

PG: Represents yeast sample type which were obtained from Gaziantep sourdough

MA, 3F, 1B, ME: Represents yeast sample type which were obtained from Mardin sourdough

PM: Represents yeast sample type which were obtained from Mardin sourdough

KD, F16: Represents yeast sample type which were obtained from Konya sourdough



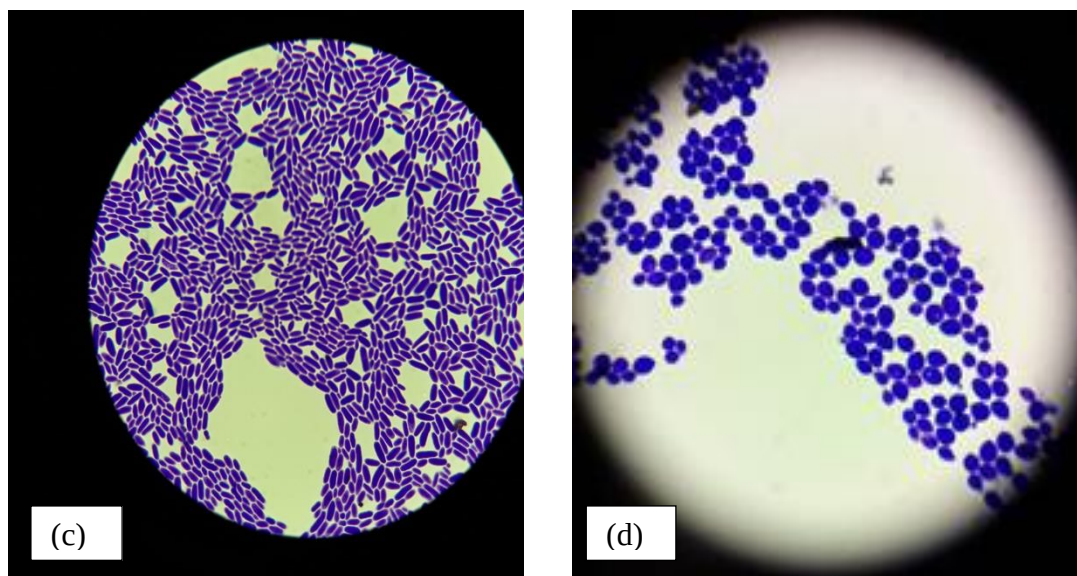


Figure 4.3 Image under microscope examination; (a): Bacilli for LAB, (b) Cocci for LAB, (c) Cylindrical for yeasts and (d) Oval for yeasts

4.3 Identification of LAB and yeasts with MALDI-TOF analysis

MALDI-TOF analysis results of LAB isolated from sourdough obtained from Konya, Gaziantep and Mardin regions are given in Table 4.3. The most LAB species diversity (8 species) was determined from Gaziantep sourdoughs, while the less LAB species (5 species) were detected from Mardin sourdoughs. Six LAB species were determined from Konya sourdoughs. Eleven types of LAB species were isolated from 36 sourdoughs.

It was determined that there are 6 different LAB types in 17 LABs isolated from Konya sourdoughs (Table 4.3). These LAB species were found to be *Pediococcus acidilactici* (*P. acidilactici*), *Levilactobacillus brevis* (previously name *Lactobacillus brevis*) (*L. brevis*), *Lactiplantibacillus plantarum* (previously name *Lactobacillus plantarum*) (*L. plantarum*), *Lactiplantibacillus pentosus* (previously name *Lactobacillus pentosus*) (*L. pentosus*), *Weissella confusa* (*W. confusa*) and *Enterococcus hirae* (*E. hirae*). It was determined that the dominant species were *P. acidilactici* (41.18%) and *L. brevis* (23.53%). One (5.88%) of *L. pentosus*, *W. confusa* and *E. hirae* was isolated from Konya sourdough.

It was determined that there are 8 different LAB out of 19 LABs isolated from Gaziantep sourdoughs (Table 4.3). These were *L. brevis*, *L. plantarum*, *P. acidilactici*, *Lactiplantibacillus paraplantarum* (previously name *Lactobacillus*

paraplantarum) (*L. paraplantarum*), *L. pentosus*, *Enterococcus faecalis* (*E. faecalis*), *Companilactobacillus paralimentarius* (previously name *Lactobacillus paralimentarius*) (*C. paralimentarius*) and *E. hirae*. Among these, the main flora was found as *L. brevis* (42.10%) and *L. plantarum* (21.05%). *P. acidilactici* from Gaziantep sourdough was determined as two species (10.52%) and the other each of five LABs was isolated as one species (5.88%).

It was determined that there are 5 different types of LAB from the 24 LABs isolated from Mardin sourdoughs (Table 4.3). These are *L. brevis*, *L. plantarum*, *P. acidilactici*, *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Leuconostoc mesenteroides* subsp. *cremoris*. Among these, *L. brevis* (58.33%), *L. plantarum* (16.66%) and *P. acidilactici* (16.66%) were found as the dominant flora. Two (8.32%) *L. mesenteroides* was determined from Mardin sourdough.

Table 4.3 Percentage of LAB with using MALDI-TOF analysis

KONYA LAB (n=17)	%
<i>Pediococcus acidilactici</i>	41.18
<i>Levilactobacillus brevis</i>	23.53
<i>Lactiplantibacillus plantarum</i>	17.64
<i>Lactiplantibacillus pentosus</i>	5.88
<i>Weissella confusa</i>	5.88
<i>Enterococcus hirae</i>	5.88
GAZİANTEP LAB (n=19)	%
<i>Levilactobacillus brevis</i>	42.1
<i>Lactiplantibacillus plantarum</i>	21.05
<i>Pediococcus acidilactici</i>	10.52
<i>Lactiplantibacillus paraplantarum</i>	5.26
<i>Lactiplantibacillus pentosus</i>	5.26
<i>Enterococcus faecalis</i>	5.26
<i>Companilactobacillus paralimentarius</i>	5.26
<i>Enterococcus hirae</i>	5.26
MARDİN LAB (n=24)	%
<i>Levilactobacillus brevis</i>	58.33
<i>Lactiplantibacillus plantarum</i>	16.66
<i>Pediococcus acidilactici</i>	16.66
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	4.16
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	4.16

MALDI-TOF analysis results of yeasts isolated from sourdough obtained from Konya, Gaziantep and Mardin regions are given in Table 4.4. The most yeast species (6 species) were determined from both, Gaziantep and Konya sourdoughs, while the less species (2 species) were detected from Mardin sourdoughs. *Pichia kudriavzevii* (*P. kudriavzevii*), *Kluyveromyces marxianus* (*K. marxianus*), *Geotrichum candidum* (*G. candidum*), *Kazachstania unispora* (*Ka. unispora*), *Galactomyces candidum* (*Gal. candidum*) and *Candida kefir* (*C. kefir*) yeast species were determined from sourdoughs obtained from Konya province. It was determined that the most yeasts were *P. kudriavzevii* (38.88%) and *K. marxianus* (22.22%). On the other hand, one species was isolated as *Galactomyces candidum* (5.55%) and *C. kefir* (5.55%). *P. kudriavzevii*, *Saccharomyces cerevisiae* (*S. cerevisiae*), *Wickerhamomyces anomalus* (*W. anomalus*), *Kazachstania humilis*, *C. glabrata* and *C. tropicalis* yeasts were isolated from sourdoughs obtained from Gaziantep province. Among these yeasts, the common flora was *P. kudriavzevii* (23.07%) and *S. cerevisiae* (23.07%). On the other hand, only one species was found to be *C. tropicalis* (7.69%).

Table 4.4 Percentage of yeasts with using MALDI-TOF analysis

Konya Yeasts (n=18)	%
<i>Pichia kudriavzevii</i>	38.88
<i>Kluyveromyces marxianus</i>	22.22
<i>Geotrichum candidum</i>	16.66
<i>Kazachstania unispora</i>	11.11
<i>Galactomyces candidum</i>	5.55
<i>Candida kefir</i>	5.55
Gaziantep Yeasts (n=13)	%
<i>Pichia kudriavzevii</i>	23.07
<i>Saccharomyces cerevisiae</i>	23.07
<i>Wickerhamomyces anomalus</i>	15.38
<i>Kazachstania humilis</i>	15.38
<i>Candida glabrata</i>	15.38
<i>Candida tropicalis</i>	7.69
Mardin Yeasts (n=9)	%
<i>Saccharomyces cerevisiae</i>	88.88
<i>Kluyveromyces marxianus</i>	11.11

As a result of MALDI-TOF analysis, yeast diversity was found to be less in sourdoughs obtained from Mardin province. Only two types of yeast (*S. cerevisiae* and *K. marxianus*) have been identified. Eleven types of yeast species were isolated from 36 sourdoughs. The results obtained emphasize that the diversity of sourdough LAB and yeasts changes regionally.

4.4 Identification of LAB and yeasts with PCR-sequence analysis

For species determination from molecular analysis, approximately 1400 bp long bands were obtained from the 23S rRNA gene region of bacterial isolates and analyzed. Forward and reverse primer analyzes were evaluated in the graphic viewer FinchTV program for chromatogram files as seen Figure 4.4. The green, blue, black and red colors at the peak mean adenine, cytosine, guanine and thymine, respectively.

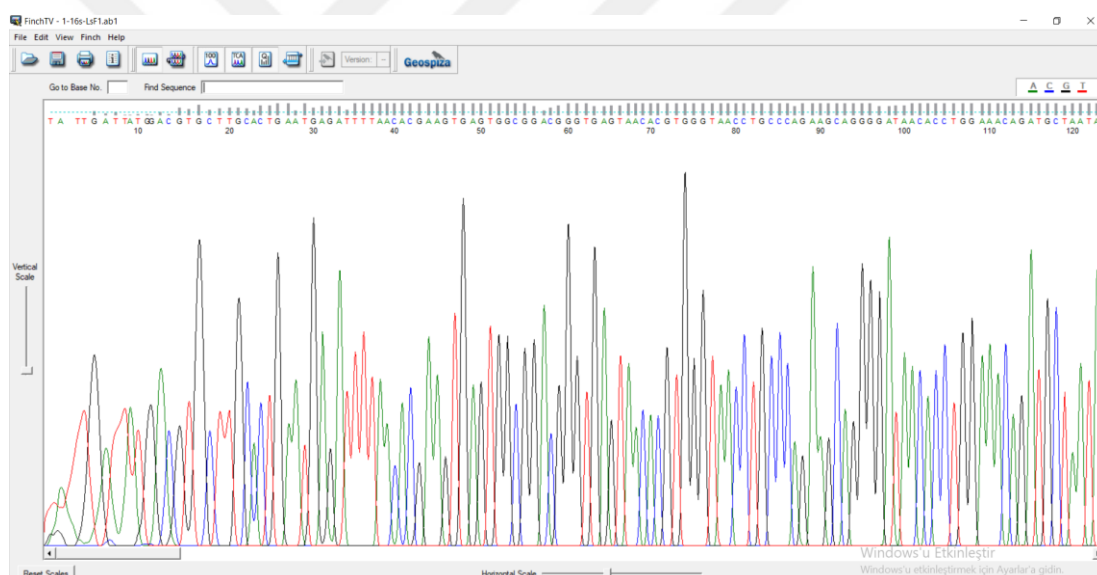


Figure 4.4 The image of the sequence peaks in the FinchTV program

Sequence data obtained were registered in Bioedit Sequence Alignment Editor Program as seen Figure 4.5 and a single sequence was obtained in FASTA format. The results were compared and matched with the nucleotide sequences of bacterial strains belonging to the genera registered in the GenBank using the NCBI (National Center for Biotechnology Information) Nucleotide BLAST program as seen Figure 4.6.



Figure 4.5 Alignment created with the Bioedit Sequence Alignment Editor program

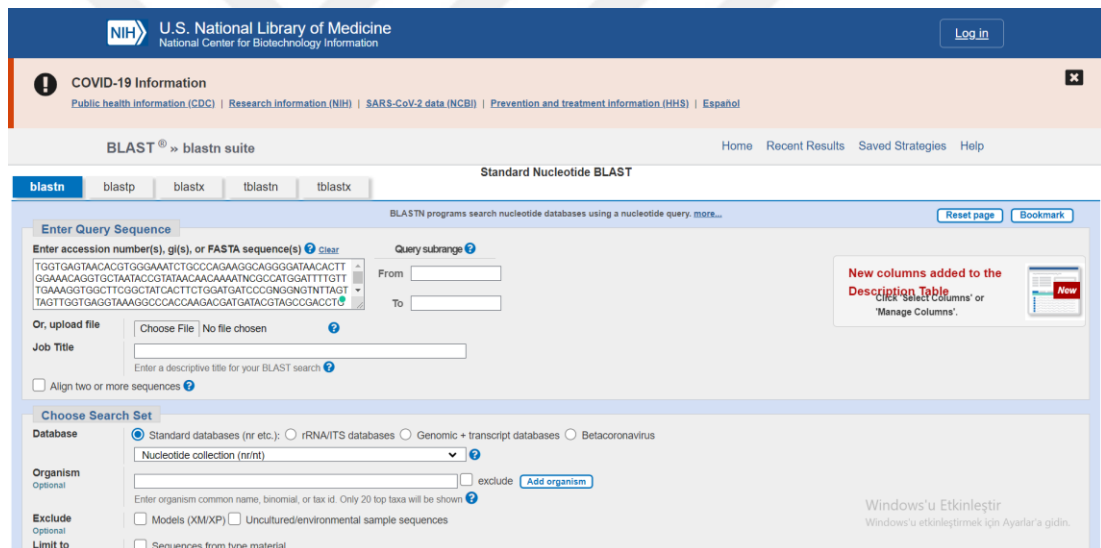


Figure 4.6 Nucleotide BLAST on NCBI website

Evolutionary analyzes of isolates with molecular species determination were performed in MEGA11 (Kumar et al., 2016). Evolutionary history was established by bootstrap consensus (1000 replicates) using the Neighbor-Joining statistical method (Saitou and Nei, 1987; Felsenstein, 1985). Evolutionary distances were calculated using the p-distance method (Nei and Kumar, 2000). In the obtained phylogenetic tree, *Bacillus* sp. is formed outgroup. In addition, the strains were obtained within the scope of our study formed similar groups in the comparative

phylogenetic tree with the reference strains found in NCBI. Phylogenetic tree analysis results of LAB are given in Figure 4.7.

For molecular species determination, bands with a length of about 450 bp were obtained from the ITS gene regions of yeast isolates and analyzed. Forward and reverse primer analyzes were evaluated in the graphic viewer FinchTV program for chromatogram files. Sequence data obtained were registered in Bioedit Sequence Alignment Editor Program and a single sequence was obtained in FASTA format. The results were compared and matched with the nucleotide sequences of yeast strains belonging to the genera registered in the GenBank using the NCBI Nucleotide BLAST program. Evolutionary analyzes of isolates with molecular species determination were performed in MEGA11 (Kumar et al., 2016). Evolutionary history was established by bootstrap consensus (1000 replicates) using the Neighbor-Joining statistical method (Saitou and Nei, 1987; Felsenstein, 1985).

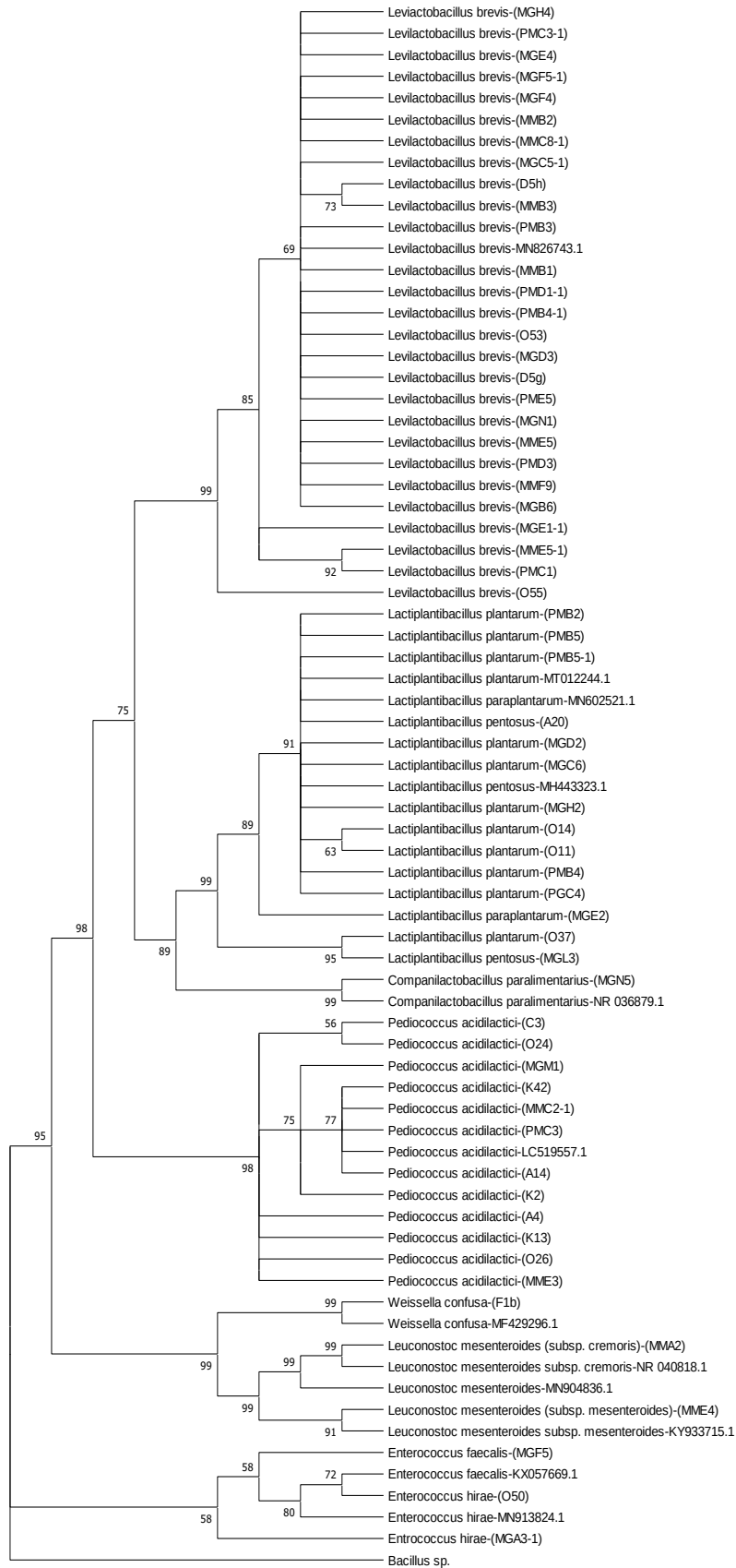


Figure 4.7 Phylogenetic tree of LAB strains

Evolutionary distances were calculated using the p-distance method (Nei and Kumar, 2000). In the obtained phylogenetic tree, *Gaffeaulyma gastricus* constitutes the outgroup. The strains were obtained within the scope of our study formed similar groups in the comparative phylogenetic tree with the reference strains found in NCBI. Phylogenetic tree analysis results of yeast are given in Figure 4.8.

4.5 Technological properties of LAB and yeasts

The homofermentative-heterofermentative, oxidative-fermentative, acid and gas formation from sugars, catalase test and growth properties in different salt and temperature environments of sourdough LAB are given in Table 4.5. All isolated bacteria showed catalase negative properties. While only *P. acidilactici* showed oxidative properties, the remaining species were determined to be fermentative. While the rate of LAB showing fermentative characteristics was 80.00%, the rate of those showing oxidative was 20.00%. In our study, homofermentative (26.66%), heterofermentative (48.33%) and facultative heterofermentative (25.00%) bacteria from the genus lactobacilli were isolated.

Temperature is important for the proliferation of LAB and yeast as in all living things. In this study, the proliferation of LAB isolated from sourdough samples at 15, 28 and 45°C is given in Table 4.5. While all the LAB showed growth at 15°C (except *P. acidilactici*) and 28°C, 45°C was important in terms of the distinctive feature of bacteria. It was determined that *P. acidilactici*, *E. faecalis* and *E. hirae* can grow at 45°C.

It was determined that all bacteria grow at 1.5 and 4.0% salt concentrations in Table 4.5. It has been observed that *P. acidilactici* is more tolerant of high salt concentrations than others. As a result of the research, it has been shown that LAB with a salt concentration of 1.5% that will be found in the production of sourdough bread can achieve a good proliferation.

It is important for the product to be produced that LAB form gas and acid as a result of proliferation and fermentation in the presence of sugars. In our research, it was determined that while all LAB produce acid from glucose, only 4 of them produce gas (Table 4.5). While 48.33 and 3.33% of LAB formed gas from maltose and sucrose, respectively. In the research, especially LAB isolates that can produce gas or

acid from sugars in sourdough production and LAB fermenting different sugars were selected.



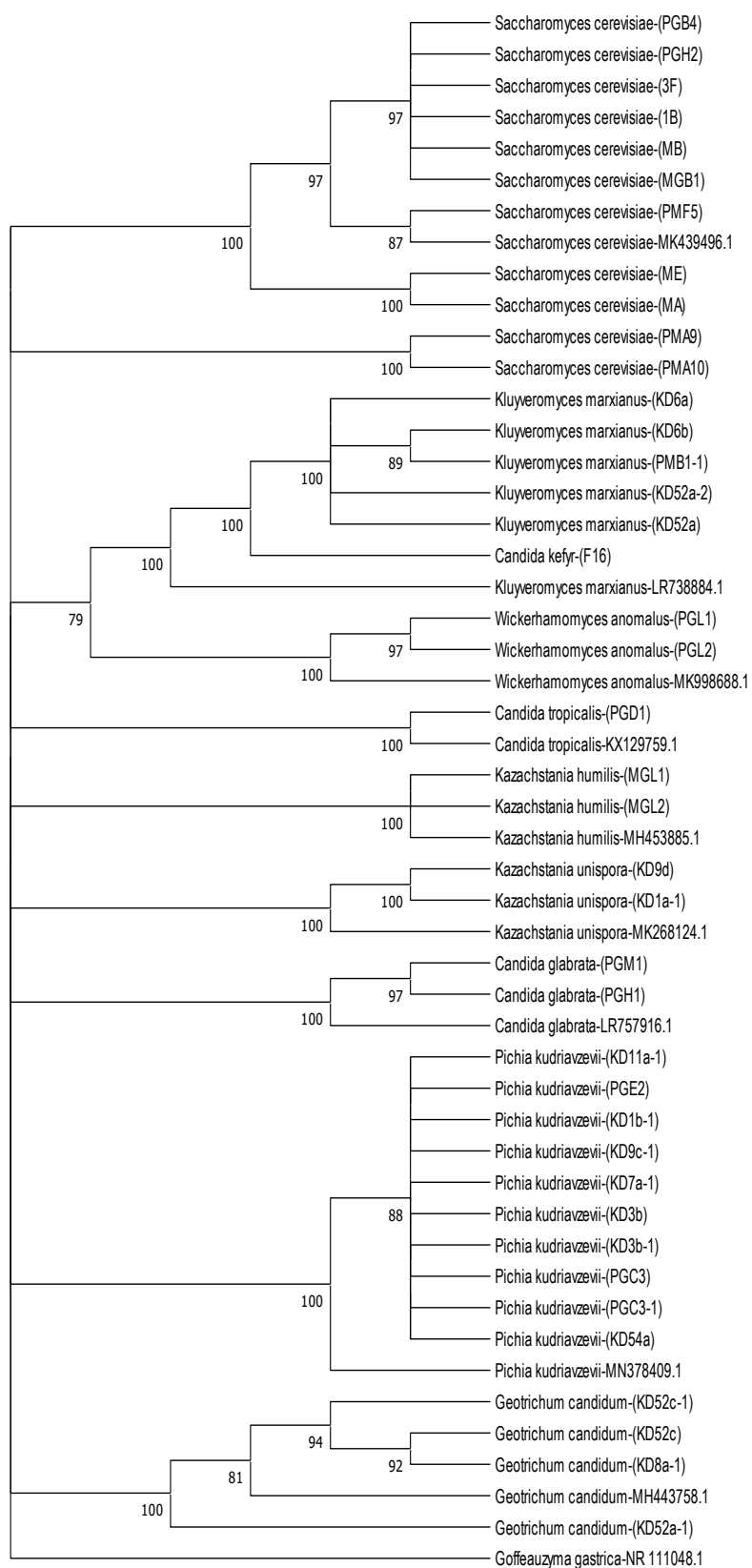


Figure 4.8 Phylogenetic tree of yeast species

The important features of LAB to be used as culture in sourdough are that they can grow well in the presence of salt 1.5% and at 28°C. Because sourdough bread production is produced around these conditions. LAB grow well under these conditions, only *L. mesenteroides* subsp. *cremoris* did not grow in 4% NaCl, but sourdough was produced with 1.5% salt, it was thought that this bacterium could reproduce under bread production conditions. Also only *P. acidilactici* cannot grow at 15°C and only *P. acidilactici* and *Enterococcus* spp. grow at 45°C. As a result of the research, all LAB can achieve good growth at the 1.5% salt concentration to be used in the sourdough industry.

In terms of bakery, carbohydrate analysis is important because LAB and yeasts contribute to properties such as gas formation and acid production from carbohydrates. In this case, the correct selection of LAB and yeasts used in bread production is an important parameter in terms of the desired taste in the final product. As a result of the symbiotic life of yeasts and LAB in sourdough fermentation, yeasts and heterofermentative LAB are responsible for the rise of dough and flavor formation, while homofermentative LAB can affect the elasticity and acidity of bread.

Growth of yeast isolated from sourdough samples at 25, 28 and 37°C is given in Table 4.6. Temperature is one of the environmental conditions effective in the growth of yeasts. Any change in temperature effects on the metabolites formed by yeasts. Therefore, growth temperatures of yeasts are important when considering their industrial use. In the study, it was determined that yeasts can grow well at 28-30°C temperatures used in sourdough bread production. Another important point in terms of technology is the ability to produce CO₂ in isolated yeasts.

For the technological properties of yeasts, their ability to grow at different temperatures and sugar environments has been investigated. In the presence of glucose, all the yeasts multiplied and 65% of them produced gas from glucose. While 82.5% of the yeasts multiplied in the environment where sucrose was present, 60.60% of them formed gas. 22.5% of the yeasts multiplied in the presence of lactose. 75% of the yeasts showed growth in the presence of maltose. From this research, yeasts capable of fermenting glucose, lactose, sucrose and maltose were isolated.

There were differences in the technological characteristics of yeast species. All yeasts grow at 25°C and 28°C. It was determined that both LAB and yeasts were grown well at 28°C used as fermentation temperature in the sourdough industry.

4.6 Technological properties of flour

The quality of the flour sample used in sourdough bread production is important. Flour quality is the ability of flour in a bread sense to produce a uniform, attractive end product with the desired properties. Moisture, ash, protein, wet gluten, extensograph and farinograph analyzes were performed to determine the flour quality. According to the Turkish Food Codex Wheat Flour Notification; the moisture content of wheat flour used in bread making should be at maximum 14.5% and the ash content (in dry matter) should be $0.7\% < - \leq 0.8\%$. It is desirable that the moisture in the flours should be lower than this value as low as possible because it will affect the water lifting capacity of the flour. Wheat flour that is at least protein content of 10.5% is considered as useable in bread production (TGK, 2013). The ash analysis made in flour and bakery products is related to the usage value of the flours in bakery and is important in terms of flour yield and quality. Therefore, flours are not desired to have high ash content. The amount of wet gluten varies depending on the production conditions and has a significant positive relationship with the protein amount (Menderis et al., 2008). There is a linear relationship between the bread making quality of wheat and the amount of protein for all types. Flours with high gluten quantity and quality have higher water absorption capacity and strong elastic dough structure; breads produced from these flours give good crumb texture with high bread volume (Bilgiçli and Soylu, 2016).

In the study, the amount of moisture, protein, ash and wet gluten in flour used in sourdough bread making was determined as seen Table 4.7. The moisture content of flour used in sourdough culture and sourdough bread making was determined as 14.2%, protein content (dry matter) 14.1%, ash content (dry matter) 0.74% and wet gluten content 33.1%. According to the results, it was determined that the flour used in making sourdough bread complies with the Turkish Food Codex Wheat Flour communiqué (TGK, 2013).

Table 4.5 Technological properties of LAB isolated from sourdough

Analysis		<i>P. acidilactici</i>	<i>L. mesenteroides subsp. mesenteroides</i>	<i>L. mesenteroides subsp. cremoris</i>	<i>L. plantarum</i>	<i>L. brevis</i>	<i>C. paralimentarius</i>	<i>L. paraplantarum</i>	<i>L. pentosus</i>	<i>E. faecalis</i>	<i>E. hirae</i>	<i>W. confusa</i>
Catalase		-	-	-	-	-	-	-	-	-	-	-
O-F		O	F	F	F	F	F	F	F	F	F	F
Ferm.		H	Het	Het	FH	Het	FH	FH	FH	H	H	H
15°C		-	+	+	+	+	+	+	+	+	+	+
28°C		+	+	+	+	+	+	+	+	+	+	+
45°C		+	-	-	-	-	-	-	-	+	+	-
%1.5 NaCl		+	+	+	+	+	+	+	+	+	+	+
%4 NaCl		+	+	-	+	+	+	+	+	+	+	+
%6.5 NaCl		+	-	-	+	+	+	+	+	+	+	-
%7 NaCl		+	-	-	+	-	-	-	-	+	+	-
%10 NaCl		+	-	-	-	-	-	-	-	-	-	-
Glucose	Acid	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	+	+	-	+	-	-	-	-	-	+
Lactose	Acid	-	+	+	+	-	-	+	+	+	+	-
	Gas	-	+	+	-	-	-	-	-	-	-	-
Sucrose	Acid	-	+	+	+	-	+	+	+	+	+	+
	Gas	-	+	+	-	-	-	-	-	-	-	-
Maltose	Acid	-	+	+	+	+	-	+	+	+	+	+
	Gas	-	+	+	-	+	-	-	-	-	-	-

*(-): No growth, no acid or no gas formation, (+): Growth

H: Homofermentative, Het: Heterofermentative, FH: Facultative heterofermentative, O: Oxidative and F: Fermentative

Table 4.6 Technological properties of yeast isolated from sourdough

Analysis		<i>S.</i> <i>cerevisiae</i>	<i>P.</i> <i>kudriavzevii</i>	<i>K.</i> <i>marxianus</i>	<i>K.</i> <i>unispota</i>	<i>C.</i> <i>tropicalis</i>	<i>C.</i> <i>glabrata</i>	<i>W.</i> <i>anomalus</i>	<i>G.</i> <i>candidum</i>	<i>K.</i> <i>humilis</i>	<i>G.</i> <i>candidum</i>	<i>C.</i> <i>kefyr</i>
25°C		+	+	+	+	+	+	+	+	+	+	+
28°C		+	+	+	+	+	+	+	+	+	+	+
37°C		-	+	+	+	±	-	-	z	-	-	-
Glucose	Growth	+	+	+	+	+	+	+	+	+	+	+
	Gas	+	-	+	+	+	+	+	-	+	-	+
Lactose	Growth	-	-	+	-	-	-	-	-	-	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	+
Sucrose	Growth	+	z	+	-	+	-	+	-	-	+	+
	Gas	+	-	+	-	+	-	+	-	-	-	+
Maltose	Growth	+	z	-	+	+	-	+	-	-	+	+
	Gas	+	-	-	+	+	-	-	-	-	-	+

*(-): No growth, no acid or no gas formation, (+): Growth (±): weakly
z: Growth observation more than seven days (week)

Table 4.7 Chemical characteristics of wheat flour (TGK, 2013)

Flour characteristics	Range (as TGK, %)	Mean (%)
Moisture	The most 14.5	14.2
Ash (dry matter)	0.7<%≤0.8	0.74
Protein (dry matter)	The least 10.5	14.1
Wet gluten	The least 27 [*]	33.1

^{*}(Bulut, 2012)

Farinograph analysis result of the flour used in the study is given in Figure 4.9. In the research, because of the farinograph analysis, it was determined that the water absorption capacity of the flour was 60.9% and the development time of the dough was 5.38 min. Farinograph analysis measures the water absorption of the flour and the resistance of a certain density of dough prepared from this flour to kneading. Particle size, primarily gluten and the amount of damaged starch are also effective in lifting water in the flour. Its resistance to kneading is related to gluten quality. Flours used for bread production should have high water absorption capacity and the kneading time should not be too long. The long kneading time is an undesirable feature in commercial ovens in terms of energy and time loss. However, the bread quality of flours with very short kneading time is generally low. The amount of water that should be given for the average of 500 consensus lines of the farinogram is specified as the amount of water uptake of that flour sample. The long development time indicates that the pulp will swell late and therefore the kneading time will be long, as well as the high amount and quality of the pulp. Low growth time causes decrease in bread volume and deterioration of the pore structure (Aydoğan et. al., 2012).

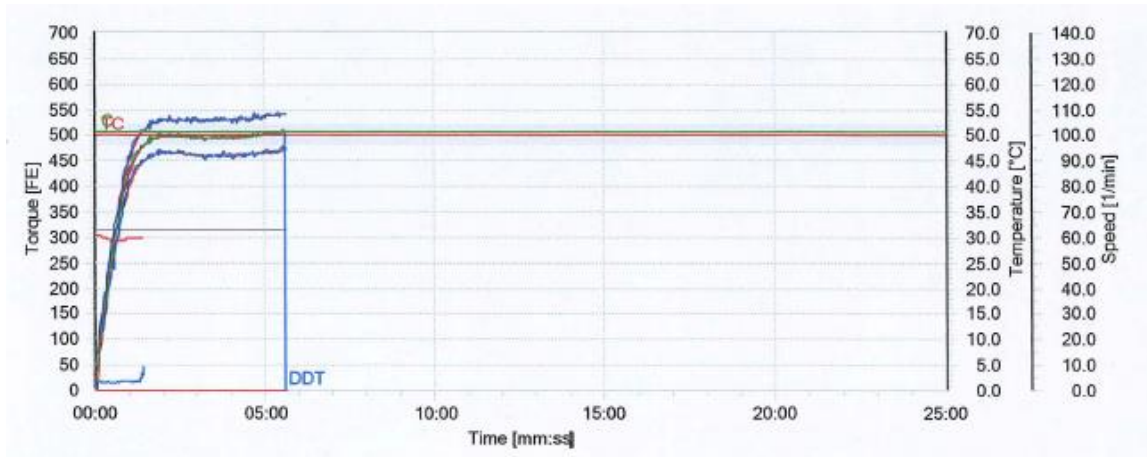


Figure 4.9 Farinograph analysis chart of flour

The result of the extensograph of the flour used in making sourdough in the research is given in Figure 4.10 and the analysis results are given in Table 4.8. Extensograph analysis measurements were made at the 45th, 90th and 135th min of the flour used in the research of the project. Dough strength was found as 367, 752 and 751 BU at 45th, 90th and 135th min, respectively. In the extensibility feature, there was a decrease in the 90th min, the best value was found to be 111 mm at 135th min. The highest maximum resistance value was observed at the 135th min and this value was determined as 832 BU. Extensograph energy values were observed as 93 cm² at the 45th min, 103 cm² at the 90th min and 115 cm² at the 135th min. Extensograph analysis is related to the dough's resistance to elongation and its ability to stretch and the gluten structure of the dough. Dough extensibility and dough strength (resistance to elongation) are widely used in determining the difference between the qualities of bread flours and choosing the appropriate raw material. Among the rheological properties, extensibility and dough strength (resistance) are used to determine the processability of wheat to different dough products. It provides important information about the extensibility value, cooking performance and final product quality (Aydoğan et al., 2013). Based on the results obtained, it was concluded that the flour was suitable for making sourdough bread and it was used in sourdough making.

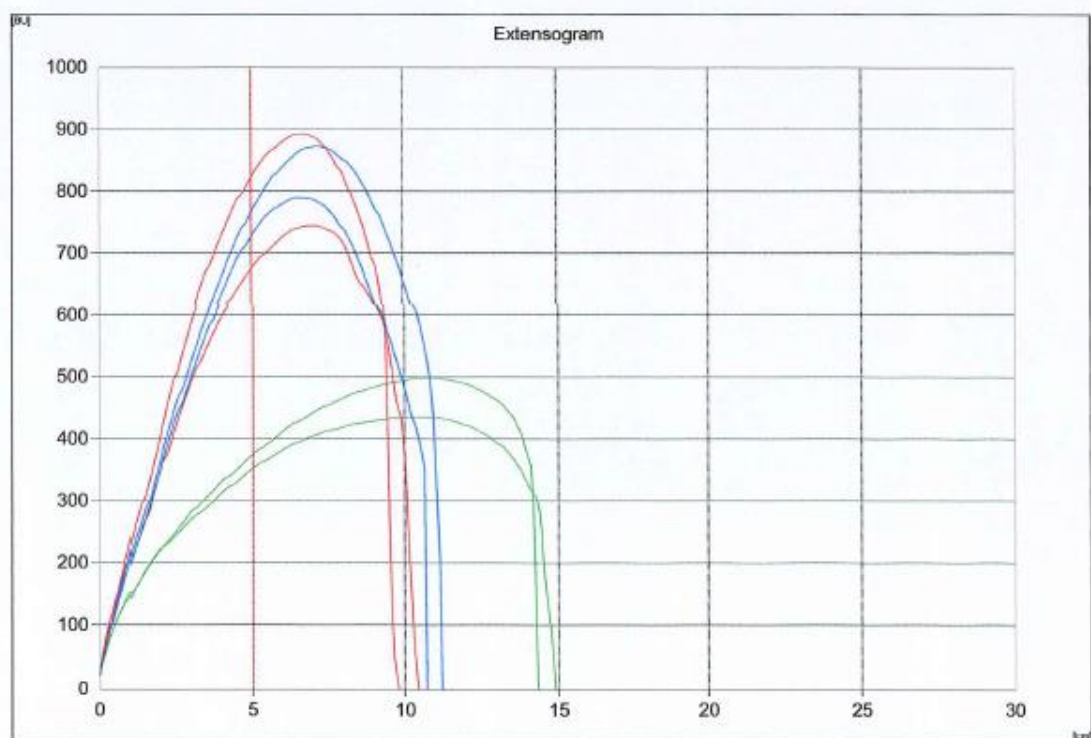


Figure 4.10 Extensogram analysis chart of flour

Table 4.8 Extensogram analysis results of the flour

Time (min)	Dough strength (BU)	Extensibility (mm)	Maximum resistance (BU)	Energy (cm ²)
45	367±5.3 ^a	148±2.6 ^a	468±6.3 ^a	93±1.3 ^a
90	752±4.8 ^b	101±1.2 ^b	820±7.3 ^b	103±3.7 ^b
135	751±3.7 ^b	111±2.5 ^c	832±8.9 ^c	115±3.2 ^c

±: Standard deviation

Means within a column with different superscripts are significantly different ($p < 0.05$)

4.7 Sourdough bread production from first combinations of LAB and yeasts

4.7.1 Determination of LAB and yeasts

W. confusa has been reported in the literature to cause bacteremia and similar diseases (Olano et al., 2001; Fairfax et al., 2014), so this bacterium was not used in the study. *C. paralimentarius* was not used to produce sourdough bread because of weak growth in

carbohydrates tests according to technological properties of LAB isolated from sourdough (as Table 4.5).

While determining the combinations of LAB to be used in bread production, technological features of microorganisms were taken into consideration. LAB and yeasts are grouped according to these biochemical properties in sourdough bread production. For LAB, features such as homofermentative, heterofermentative, gas and acid production growth at 28°C and 1.5% NaCl were taken into consideration in the production of sourdough bread. Properties of yeasts such as producing gas from sugars and growth at 28°C, have been taken into consideration in combination in the production of sourdough bread.

After the identification of LAB and yeasts at the species level, the combinations of LAB and yeasts for use in sourdough bread production were determined according to their technological properties. A total of 12 sourdough breads were produced from 12 sourdough culture combinations as seen Table 4.9. The qualities of the breads were evaluated by making physicochemical, texture and sensory analyzes of the sourdough breads. According to the sourdough bread quality, second culture combinations were made.

Combinations of sourdough cultures were determined for sourdough bread production. LAB and yeasts were selected according to homo or heterofermentative, carbohydrate fermentation properties as given in Table 4.10. *L. brevis*, *L. mesenteroides* subsp. *mesenteroides*, *L. mesenteroides* subsp. *cremoris* were chosen because of gas production, rapid acidification, fermented maltose with heterofermentative characteristics. Especially, *L. brevis* ferment glucose and maltose at a faster rate than *L. plantarum*. *P. acidilactici*, *E. faecalis*, *E. hirae* are homofermentative that were chosen due to form mainly lactic acid during fermentation. Heterofermentative LAB produce high amount of acidic and strong crust flavors on sourdough bread while homofermentative LAB produce softer and sweeter flavor (Valjakka et al., 2003). *Lpb. plantarum*, *L. paraplantarum* and *L. pentosus* were chosen due to facultative heterofermentative characteristics, they degrade hexoses by EMP pathway as homofermentative and *L. plantarum* promotes leavening.

Table 4.9 Combinations of LAB and yeasts used in the sourdough bread production

Sourdough	Sourdough culture combinations	
SD1	G a z i a n t e p	<i>L. brevis</i> + <i>L. paraplantarum</i> + <i>S. cerevisiae</i>
SD2		<i>L. pentosus</i> + <i>L. paraplantarum</i> + <i>P. kudriavzevii</i>
SD3		<i>L. brevis</i> + <i>L. plantarum</i> + <i>E. hirae</i> + <i>W. anomalus</i>
SD4		<i>L. plantarum</i> + <i>P. acidilactici</i> + <i>E. faecalis</i> + <i>C. tropicalis</i> + <i>C. glabrata</i>
SD5	K o n y a	<i>L. brevis</i> + <i>L. pentosus</i> + <i>P. kudriavzevii</i>
SD6		<i>L. plantarum</i> + <i>L. pentosus</i> + <i>G. candidum</i>
SD7		<i>L. brevis</i> + <i>P. acidilactici</i> + <i>E. hirae</i> + <i>Ka. unispora</i> + <i>C. keyfr</i>
SD8		<i>L. brevis</i> + <i>L. pentosus</i> + <i>L. plantarum</i> + <i>E. hirae</i> + <i>P. kudriavzevii</i> + <i>K. unispora</i> + <i>K. marxianus</i>
SD9	M a r d i n	<i>L. brevis</i> + <i>L. mesenteroides</i> subsp. <i>cremoris</i> + <i>S. cerevisiae</i>
SD10		<i>L. plantarum</i> + <i>L. brevis</i> + <i>K. marxianus</i>
SD11		<i>P. acidilactici</i> + <i>L. plantarum</i> + <i>L. mesenteroides</i> subsp. <i>cremoris</i> + <i>K. marxianus</i>
SD12		<i>L. brevis</i> + <i>L. mesenteroides</i> subsp. <i>mesenteroides</i> + <i>P. acidilactici</i> + <i>L. plantarum</i> + <i>S. cerevisiae</i> + <i>K. marxianus</i>

G. candidum and *K. humilis* were not used to produce sourdough bread because of weak growth in carbohydrates tests according to technological properties of yeasts isolated from sourdough (as Table 4.6). The yeast species were seen different gas producing capacities. *S. cerevisiae* ferments glucose, sucrose and maltose and improves gas production. *S. cerevisiae* is effective in removing the amino acids from the dough and have high gas production. *W. anomalus* produce gas high amount of to increase volume of sourdough bread than *P. kudriavzevii*. *K. marxianus* can be used alternative for *S. cerevisiae* in sourdough bread production. In line with this information, *S. cerevisia*, *W. anomalus*, *K. marxianus* and *P. kudriavzevii* are used more than other isolated sourodugh yeasts in the sourdough bread production.

Table 4.10 Selection characteristics of sourdough cultures

Characteristics of sourdough cultures	
LAB	<i>L. brevis</i> is responsible with fermentation of maltose and produces gas and acid. <i>L. paraplantarum</i> produces acid as main product from carbonhydrates (glucose, lactose, sucrose, maltose), but <i>L. brevis</i> does not produce acid and gas from lactose and sucrose. <i>L. pentosus</i> and <i>L. paraplantarum</i> produce acid as main product from glucose, sucrose, maltose and lactose. <i>L. plantarum</i> is facultative heterofermentatif and is selected based on its ability to produce acid from glucose, lactose, sucrose and maltose. <i>E. hirae</i> is selected due to acid production as homofermentative. <i>P. acidilactici</i> ferments glucose and produce lactic acid as main product. <i>E. faecalis</i> produces acid during fermentation. <i>L. pentosus</i> produces acid from carbonhydrates such as glucose, sucrose, maltose and lactose. <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> is selected based on its lactic acid, acetic acid and ethanol production during fermentative. <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> is selected based on its producing lactic acid, acetic acid and ethanol as fermentative end products.
Yeasts	<i>S. cerevisiae</i> is responsible with CO₂ production. <i>P. kudriavzevii</i> is selected due to grow in acidic environment and production of gas. <i>C. tropicalis</i> and <i>C. glabrata</i> ferment maltose to produce CO₂. <i>G. candidum</i> secretes proteases and lipases due to degrade proteins and lipases. <i>Ka. unispora</i> ferments maltose to produce CO₂. <i>C. keyfr</i> is selected due to grow in acidic environment and producing gas. <i>K. marxianus</i> responsible with acid production growing at low and moisture (Dimitrellou et al., 2009). <i>W. anomalus</i> is selected due to fermenting many carbon sources such as glucose, lactose, sucrose and maltose.

4.7.2 Microbiological characteristics of sourdough

LAB (results according to MRSA), MAB (mesophilic aerobic bacteria) and yeast counts were performed in sourdough as given in Figure 4.11. As a result of the 3-day fermentation (back-slopping) of sourdoughs, the number of LAB varied between 6.32 and 8.02 log colony forming unit (cfu)/g. LAB of SD4 sourdough was 7.64 log cfu/g, LAB of SD8 sourdough was 7.48 log kob/g and LAB of SD12 sourdough was 8.02 log cfu/g. In the research, adding higher number of LAB to sourdough resulted in a higher LAB number in sourdough as expected. This situation was found as in the SD4, SD8 and SD12 sourdoughs ($p < 0.05$) compared to other sourdoughs.

As a result of sourdough fermentation, the number of MAB varied between 3.91 and 5.51 log cfu/g. MAB of SD1 sourdough was 5.51 log cfu/g, MAB of SD5 sourdough was 5.03 log kob/g and MAB of SD2 sourdough was 4.95 log cfu/g ($p < 0.05$). The MAB number was found to be lower in sourdoughs in higher LAB used. Lactic acid production by LAB was affected the growth of MAB. The MAB count was higher in the control sourdough due to lower number of LAB. The reason for this was that not enough acid was formed during fermentation.

Yeasts in sourdough were ranged from 5.27 to 6.98 log cfu/g (Figure 4.11). Yeasts of SD4 sourdough were 6.53 log cfu/g, yeasts of SD8 sourdough were 6.78 log cfu/g and yeasts of SD12 sourdough were 6.98 log cfu/g. The LAB and yeast numbers of the sourdoughs were found to be significantly ($p < 0.05$) higher than the control dough. The MAB numbers of the sourdoughs were determined lower than control dough.

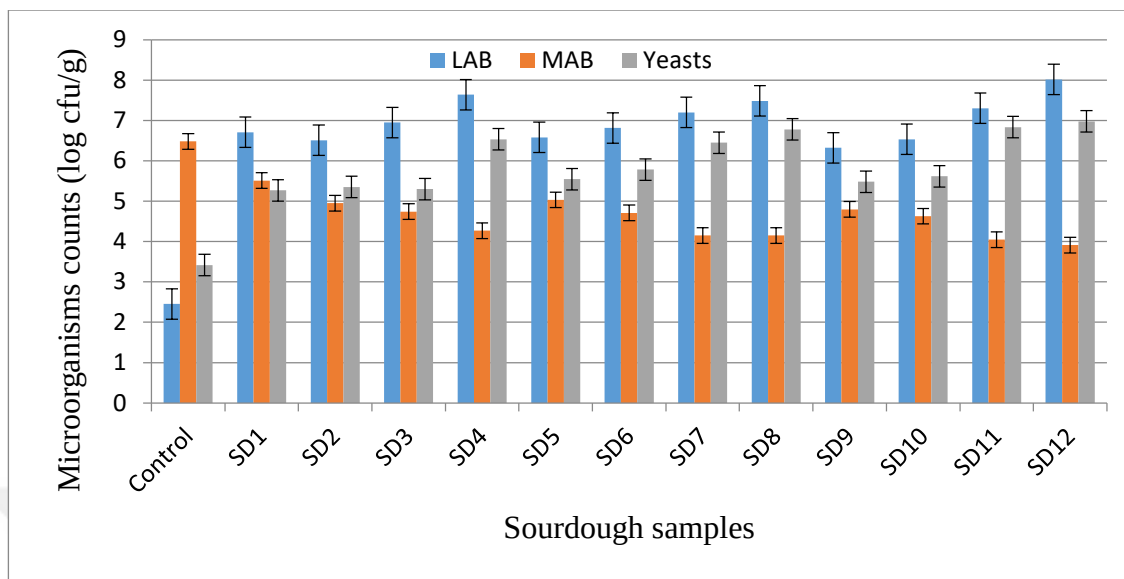


Figure 4.11 Microbiological analysis results of sourdoughs

SD1: *L. brevis*+*L. paraplantarum*+*S. cerevisiae*, SD2: *L. pentosus*+*L. paraplantarum*+*P. kudriavzevii*, SD3: *L. brevis*+*L. plantarum*+*E. hirae*+*W. anomalus*, SD4: *L. plantarum*+*P. acidilactici*+*E. faecalis*+*C. tropicalis*+*C. glabrata*, SD5: *L. brevis*+*L. pentosus*+*P. kudriavzevii*, SD6: *L. plantarum*+*L. pentosus*+*G. candidum*, SD7: *L. brevis*+*P. acidilactici*+*E. hirae*+*K. unispora*+*C. keyfr*, SD8: *L. brevis*+*L. pentosus*+*L. plantarum*+*E. hirae*+*P. kudriavzevii*+*K. unispora*+*K. marxianus*, SD9: *L. brevis*+*L. mesenteroides subsp. cremoris*+*S. cerevisiae*, SD10: *L. plantarum*+*L. brevis*+*K. marxianus*, SD11: *P. acidilactici*+*L. plantarum*+*L. mesenteroides subsp. cremoris*+*K. marxianus*, SD12: *L. brevis*+*L. mesenteroides subsp. mesenteroides*+*P. acidilactici*+*L. plantarum*+*S. cerevisiae*+*K. marxianus*

4.7.3 pH and titratable acidity of sourdough

The pH and titratable acidity values of sourdoughs during fermentation are given in Figure 4.12. During the fermentation periods of sourdough, the pH value varied between 4.05 and 4.80. Low pH was determined in sourdough SD4 (4.08), SD8 (4.10) and SD12 (4.05) where both of heterofermentatives and homofermentatives were used as mixed culture to produce sourdough. These sourdoughs (SD4, SD8 and SD12) were significantly ($p < 0.05$) different from other sourdoughs. Besides, the presence of *S. cerevisiae* in the starter cultures reduces acidification in doughs and breads especially in associations of *S. cerevisiae* and *L. plantarum* or *L. brevis* (Martinez-Anaya, 2003). In present study, these combinations were seen in SD1, SD9 and SD12 sourdoughs. However, SD1 (4.72) and SD9 (4.80) sourdoughs were not determined low pH as SD12

(4.05) sourdough. Because it was that the presence of more homofermentative and facultative heterofermentative bacteria in SD4, SD8 and SD12 sourdoughs. LAB has influenced the pH from producing a significant amount of lactic acid. Homofermentative LAB produce only lactic acid by glucose fermentation while heterofermentative LAB produce ethanol, acetic acid and CO₂ besides lactic acid from carbohydrates (Erkmen and Bozoğlu, 2016). Facultative heterofermentative LAB has an impact on low pH and high acidification would be produced by homofermentative fermentation. The statistical results showed that sourdoughs pH values were significantly lower than control dough ($p < 0.05$). So that, the main change of pH in sourdough is caused by LAB that are homofermentative and facultative heterofermentative as seen in SD4, SD8 and SD12.

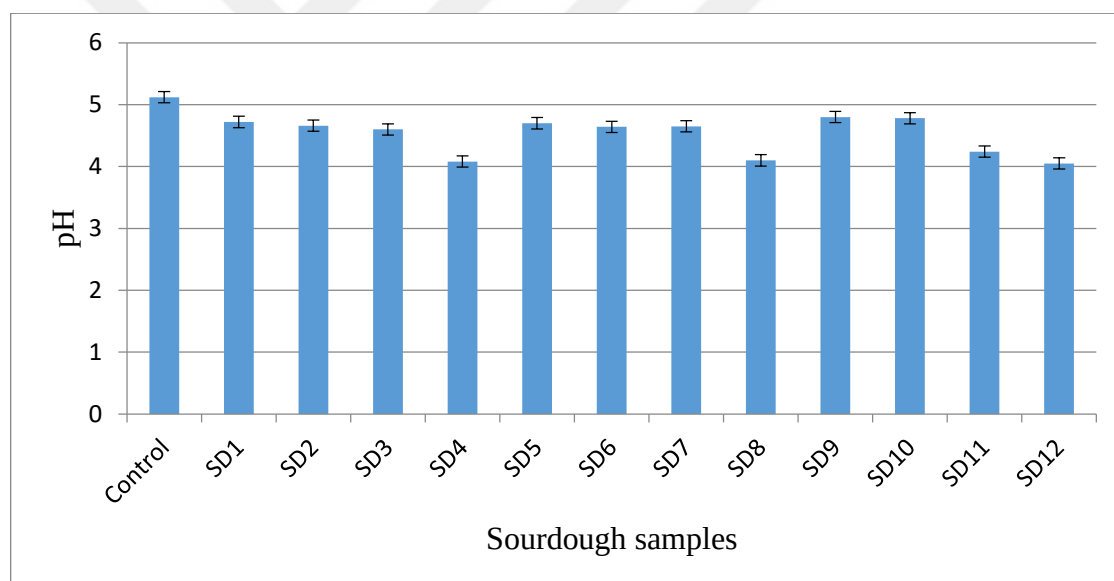


Figure 4.12 pH values of sourdoughs

Titrateable acidity in sourdough varied between 3.00 and 3.50% as seen in Figure 4.13. In the research, titrateable acidity was found lower in control dough than in sourdoughs. As pH increases resulted with decreases in acidity. These results confirmed by TTA as in sourdoughs SD4, SD8 and SD12 (3.45, 3.40 and 3.50%, respectively). They gave the highest levels of TTA as they have low pH. It may be due to the presence of homofermentative and heterofermentative LAB in sourdoughs. The statistical results showed that SD4, SD8 and SD12 TTA values significantly different from control dough

($p < 0.05$). Sourdoughs SD5, SD9 and SD10 were not significantly different from control dough ($p > 0.05$).

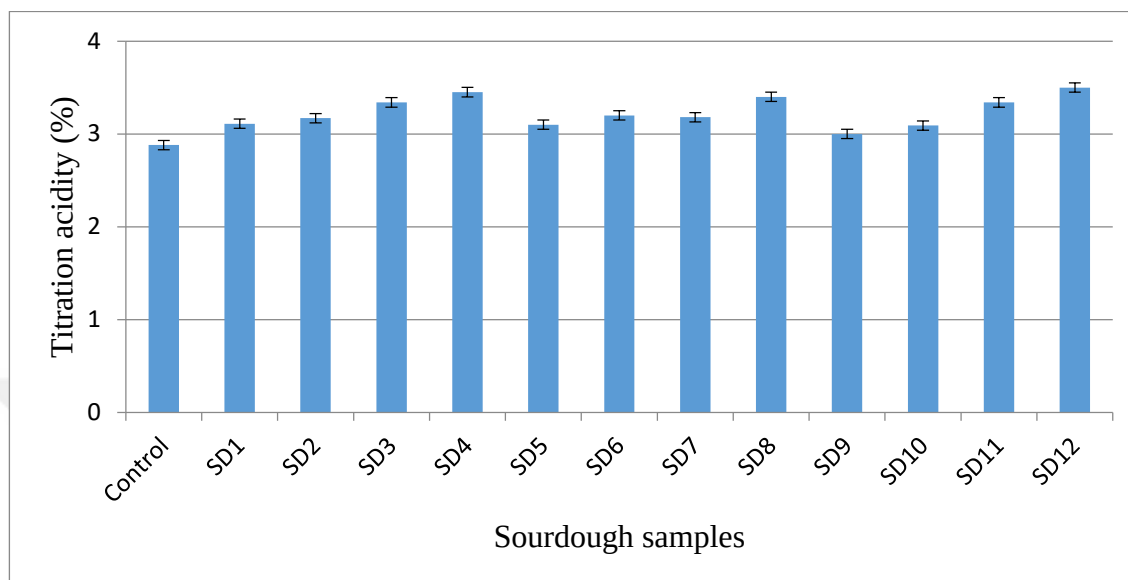


Figure 4.13 Titration acidity values of sourdoughs

*Lactic acid

Volume (dough swelling power) values of sourdoughs during fermentation are given in Figure 4.14. Volume varied between 165.10 and 171.65%. In the research, it was determined that higher volume containing obligate heterofermentative LAB sourdoughs (SD8, SD9 and SD12). Similar results were reported by Valjakka et al. (2003) that obligate heterofermentative LAB (such as *L. brevis*) produces CO_2 outside of yeast and cause noticeable swelling and aroma formation in sourdough. In present study, control dough swelled less than sourdoughs ($p < 0.05$). The reasons that heterofermentative LAB produced gas in addition to yeasts and they convert usable sugars into CO_2 . The highest volume was determined in SD12 sourdough that produced with *S. cerevisiae* and heterofermentative LAB. Sourdoughs that produced in *S. cerevisiae* were determined more volume than other yeasts used in sourdoughs. But, among sourdoughs were not significantly difference ($p > 0.05$). There were significant ($p < 0.05$) difference between sourdough volume and control dough volume, only SD2 (165.10%) sourdough were not significantly ($p > 0.05$) different from control dough. SD9 (170.30%) and SD12 (171.65%) sourdoughs were included high volume values.

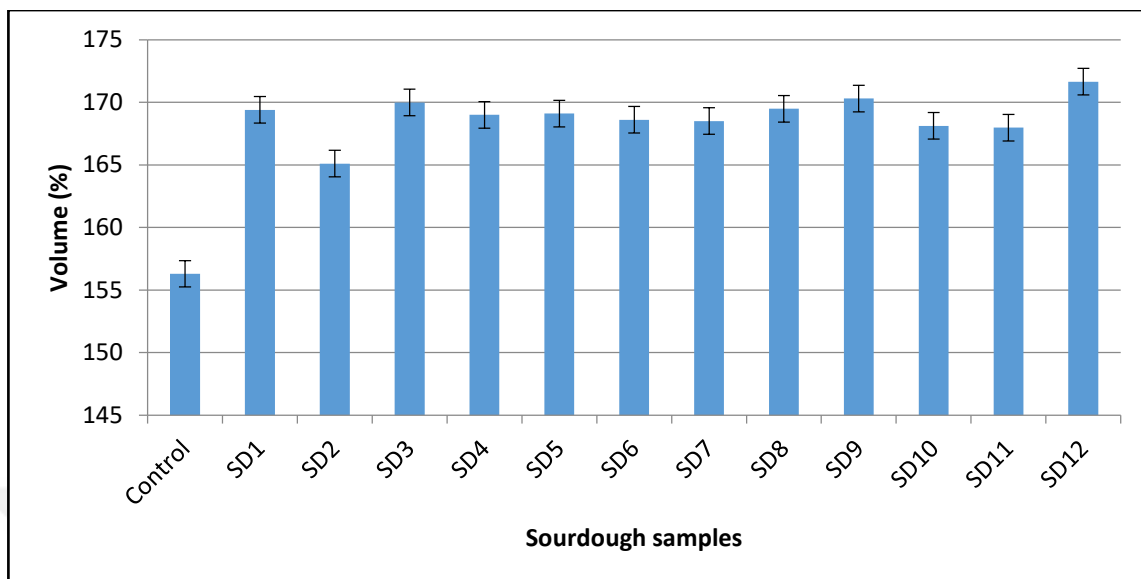


Figure 4.14 Volume changes on the of sourdoughs

4.7.4 pH and titratable acidity of sourdough bread

pH and titratable acidity analysis were made in sourdough bread samples and the results are given in Figure 4.15. The pH of sourdough breads varied between 4.21 and 5.18. It was determined that the breads with the lowest pH were SD4, SD8 and SD12.

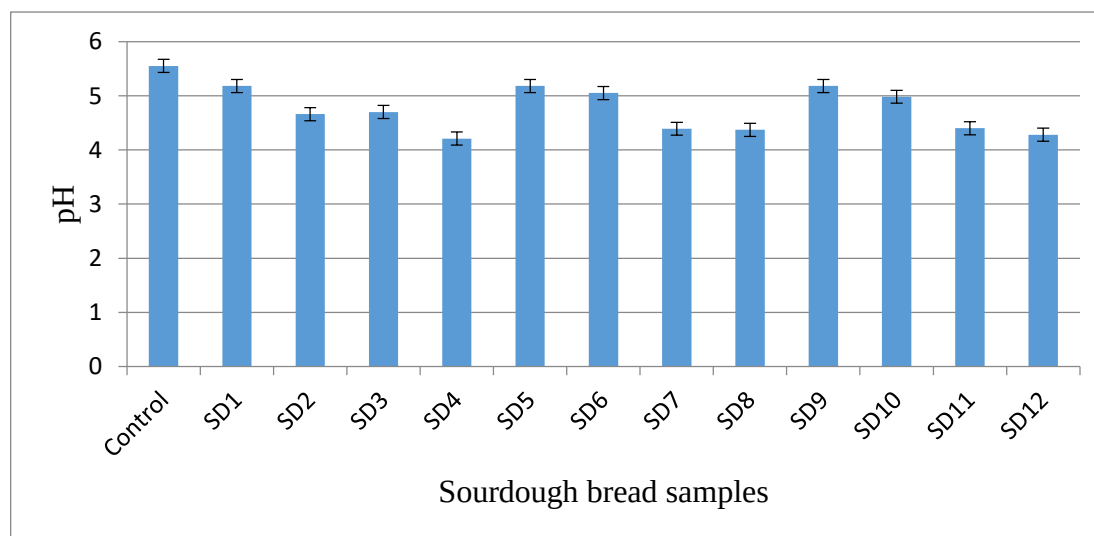


Figure 4.15 pH values of sourdough breads

Titratable acidity of sourdough breads varied between 2.35 and 3.10% as seen in Figure 4.16. The decrease in titratable acidity of sourdough breads related to effect of baking.

Corsetti et al. (1998) indicated similar results and titratable acidity was decreased after baking. In their study, sourdough TTA were between 3.30 and 6.65; breads TTA were between 2.00 and 4.20. The pH of control bread was higher than that of sourdough breads while TTA of control bread was lower than that of sourdough breads. The reasons for this can be stated as the low number of natural LAB contaminations. Lower numbers of LAB were detected in sourdough controls. Sourdough breads pH and TTA were significant different ($p < 0.05$) from control bread. There were no significant ($p > 0.05$) difference between SD1, SD5, SD9 and control bread for TTA.

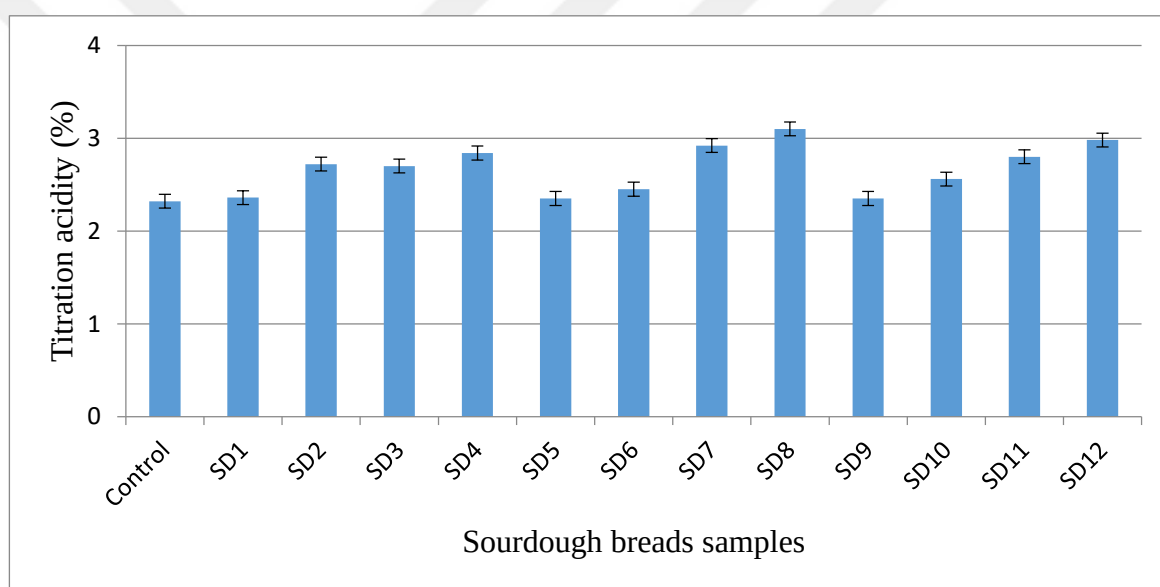


Figure 4.16 Titration acidity values of sourdough breads

4.7.5 Moisture, total dry matter and loaf height of sourdough breads

The moisture values in sourdough breads are given in Figure 4.17. Analyses were made on the 0., 3. and 7. days after the sourdough breads were produced. A decrease in the moisture content of the sourdough breads at 4°C during 7 days of storage was determined. In other words, the moisture content of all sourdough breads decreased during the sourdough bread storage. The moisture content of the breads varied between 21.95 and 30.05% at the end of 7 days storage. The reason for this may have been the removal of water from the sourdough breads in the form of vapor during storage. Because the breads were kept in vapor-proof, unpackaged containers wrapped in thick

cheesecloths. For this reason, the sourdough breads may have low moisture. In the study, it was determined that when heterofermentative and homofermentative bacteria are used together, sourdough bread retains more moisture after 7 days storage. This is because different groups of microorganisms have different metabolic activities. It has been determined that different microorganisms increase the water retention capacity of the sourdough bread structure as they break down proteins and polysaccharides more. After 7 days, SD4 (28.63%), SD7 (28.41%), SD8 (29.74%) and SD12 (30.05%) sourdough breads were significantly ($p<0.05$) different compared to other sourdough breads. These sourdough breads had more water holding capacity during 7 days. It may be about more exopolysaccharides (EPS) that produced LAB and yeasts. EPS increase water retention capacity. The statistical results showed that there were significant ($p<0.05$) difference between sourdoughs and control bread after 7 days. Statistical results showed that there were significant ($p<0.05$) difference among storage days.

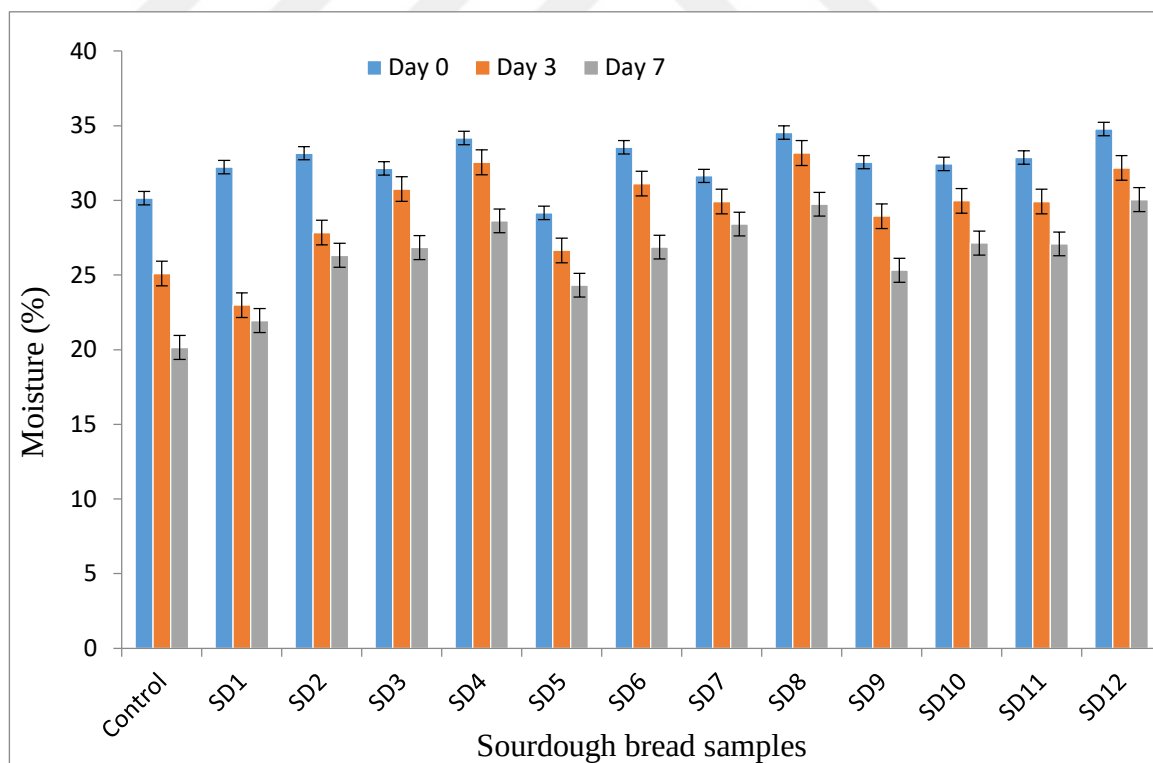


Figure 4.17 Moisture contents of sourdough breads

The total amount of dry matter in breads varied between 69.95 and 78.05% at the end of 7th day as seen in Figure 4.18. The highest dry matter was determined in control bread

(79.85%) and SD1 sourdough bread (78.05%) while the lowest dry matter was determined in SD12 (69.95%) and SD8 sourdough breads (70.26%). The amount of dry matter varied depending on the moisture content. Dry matter content was found to be low as the moisture holding capacity increased (SD8 and SD12). Statistical results showed that there were significant ($p < 0.05$) difference between sourdoughs and control bread. The statistical results showed that there were significant ($p < 0.05$) difference among days with respect to dry matter.

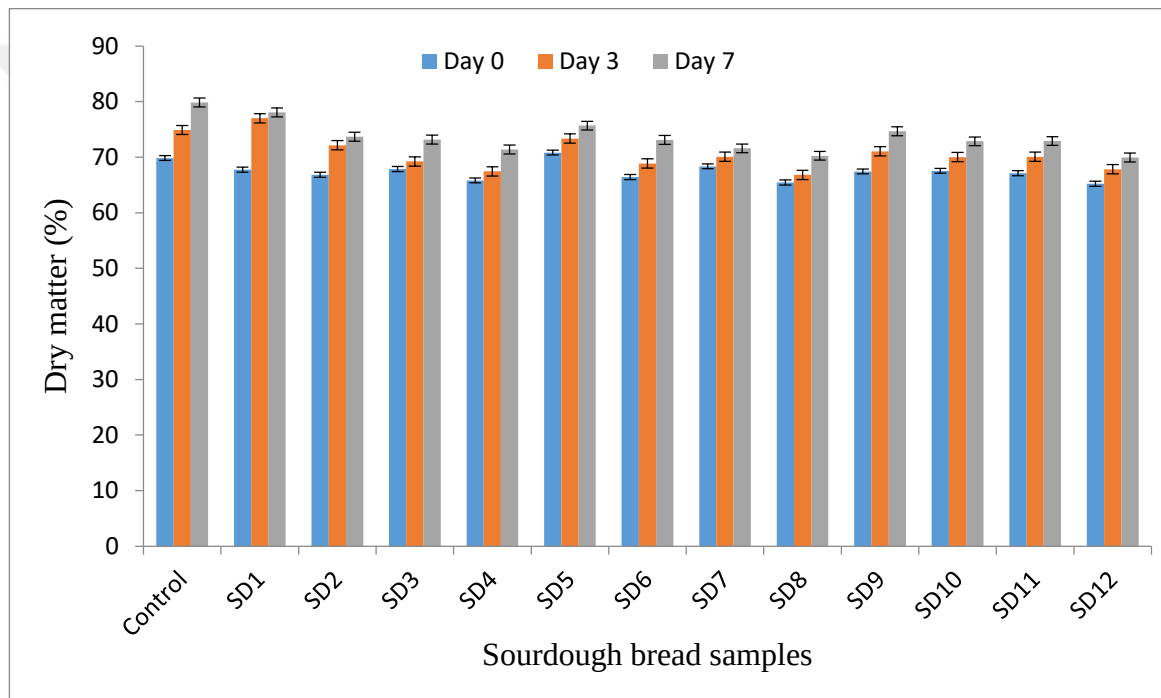


Figure 4.18 Dry matter values of sourdough breads

Loaf height of sourdough breads varied between 6.3 and 7.2 cm as seen Table 4.11. The loaf height was measured based on the highest point of the sourdough bread. The highest loaf height was determined in sourdough breads produced from *S. cerevisiae* and other yeast cultures together with heterofermentative LAB. These were determined on the SD1 (7.0 cm), SD9 (6.9 cm), SD4 (6.9 cm), SD8 (7.1 cm) and SD12 (7.2 cm). In these breads, generally higher height were determined with the heterofermentative LAB produced gas together with yeasts. The results of the research were realized as expected more gas production by using heterofermentative LAB besides yeasts. Control bread was produced using commercial yeast only. The loaf height of control bread has been

determined to be lower than the sourdough breads. Statistical results showed that there were significant ($p<0.05$) difference between sourdough breads and control bread.

Table 4.11 Loaf height values of sourdough breads

	Sourdough bread												
	Control	SD1	SD2	SD3	SD4	SD5	SD6	SD7	SD8	SD9	SD10	SD11	SD12
Height (cm)	6.0±0.03 ^a	7.0±0.08 ^f	6.3±0.05 ^c	6.5±0.05 ^d	6.9±0.06 ^g	6.5±0.04 ^d	6.8±0.07 ^e	6.2±0.04 ^b	7.1±0.07 ^h	6.9±0.07 ^f	6.8±0.06 ^e	6.2±0.03 ^b	7.2±0.08 ⁱ

±: Standard deviation

Means within a row with different small letters indicates significant difference ($p<0.05$)

4.7.6 Crust and crumb color characteristics

Crust color values of breads were determined after baking. Analysis results of L^* , a^* and b^* crust values are given in Figure 4.19. According to these values, bread crust with the highest brightness value (L^*) was found as 47.23 in bread SD6 and the lowest value was found as 30.56 in bread SD2. The redness parameter a^* value was found to be 13.92 as the highest value for SD2 bread, while the lowest value was found as 11.23 for the SD3 bread. For the yellowness indicator b^* value of crust, bread SD2 has the highest value with 21.42, while bread SD6 has the lowest value with 12.45. In the study, it was determined that the L^* values in crust of control breads were not different ($p>0.05$) from SD1, SD4, SD5, SD7, SD8, SD9 and SD10 sourdough breads. There was no difference ($p>0.05$) between control bread and SD4 sourdough breads for a^* values. There was no difference ($p>0.05$) between control bread and SD6 sourdough breads for b^* values. Other sourdough breads were significantly ($p<0.05$) different from each other for L^* , a^* and b^* values.



Figure 4.19 Crust color values of sourdough breads

Analysis results of L*, a* and b* crumb values are given in Figure 4.20. According to the crumb color of the bread, the highest L* value was determined as 60.36 for the bread SD10 and the lowest L* value was determined as 47.26 for the SD2 bread. The redness parameter a* value was found to be 6.48 as the highest for the SD1 bread, while the lowest value was found as 3.45 the SD7 bread. The yellowness indicator b* value of crumb, bread SD6 has the highest value with 19.97, while bread SD12 has the lowest value with 15.95. In the study, it was determined that the crust values of control breads were not different from SD1, SD4, SD7, SD11 and SD12 sourdough breads for L* values ($p>0.05$). There were no difference ($p>0.05$) among control bread and SD1 and SD3 sourdough breads for a* values. There were no difference ($p>0.05$) among control bread and SD1, SD3, SD5, SD8 and SD10 sourdough breads for b* values. Among other sourdough breads, there were significant difference ($p<0.05$) for L*, a* and b* values.

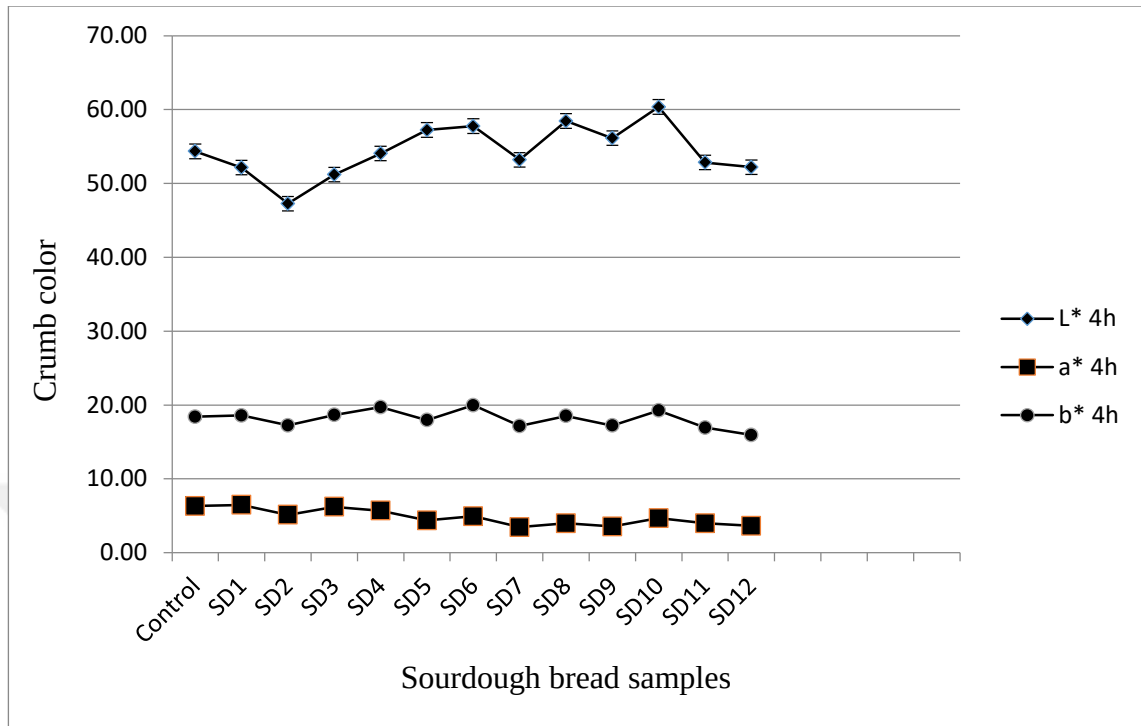


Figure 4.20 Crumb color values of sourdough breads

4.7.7 Volume and specific volume of sourdough breads

The volume of sourdough bread is the space on the sourdough bread. There should be an ideal relationship between sourdough bread weight and volume. In other words, excessive or insufficient volume indicates low quality (Bircan et al., 2017). The volume and specific volume were determined from the sourdough breads are given in Table 4.12. The highest volume in sourdough bread was found on SD4, SD8 and SD12. The highest volume of bread is 1370 cm³ on SD12 and 1360 cm³ on SD8 and the lowest volume obtained for SD2 sourdough bread as 1240 cm³. In the study, more volume from breads were obtained by using LAB and yeasts.

The specific volume was found by dividing the sourdough bread volume with the weight of the sourdough bread. The highest and lowest specific volumes were determined on SD12 (4.35 cm³/g) and SD2 (3.99 cm³/g). Statistical results showed that there were significant difference ($p < 0.05$) between control bread and sourdough breads with respect to volume. There were no differences ($p > 0.05$) among SD2, SD3, SD4, SD7 sourdough and control bread with respect to specific volume while there was significant difference ($p < 0.05$) between control bread and other sourdough breads. It may be oven spring that

is expansion of dough in the oven. It is related to production of CO₂ and vaporization of ethanol and water during heating in the baking. So that, air cells in dough expands (Khalid et al., 2017).

Table 4.12 Results of volume and specific volume of sourdough breads

Bread	Volume (cm³)	Specific volume (cm³/g)
Control	1210±24.89 ^a	3.87±0.1 ^a
SD1	1320±83.76 ^{cd}	4.27±0.4 ^c
SD2	1240±33.17 ^b	3.99±0.6 ^{abc}
SD3	1250±48.45 ^{bc}	4.03±1.0 ^{abc}
SD4	1270±33.17 ^b	4.06±1.1 ^{abc}
SD5	1250±47.12 ^{bc}	4.01±1.9 ^{bc}
SD6	1260±33.17 ^{bc}	4.05±1.0 ^{bc}
SD7	1270±64.77 ^b	4.08±0.6 ^{ab}
SD8	1360±56.55 ^d	4.34±0.1 ^c
SD9	1300±47.29 ^{bc}	4.15±0.8 ^{bc}
SD10	1265±18.71 ^{bcd}	4.04±0.4 ^{bc}
SD11	1275±56.68 ^{bc}	4.10±0.2 ^{bc}
SD12	1370±78.47 ^d	4.35±0.1 ^c

± : Standard deviation

Means within a column with different small letters indicates significant difference ($p < 0.05$)

4.7.8 Texture characteristics of sourdough breads

The hardness, cohesiveness, springiness, gumminess and chewiness values were obtained from the texture analysis of sourdough breads at the 4th and 24th hours. Hardness can be defined as the force required for physical deformation and sensory for compressing the material between the anterior teeth. In the calculation, it can be expressed as the peak power (height of the first curve) during the first compression cycle (Gerçekaslan et al., 2007). The increase in hardness in bread is usually associated with staling (Elgün and Ertugay, 2002). Analysis results of hardness values at the 4th and 24th hours are given in Figure 4.21. In the research, an increase in the hardness of the bread was observed at the end of the 24th hours compared to 4th hours in sourdough breads. The hardness values of sourdough breads varied from 1070.4 to 2130.4 g after 4

hours. After 24th hours, the sourdough breads with the lowest hardness were determined as on SD8 and SD12. The highest hardness was found in SD2, SD3 and SD5 sourdough breads. Statistical results showed that there were significant ($p<0.05$) difference among sourdough breads and control bread.

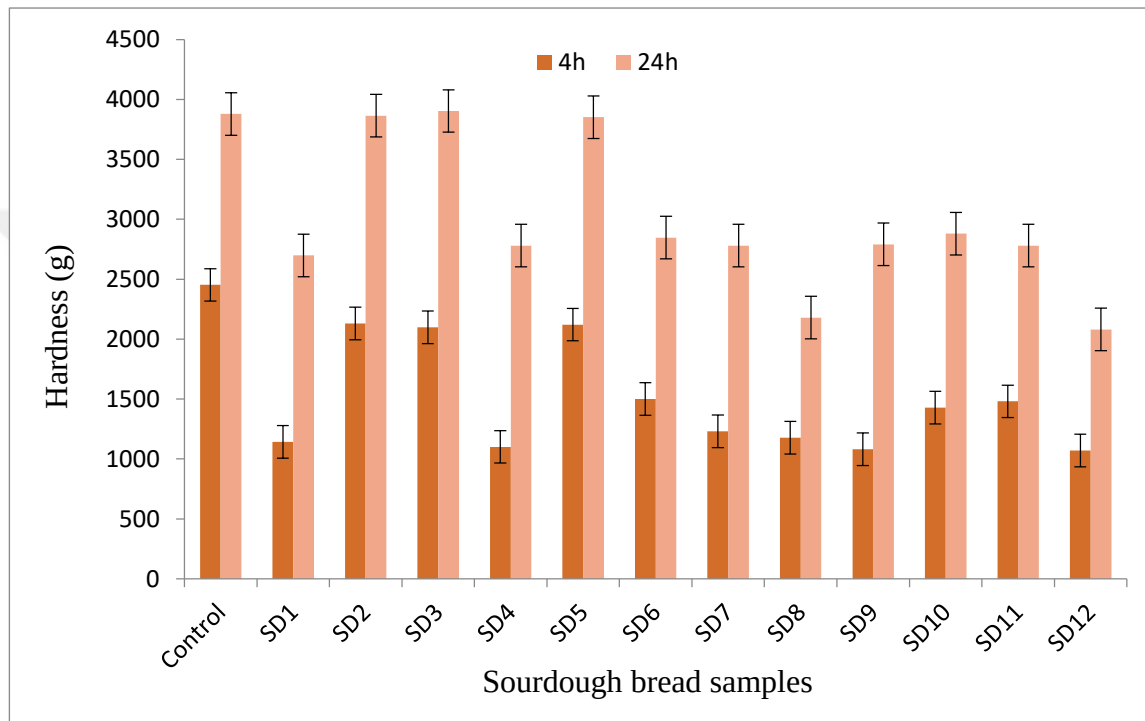


Figure 4.21 Hardness results of sourdough breads

Cohesiveness indicates the ability of the material to deform before its internal structure deteriorates. The results of sourdough breads are shown in Figure 4.22. The cohesiveness values of sourdough breads after 24th hours varied between 0.53 and 0.73. In the research, decrease in the cohesiveness of the bread was observed at the end of the 24th hour compared to after the 4th hours. After 24th hours, the breads with the lowest cohesiveness were determined for SD7 and SD11. The highest cohesiveness was found in sourdough bread SD2. However, statistical results showed that there were no significant different among SD2, SD3, SD4, SD9 and SD11 sourdough breads and control bread after 4 hours ($p>0.05$) while there were significant difference ($p<0.05$) among other sourdough breads. There were no significant difference ($p>0.05$) between

SD2 sourdough bread and control bread after 24 hours while there were significant difference ($p<0.05$) with of the sourdough breads.

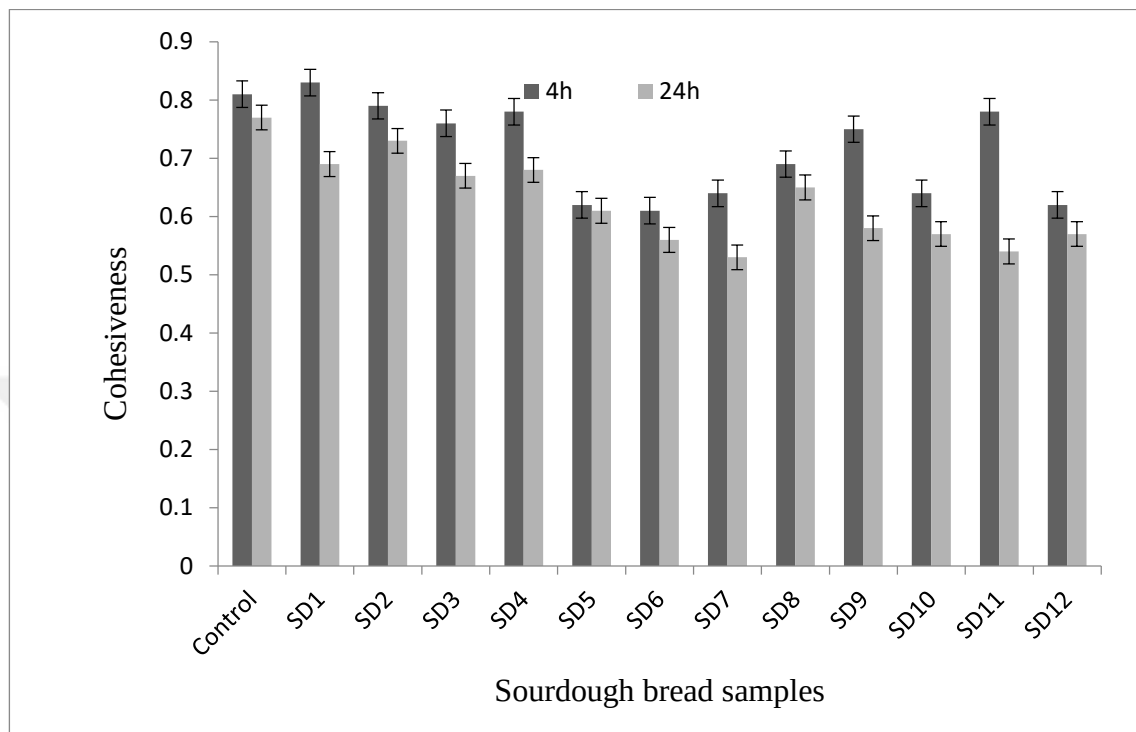


Figure 4.22 Cohesiveness results of sourdough breads

Springiness refers to the rate at which a deformed product returns to its undeformed state when the deforming force is removed, while chewing is the energy required to chew solid food to make it ready for swallowing and it is related to hardness and chewiness (Eriksson et al., 2014). The results of breads are given in Figure 4.23. There was statistically significant difference ($p<0.05$) between sourdough breads springiness after 4th and 24th hours. The springiness of sourdough breads varied from 0.78 to 0.90 after 4 hours while it was determined from 0.70 to 0.82 after 24th hours. The porous and elastic texture effected in sourdough bread with the presence of insoluble gluten in flour and a surfactant protein globulin (Bhol and Bosco, 2014).

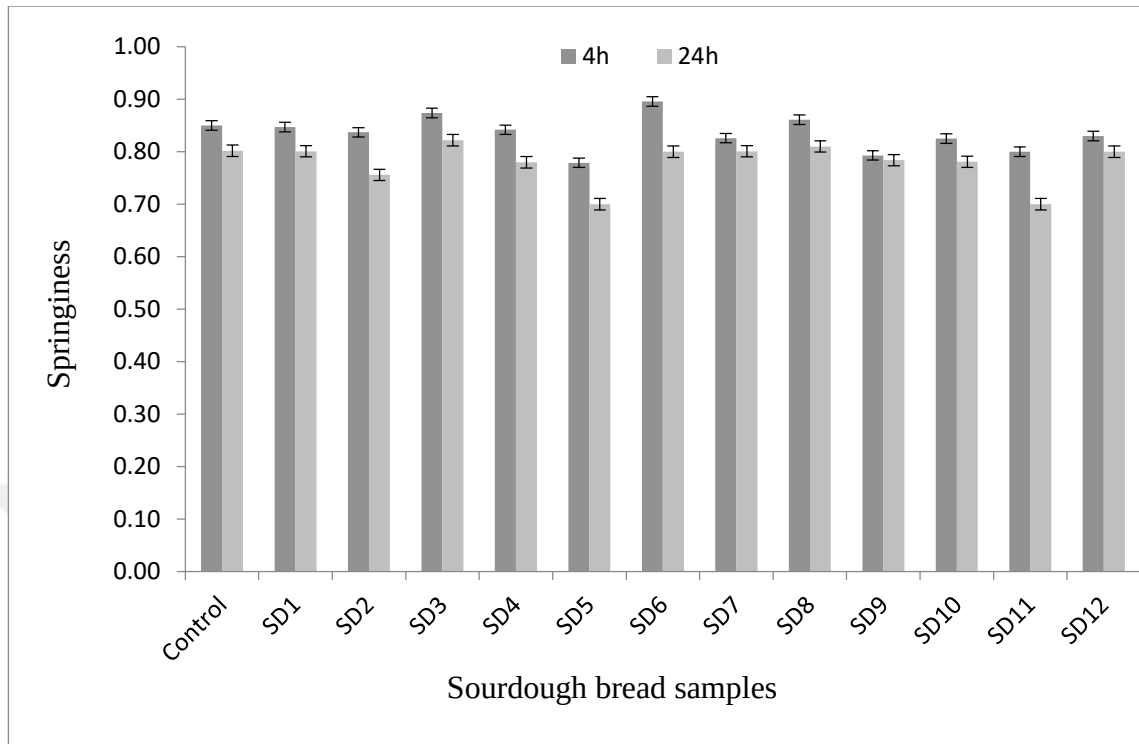


Figure 4.23 Springiness results of sourdough breads

Gumminess is expressed as the effect of hardness and stickiness values (Gerçekaslan et al., 2007). The results obtained in the study were determined to increase the chewing gum value of sourdough breads with the extension of the storage period as seen Figure 4.24. The increase in the gummy value increased the hardness value of the breads. With the use of heterofermentative LAB, a decrease in the hardness of the breads was observed during storage and the gumminess values also decreased. The lowest gummy was determined in SD4, SD8 and SD12 breads after 4th and 24th hours. The highest gummy was determined SD2, SD3 and SD5 sourdough breads. It was found that the gumminess value of the control bread was significantly higher ($p < 0.05$) than sourdough breads.

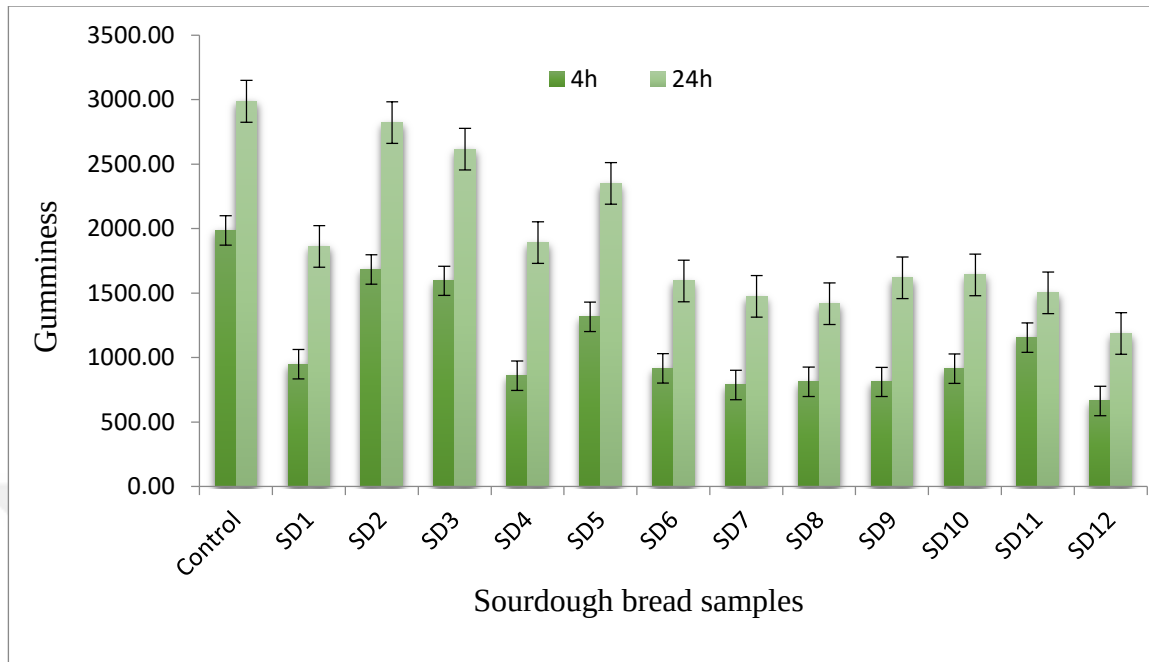


Figure 4.24 Gumminess results of sourdough breads

The results of chewiness are given in Figure 4.25. Chewiness is defined as the energy required to physically break down solid bread and bring it into swallowing state while it can be defined as the number of chewing required sensorially until the substance is ready to be swallowed and the speed and strength of chewing per second. In the calculation of chewiness, it is expressed as the value obtained by multiplying the hardness, stickiness and flexibility values (Gerçekaslan et al., 2007). The chewiness values were determined between 550.83 and 1408.68 g after 4 hours while it was determined between 948.71 and 2144.85 after 24 hours. The statistical results showed that there were significant difference ($p < 0.05$) among sourdough breads from control bread.

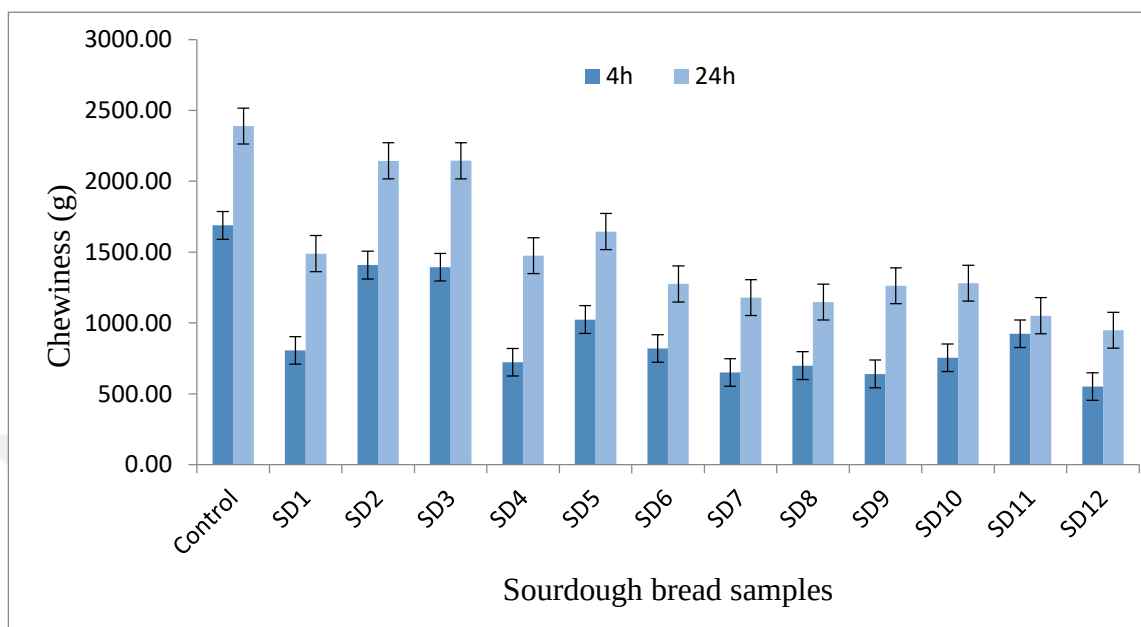


Figure 4.25 Chewiness results of sourdough breads

4.7.9 Determination of calorimetric properties of breads

Values belonging to the 0th, 3rd and 7th days obtained from the thermal analysis results are given in Appendix B (Table B.9). The enthalpy value (ΔH) of the sourdough breads were indicated as calorimetric properties. It indicates the enthalpy changes in the gelatinization value of bread. In the study, endothermic transition thermograms were seen in all bread samples.

As the storage period increases, enthalpy value of breads also increased. The enthalpy value of the DSC graphics indicates the amount of energy measuring during the gelatinization transition. Enthalpy values varied from 80.5 to 103.7 on day 0, from 89.7-187.4 on day 3 and from 165.7 to 230.3 on day 7 as seen in Figure 4.26. Increased ΔH value indicates increased recrystallization of amylopectin, increased enthalpy value during storage indicates staling of bread. In the research, enthalpy values of breads were increased. However, this value differences were observed with combinations of LAB and yeasts. In other words, the use of more LAB varieties resulted in less enthalpy value and less stale. There was significant difference ($p < 0.05$) among sourdough breads.

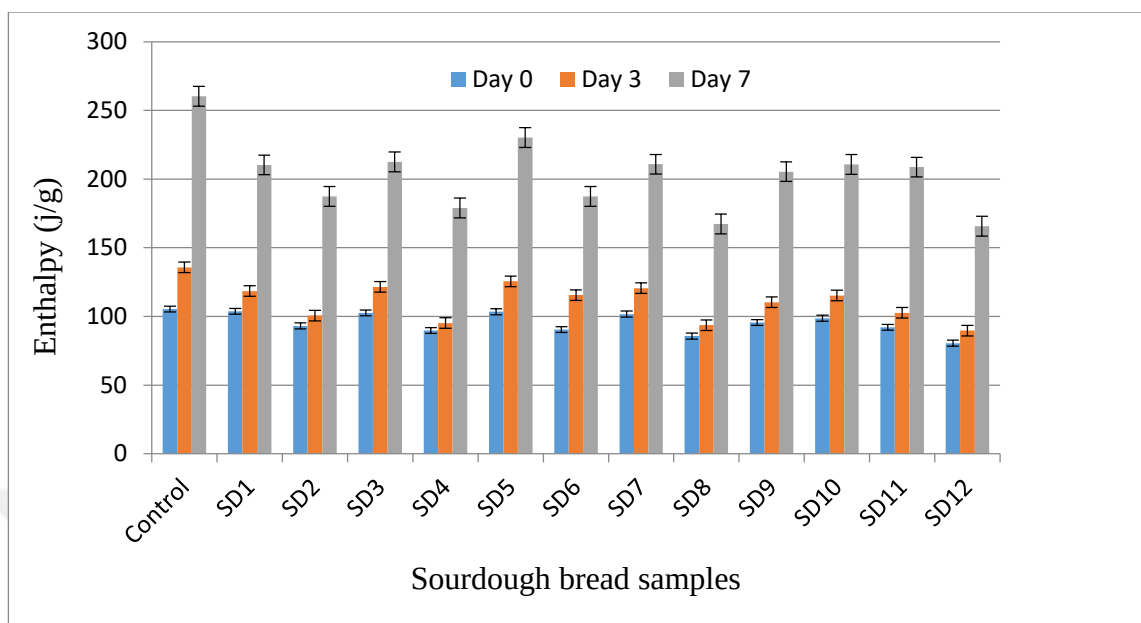


Figure 4.26 Enthalphy results on sourdough breads

4.7.10 Sensory analysis of sourdough breads

Sensory properties of sourdough breads are given in Table 4.13. After the bread was baked, an analysis was made to determine the sensory properties of the panelists consisting of 10 people at the 4th and 24th hours and 7th day of storage. The breads were sliced and grading was requested for the external and internal characteristics of the bread. Although the results of the sensory properties of the breads at the 4th, 24th hour and the 7th day were parallel, there was a decrease in taste with the increasing storage period. As a result of the sensory test, breads SD4, SD8 and SD12 were the most preferred breads.

In the research, SD4, SD8 and SD12 sourdough breads were different ($p < 0.05$) from other sourdough breads according to the panelist evaluations after 4th and 24th hours and the 7th day. These sourdough breads were more preferred because it was related to sour taste, high volume, good chewability and swallowability, low hardness, little moisture loss. In the study, while sensory value of SD6 and SD7 were not different ($p > 0.05$) from control bread, other breads differ ($p < 0.05$) from control bread after 24th hour and 7th day.

Table 4.13 Sensory score scale of 12 sourdough breads

SD bread	4h	24h	7d
Control	35±2.87 ^{aA}	30±1.69 ^{aB}	28±2.95 ^{abC}
SD1	61±5.03 ^{fA}	51±3.02 ^{cB}	42±3.71 ^{efC}
SD2	53±4.47 ^{deA}	42±3.25 ^{bB}	38±2.10 ^{deC}
SD3	51±3.62 ^{cdA}	42±3.10 ^{bB}	36±3.11 ^{cdC}
SD4	79±6.05 ^{gA}	68±3.87 ^{dB}	60±3.56 ^{hC}
SD5	58±2.59 ^{efA}	53±4.37 ^{cB}	43±3.57 ^{fC}
SD6	46±3.65 ^{bcA}	35±3.38 ^{aB}	31±4.42 ^{bC}
SD7	65±3.52 ^{fA}	63±3.02 ^{dB}	52±3.42 ^{gC}
SD8	77±4.26 ^{gA}	65±7.30 ^{dB}	57±3.43 ^{hC}
SD9	58±3.72 ^{efA}	45±3.05 ^{bB}	32±2.14 ^{bcC}
SD10	60±4.86 ^{fA}	52±3.71 ^{cB}	48±3.46 ^{gC}
SD11	63±3.91 ^{fA}	55±3.32 ^{cB}	50±3.06 ^{gC}
SD12	80±4.44 ^{gA}	70±4.27 ^{dB}	62±3.68 ^{hC}

Evaluation was made on 10 panelists, d=day 4th= 4 hour, 24th = 24 hour, 7d= 7 day SD: Sourdough
 ±: Standard deviation

Means within column small letters indicate significantly different ($p<0.05$)

In the rows, different capitilized letters represent significant ($p<0.05$) sensory value among sourdough breads.

4.8 Sourdough bread production of second combinations

Following cultures were selected from first sourdough bread depending on sourdough bread characteristics. Good quality (chemical, sensory and texture) LAB and yeasts were used in combination. In the study, the panelist evaluations of SD4, SD8 and SD12 sourdough breads indicate that the use of more LAB and yeast species caused statistically difference among sourdough breads ($p<0.05$). These breads gained more acclaim. For these reasons, cultures were selected according to these properties of breads. All types of cultures were used in combinations because quality of bread is better due to the use of more cultures. In second combinations, it has been tried to choose those that form acid and gas by using especially two sugars (glucose and maltose). Because the important feature of sourdough is that it creates enough acid besides gas. pH, which directly affects the fermentation rate of the dough, is an important technological criterion. One of the important issues limiting the use of sourdough in bread production is the slow acid production or slow metabolic activities of LAB. Considering this

negative situation, selection was made according to parameters such as rapid decrease in pH and rapid increase in acidity value of the LAB used in the study. In the study, it was determined by the panelists that better quality bread was produced their LAB and yeasts combinations were used (combinations from SD4, SD8 and SD12). In combinations, especially by using maltose in addition to glucose, it has gained more appreciation with acid and gas formation. With these combinations, second group sourdough breads were produced.

The second culture combinations (5 combinations) were given in Table 4.14. They were used in the production of bread (5 types of bread). The qualities of the sourdough breads were evaluated by making physicochemical, texture, and sensory analyzes.

Table 4.14 Combinations of LAB and yeasts used in the second sourdough bread production

Sourdough	Sourdough culture combinations
SD13	<i>L. brevis</i> + <i>L. plantarum</i> + <i>L. paraplantarum</i> + <i>P. kudriavzevii</i> + <i>W. anomalus</i>
SD14	<i>L. plantarum</i> + <i>P. acidilactici</i> + <i>E. faecalis</i> + <i>C. tropicalis</i> + <i>C. glabrata</i> + <i>K. unispora</i>
SD15	<i>L. brevis</i> + <i>L. mesenteroides</i> subsp. <i>cremoris</i> + <i>S. cerevisiae</i> + <i>K. unispora</i>
SD16	<i>P. acidilactici</i> + <i>L. plantarum</i> + <i>L. mesenteroides</i> subsp. <i>mesenteroides</i> + <i>K. marxianus</i>
SD17	<i>L. brevis</i> + <i>L. mesenteroides</i> subsp. <i>mesenteroides</i> + <i>E. hirae</i> + <i>L. plantarum</i> + <i>P. acidilactici</i> + <i>L. pentosus</i> + <i>S. cerevisiae</i> + <i>K. marxianus</i>

Selection of second sourdough bread cultures (Table 4.14) were determined according to 12 sourdough breads results in Table 4.15. It can be seen combinations of homofermentative and heterofermentative LAB are significant because of synergistic effect. *C. paralimentarius*, *K. humilis*, *G. candidum* and *W. confusa* did not used in sourdough bread production in these groups as seen in the first combination. *K. humilis*, *G. candidum* and *C. paralimentarius* growth weak in culture medium. *G. candidum* and *C. keyfr* did not used to produce second combination breads because of sourdough breads were not preferred by panelist.

Table 4.15 Selection characteristics of second sourdough cultures

Sourdough	Selection characteristics of sourdough cultures
LAB	<i>L. brevis</i> is responsible for fermented maltose and produce gas. <i>L. plantarum</i> produces well acid from glucose, sucrose, maltose and lactose. <i>P. acidilactici</i> is fermented glucose to produce mainly lactic acid. <i>E. faecalis</i> is selected due to acidity the dough and grow during fermentation. <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> is selected based on its producing lactic acid, acetic acid and ethanol as fermentative end products. <i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> is selected based on its producing lactic acid, acetic acid and ethanol as fermentative end products. <i>E. hirae</i> is selected due to acidity the dough and grow during fermentation. <i>L. pentosus</i> did not use to produce sourdough bread for second combinations.
Yeasts	<i>P. kudriavzevii</i> is selected due to grow in acidic environment and produce gas. <i>W. anomalus</i> is selected due to ferment many carbon sources. <i>K. unispora</i> , <i>C. tropicalis</i> and <i>C. glabrata</i> ferment maltose to produce CO ₂ . <i>S. cerevisiae</i> is responsible for CO ₂ production to have better bread volume. <i>K. marxianus</i> is responsible for lower pH and moisture loss during storage to extension of shelf life according to study in literature (Dimitrellou et. al., 2009). <i>G. candidum</i> and <i>C. keyfr</i> did not use to produce sourdough bread for second combinations.

4.8.1 Microbiological properties of sourdough

The microbiological results of the sourdoughs obtained by the second combination of cultures in the study are given in Figure 4.27. LAB counts varied between 6.58 and 9.12 log cfu/g, MAB counts varied between 4.10 and 5.16 log cfu/g and yeast counts ranged from 5.89 to 7.88 log cfu/g. Statistical results showed that there were significant difference ($p < 0.05$) between sourdoughs and control dough. The reason for the high MAB number was due to the low numbers LAB and the high pH value compared to

higher number of LAB. It can be said that the reason for the higher number of MAB in the control dough is due to the low number as LAB that will compete with the microflora of the dough. In the study, It was determined that the LAB and yeast growth in the second sourdoughs were higher than produced in the first combinations. Accordingly, the number of MAB was found to be lower in the sourdough breads produced from second combinations than first combinations.

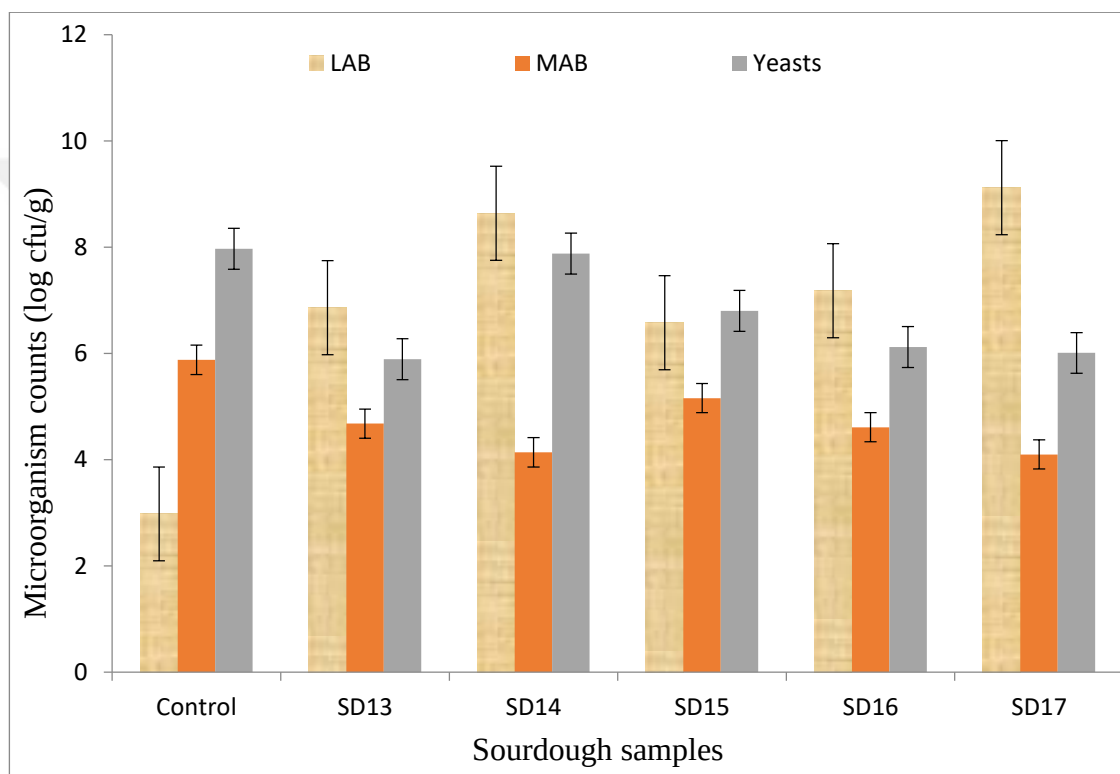


Figure 4.27 Microbiological results of sourdoughs produced in the second combinations

SD13; *L. brevis*+*L. plantarum*+*L. paraplantarum*+*P. kudriavzevii*+*W. anomalus*, SD14; *L. plantarum*+*P. acidilactici*+*E. faecalis*+*K. unispora*+*C. tropicalis*+*C. glabrata*, SD15; *L. brevis*+*L. mesenteroides* subsp. *cremoris*+*S. cerevisiae*+*K. unispora*, SD16; *P. acidilactici*+*L. plantarum*+*L. mesenteroides* subsp. *mesenteroides*+*K. marxianus*, SD17; *L. brevis*+*L. mesenteroides* subsp. *mesenteroides*+*E. hirae*+*L. plantarum*+*P. acidilactici*+*L. pentosus*+*S. cerevisiae*+*K. marxianus*

4.8.2 pH and titratable acidity of sourdough in second combinations

pH results of the sourdoughs produced with the second combinations are given in Figure 4.28. In the study, it was determined that the pH values of sourdoughs varied between 3.43 and 4.15. It was determined that the pH value of the control dough was higher

(5.62) than sourdoughs. It was found that the pH value of the sourdoughs were significantly difference ($p < 0.05$) from the pH of the control dough.

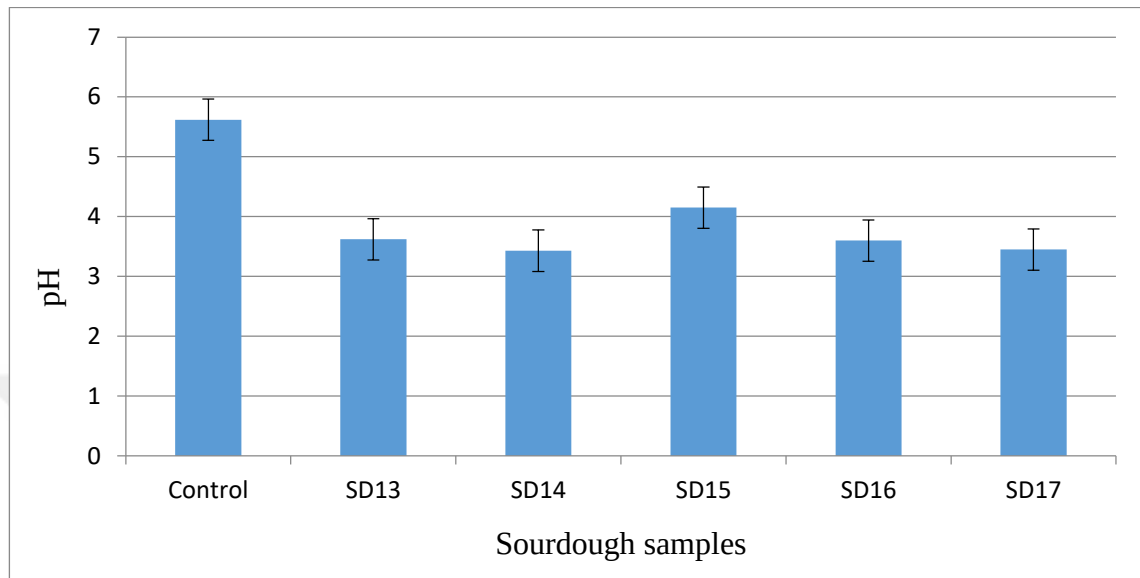


Figure 4.28 pH results of sourdoughs produced with second combinations

TTA values of sourdough varied between 3.25 and 3.95 as seen in Figure 4.29. The least TTA value was observed in sourdough SD15 and the most titratable acidity was determined in sourdough SD17. The statistical results showed that there was significant difference ($p < 0.05$) between sourdoughs and control dough TTA.

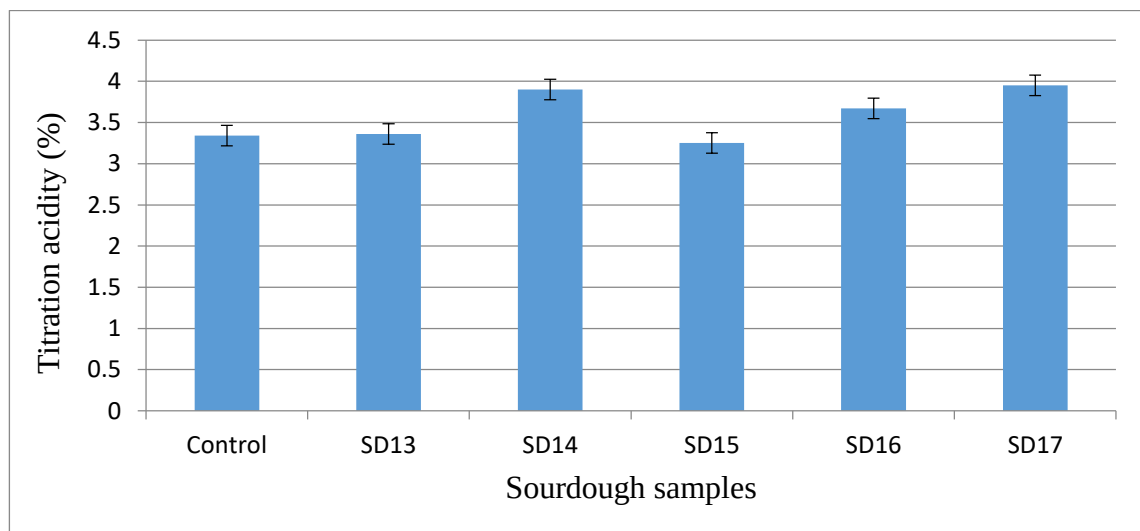


Figure 4.29 Titration acidity results of sourdoughs produced with second combinations

Volume (dough swelling power) results of sourdoughs produced with second combinations are given in Figure 4.30. Control dough volume was less than sourdough volumes ($p<0.05$). A little more sourdough volume was detected in the SD13 and SD15 than others but as statistical analysis, there was no difference ($p>0.05$) between SD13 and SD15. Among the sourdoughs, the highest volume ($p<0.05$) was determined in SD17 sourdough. The content of more heterofermentative LAB species and multiple initial LAB species may have been caused higher volume increase due to heterofermentative LAB, since that can produce additional gas together with yeasts which resulted in a high volume content of sourdough.

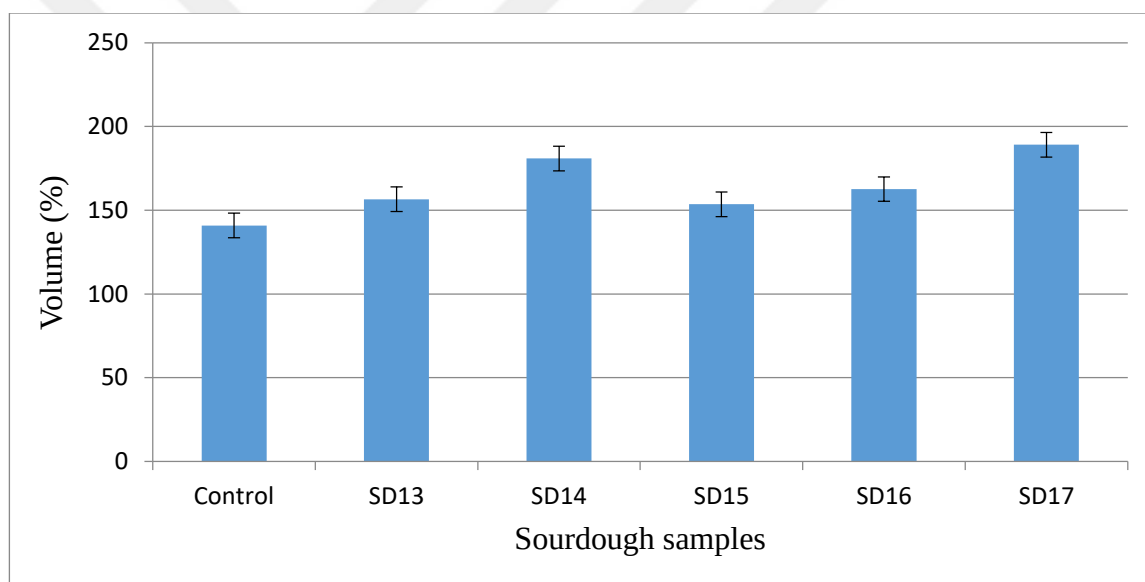


Figure 4.30 Volume results of sourdoughs produced with second combinations

4.8.3 pH and titration acidity of sourdough bread in second combinations

The pH values of sourdough breads obtained with the second combination cultures in the study are given in Figure 4.31. In the study, it was determined that the pH values of sourdough breads varied between 3.70 and 4.32. It was determined that the sourdough breads with the lowest pH were SD14 and SD17. The pH value of the sourdoughs was found to be significantly lower than the control dough pH ($p<0.05$).



Figure 4.31 pH of sourdough breads produced with second combinations

The TTA values of sourdough breads varied between 2.34 and 3.21 as seen in Figure 4.32. Statistical results showed that titratable acidity values were significantly different from control ($p < 0.05$). More homofermentative LAB were used together in the combination, so a greater increase in acidity was observed. The pH of control bread was higher than that of sourdough breads while TTA of control bread was lower than that of sourdough breads. Sourdough breads were significantly different ($p < 0.05$) from control bread with respect to pH and TTA. There was no difference ($p > 0.05$) between SD15 and control bread for TTA statistical analysis.

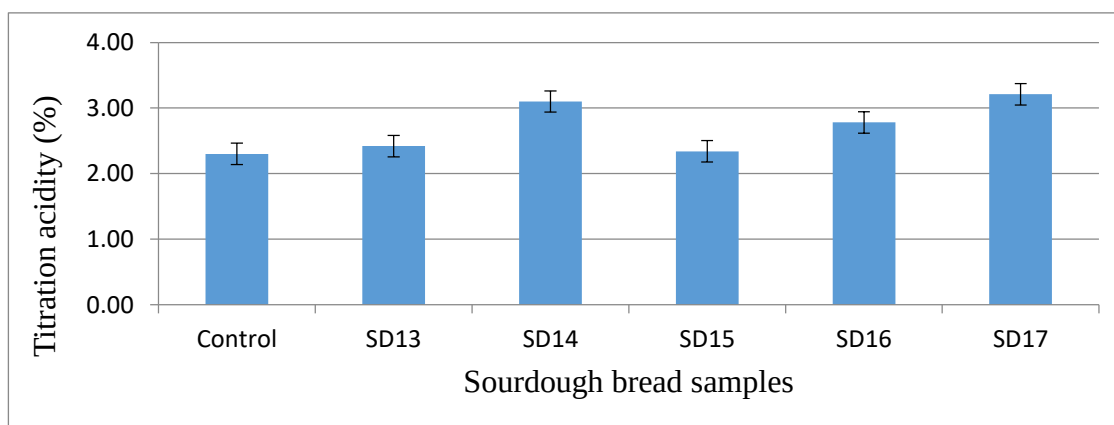


Figure 4.32 Titration acidity of sourdough breads produced with second combinations

4.8.4 Moisture, total dry matter and loaf height in second combinations

Moisture, total dry matter and loaf height analysis results in sourdough breads obtained with the second combination cultures in the study are given in Figure 4.33. Moisture content of sourdough breads varied between 27.89 and 31.67% after 7 days. It was determined that sourdough breads contained more moisture than control breads ($p < 0.05$), but there were no significant difference ($p > 0.05$) between SD14, SD16 and SD17 sourdough breads depending on storage times. This was due to more hydrolysis of organic compounds in sourdough bread. Complex organic compounds in sourdoughs are more hydrolyzed and this was increased water holding capacity of organic compounds (Erkmen and Bozoglu, 2016).

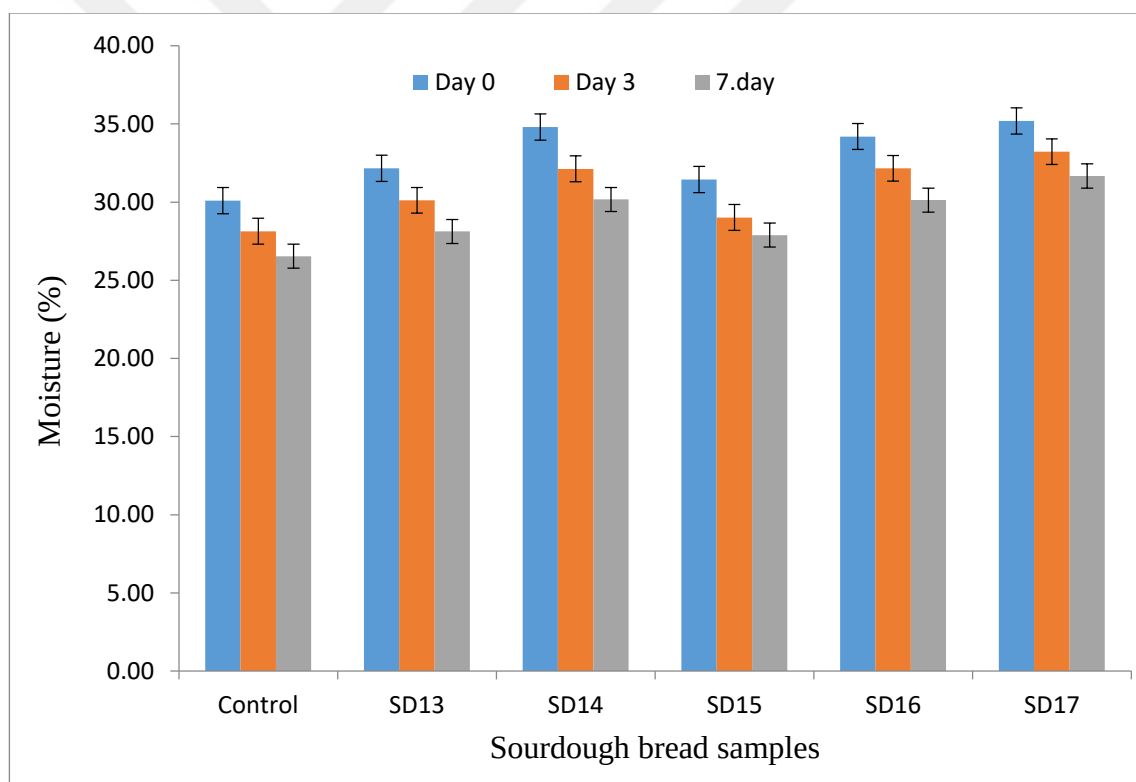


Figure 4.33 Moisture of sourdough breads produced with second combinations

There was small difference between the dry matter values of the sourdough breads as seen in Figure 4.34. The total dry matter values of sourdough breads varied between 68.33 and 72.11% after 7 days. There was no statistically significant difference ($p > 0.05$) in total dry matter content compared with control bread, but SD17 was significantly different ($p < 0.05$) from control bread and other sourdough breads at 0 day. There was no

significant difference ($p>0.05$) among sourdough breads on the 3rd and 7th days. Besides, according to statistical results, there were significant differences ($p<0.05$) between days in to the dry matter values of sourdough bread.

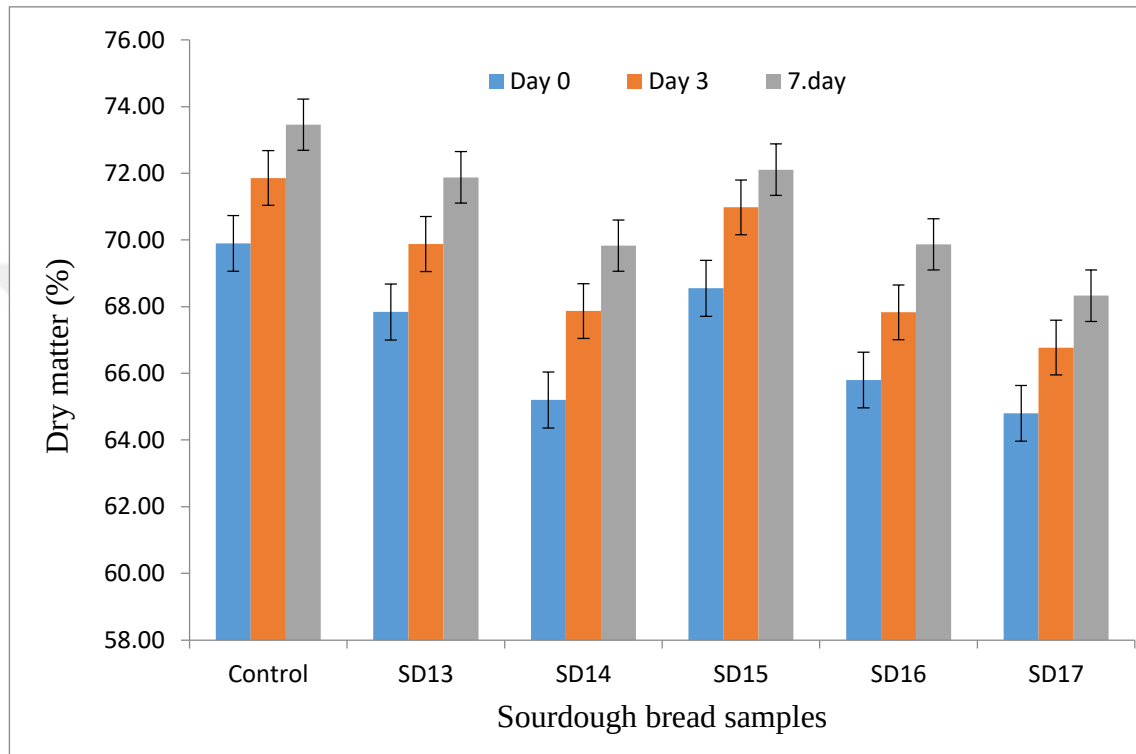


Figure 4.34 Dry matter of sourdough breads produced with second combinations

In the study, the loaf height of sourdough breads varied between 6.5 and 7.3 cm as seen in Table 4.16. It was determined that the loaf height of the breads produced using combination of LAB and yeasts was significantly higher ($p<0.05$). It was observed that the loaf height of the control bread was less than that of the sourdough breads. The loaf height was higher ($p<0.05$) in SD15 and SD17 than the others. It has been shown that more CO_2 production leads to increased loaf height because the higher number of homofermentative and heterofermentative LAB was used in the production of these sourdough breads than the others and the number of yeast species is also higher.

Table 4.16 Loaf height of sourdough breads produced from second combinations

Sourdough bread	Control	SD13	SD14	SD15	SD16	SD17
Height (cm)	6.1±0.06 ^a	6.8±0.09 ^c	6.5±0.08 ^b	7.2±0.10 ^e	7.0±0.12 ^d	7.3±0.13 ^e

±: Standard deviation

Means within a row with different small letters indicates significant different ($p < 0.05$)

4.8.5 Crust and crumb color characteristics in second combinations

Crust properties of bread; normal shell color ranges from dark golden-brown (tops) to light golden-brown (sides) (Bircan et al., 2017). L^* value indicates on a vertical axis with values from 0 (black) to 100 (white). The $+a^*$ and $-a^*$ values indicate red and green component of a color, respectively. The yellow and blue components are represented on the $+b^*$ and $-b^*$ axis (Ly et al., 2020). The results of crust color analysis of the sourdough breads produced with the second combinations are given in Figure 4.35. L^* crust values of breads varied between 36.40 and 42.40. a^* values of sourdough breads varied between 11.16 and 14.94. b^* values of sourdough breads varied between 14.86 and 18.94. The control sourdough bread L^* , a^* and b^* crust color values were determined as 43.50, 13.12 and 15.62, respectively. SD14 and SD17 has a lower L^* values and higher a^* and b^* values. This means that SD14 and SD17 were darker than other sourdoughs. It may be related to some maillard reaction product (MRP) can be found in the crumb and likely come from crust-to-crumb transfer. Because the internal temperature of the dough and the hydration rate are not suitable for starting the maillard reaction, very little MRP is formed in the crumb (Troade et al., 2022). There was no difference ($p > 0.05$) in crust color L^* values compared to the control bread depending on the culture use but SD17 sourdough bread was different ($p < 0.05$) from other sourdough breads. There was no difference ($p > 0.05$) in crust color a^* values between control bread and sourdough breads. For b^* values, there was difference ($p < 0.05$) between sourdough breads and control bread but there was no difference ($p > 0.05$) among SD13, SD14, SD15, SD16 sourdough breads.

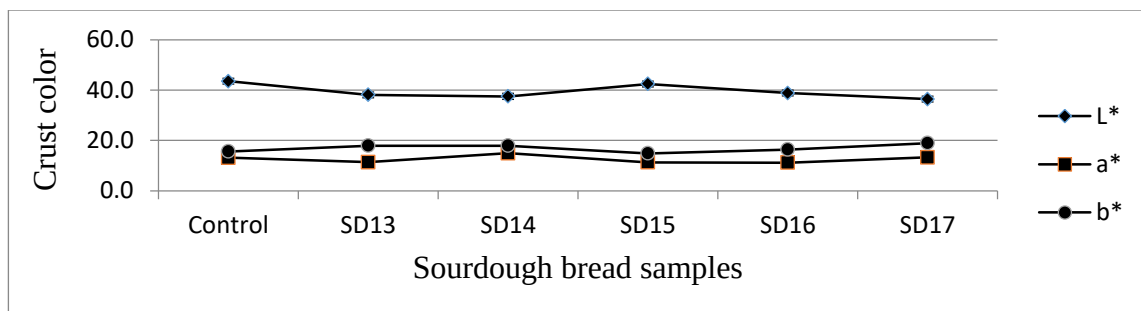


Figure 4.35 Crust color values of sourdough breads produced from second combinations

The results of crumb color analysis of the sourdough breads produced with the second combinations are given in Figure 4.36. L* crumb values of breads varied between 52.60 and 58.30. a* crumb values of breads varied between 3.58 and 5.43. The results of b* crumb color analysis of the sourdough breads produced with the second combinations are ranged from 15.41 to 20.93. There was no difference ($p>0.05$) in L* values compared to the control bread except SD14, SD15 and SD17 sourdough breads ($p<0.05$). There was no significant difference ($p<0.05$) between sourdough breads and control bread for a* values. There was no difference ($p>0.05$) between SD15, SD16 and SD17 sourdough breads for b* values.

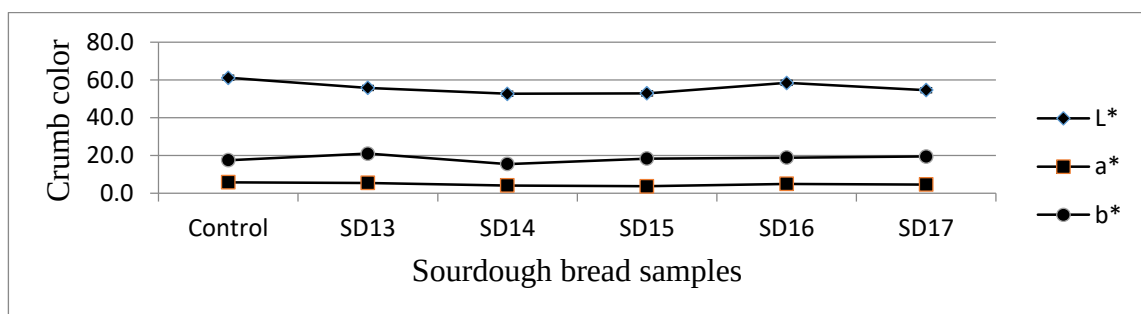


Figure 4.36 Crumb color values of sourdough breads produced from second combinations

4.8.6 Volume and specific volume of sourdough breads from second combinations

Volume and specific volume of sourdough breads produced with the second combinations are given in Table 4.17. The volumes of the sourdough breads varied between 1355 and 1390 cm³, while the volume of the control bread was 1220 cm³. Specific volume results of sourdough breads were determined between 4.31 and 4.47

cm³/g. The volume and specific volumes were higher on SD17 (1390 cm³ and 4.47 cm³/g) while they were lower, respectively (1355 cm³ and 4.31 cm³/g) in SD15. These results indicate that LAB in sourdough breads produce CO₂ apart from yeasts. The statistical results showed that there was significant difference ($p<0.05$) among sourdough breads, but between control bread and SD15 were no difference ($p>0.05$) for volume values. For specific volume, there was difference ($p<0.05$) between sourdough breads and control bread except SD13 and SD15. No difference ($p>0.05$) was determined among the volumes and specific volumes of sourdough breads.

Table 4.17 Volume and specific volume results of bread produced with the second combinations

Sourdough bread	Volume (cm ³)	Specific volume (cm ³ /g)
Control	1220±21.25 ^a	3.90±0.5 ^a
SD13	1360±41.68 ^b	4.37±0.8 ^{ab}
SD14	1380±39.19 ^{cd}	4.43±0.4 ^b
SD15	1355±18.24 ^a	4.31±0.4 ^{ab}
SD16	1375±49.12 ^c	4.39±0.5 ^b
SD17	1390±31.83 ^d	4.47±0.6 ^b

±: Standard deviation

Means within a column with different small letters are significantly different ($p<0.05$)

4.8.7 Texture characteristics in second combinations

The hardness analysis results of sourdough breads produced with the second combinations are given in Figure 4.37. The hardness of the sourdough breads changed between 1120.5 and 1360.7 g and 1980.7 and 2789.5 g after 4th and 24th hours, respectively. Statistical results showed that there was significant difference ($p<0.05$) among sourdough breads for 4th and 24th hours. Research results showed that the hardness values of sourdough breads numbered SD14 and SD17 were lower than the other sourdough breads. The hardness of the sourdough breads increased during storage and it was determined that this increase was significant ($p<0.05$).

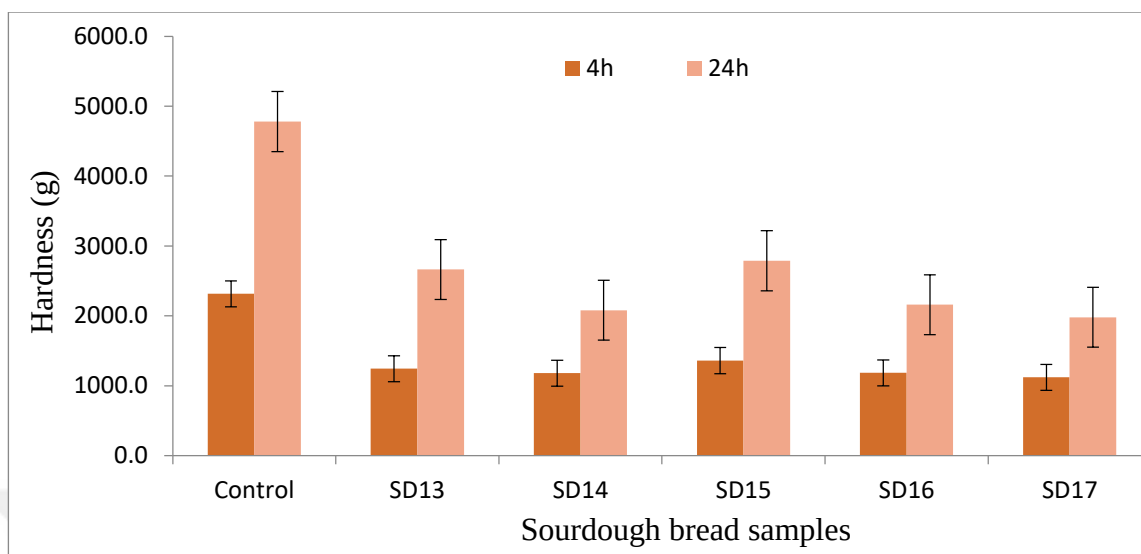


Figure 4.37 Hardness results of sourdough breads produced with second combinations

Cohesiveness results of sourdough breads from the second LAB and yeast combinations are given in Figure 4.38. The cohesiveness values varied from 0.65 to 0.78 and from 0.60 to 0.73 for 4th and 24th hours, respectively. No significant difference ($p > 0.05$) was observed between the cohesiveness values of the sourdough breads, but only SD17 sourdough bread was different ($p < 0.05$) from other sourdough breads for 4th and 24th hours.

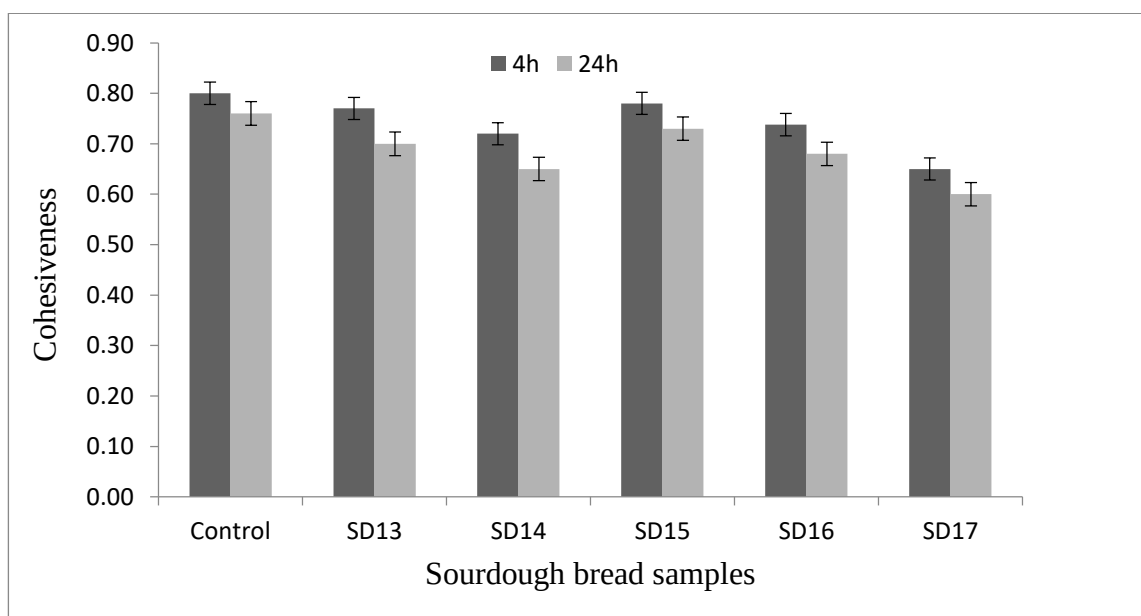


Figure 4.38 Cohesiveness results of sourdough breads from the second combinations

Springiness values of sourdough breads after 4th and 24th hours varied from 0.72 to 0.83 and from 0.73 to 0.81 as seen in Figure 4.39, respectively. No significant differences ($p>0.05$) were determined among springiness values of the breads during storage. Sourdough breads have softened during storage, because they lose moisture from the inside to or because the bread crust gets moisture from the outside, the breads become soft. There was difference ($p<0.05$) between springiness values of 4th and 24th hours.

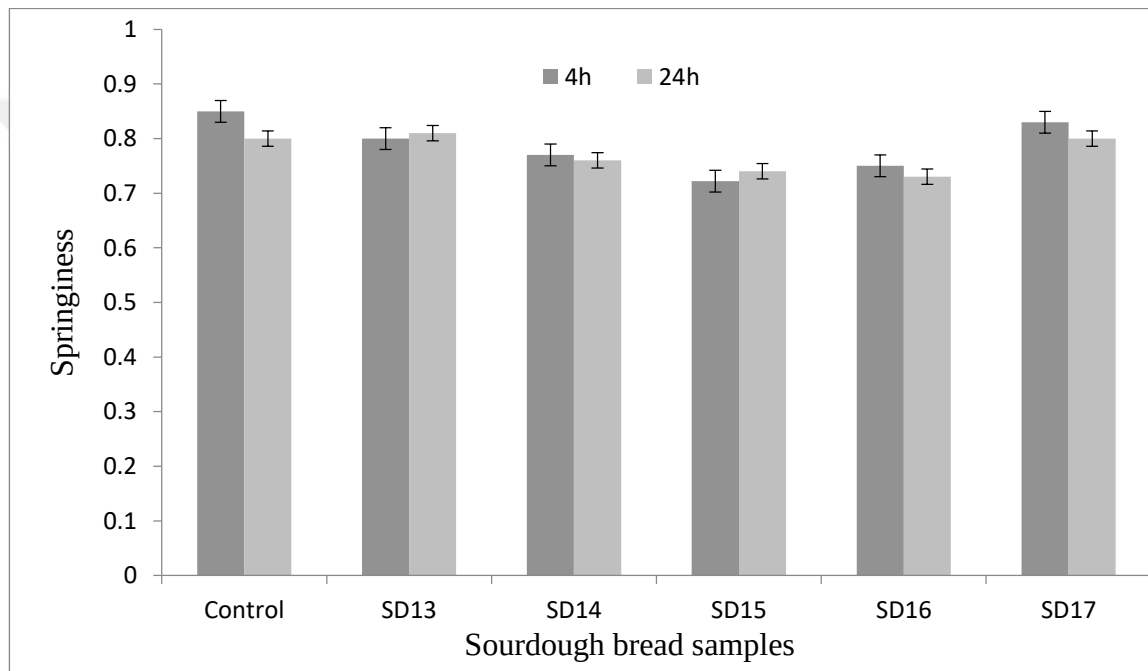


Figure 4.39 Springiness results of sourdough breads produced with second combinations

Gumminess results of sourdough breads produced with second combinations are given in Figure 4.40. Gumminess values of sourdough breads after 4th and 24th hours varied from 728.33 to 1061.35 g and from 1188.42 to 2036.34 g, respectively. The gumminess of sourdough breads increased during storage. The use of more than one culture species decreased the gumminess value. There was significant difference ($p<0.05$) among sourdough breads and control bread. There was a significant difference ($p<0.05$) between 4th and 24th hours.



Figure 4.40 Gumminess results of sourdough breads produced with second combinations

Chewiness results of sourdough breads produced with second combinations are given in Figure 4.41. Chewiness values of sourdough breads after 4th and 24th hours varied between from 604.51 to 767.10 g and from 950.74 to 1510.09 g, respectively. The chewiness value of the control bread was significantly higher ($p < 0.05$) than the sourdough breads. The chewiness values of sourdough breads increased during storage. There was significant difference ($p < 0.05$) between 4th and 24th days sourdough breads chewiness values.

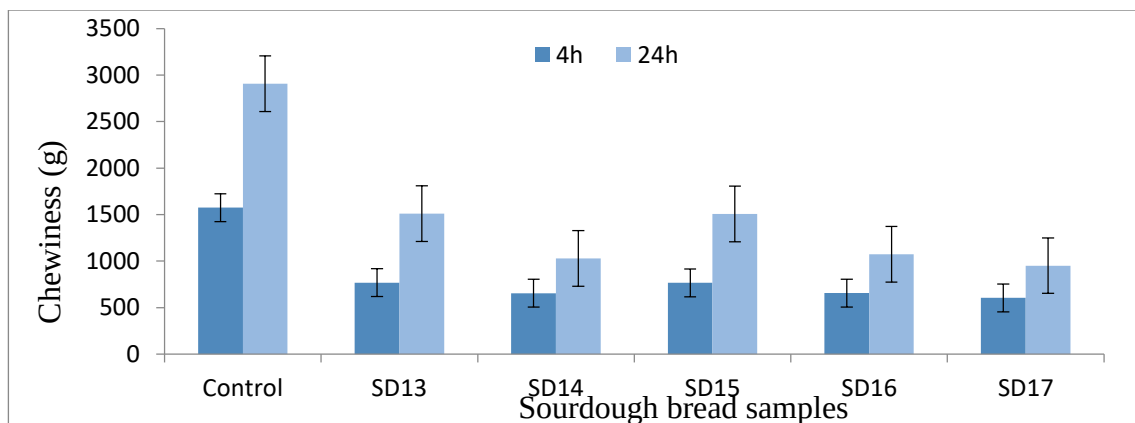


Figure 4.41 Chewiness results of sourdough breads produced with second combinations

4.8.8 DSC analysis results of sourdough breads for second combinations

Enthalpy results from DSC thermal analysis of sourdough breads produced with the second combinations are given in Figure 4.42. The enthalpy values varied from 75.6 to 109.7 on day 0, from 87.5 to 113.6 on day 3 and from 145.8 to 243.1 on the day 7. The enthalpy value of the sourdough breads were given as calorimetric properties. As the storage time increased, the enthalpy values increased. The increase in enthalpy (ΔH) values in sourdough breads indicates that the recrystallization of amylopectin is increased, that is, the increase in enthalpy value during storage indicates staling. Enthalpy values increased depending on the storage time as seen in first combinations. The enthalpy values of sourdough breads were lower than the control bread ($p < 0.05$). The enthalpy values were found to be lower in the second group combinations, compared to the results obtained from the first combinations with the use of more LAB and yeast species.

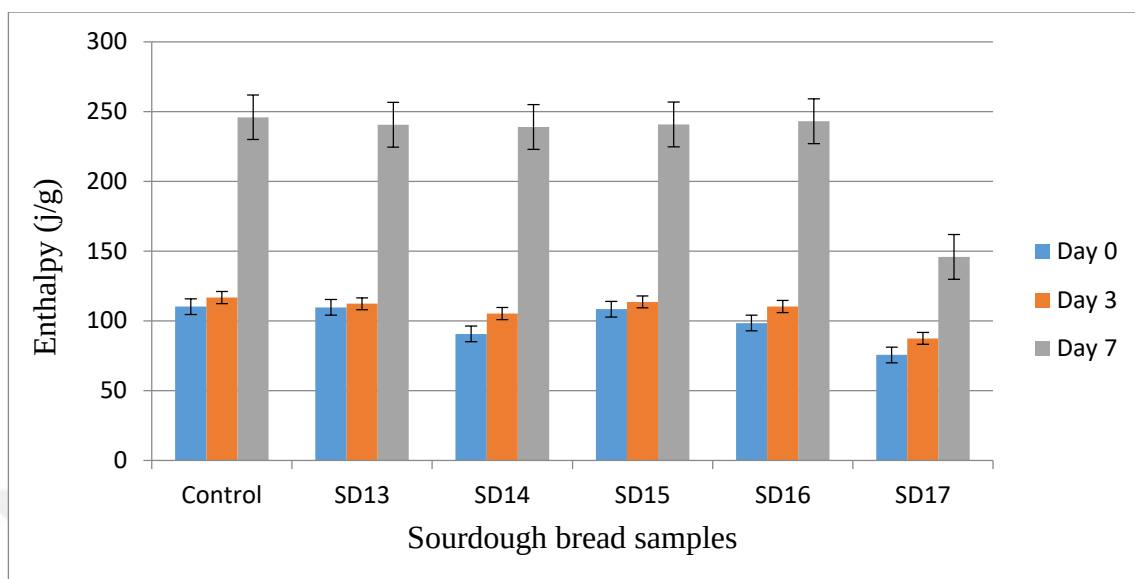


Figure 4.42 Enthalpy results on sourdough breads produced with second combinations

4.8.9 Sensory analysis of second combinations

The sensory properties of sourdough breads produced with the second combinations are given in Table 4.18. At the 4th and 24th hours and 7th day after the sourdough bread was baked, 10 panelists were analyzed to determine the sensory characteristics. Sourdough breads were sliced and a rating was requested for the external and internal properties of the bread. Although the results of the sensory properties of the sourdough breads were parallel at the 4th and 24th hours, and 7th day, a decrease in taste was observed with the storage time. As a result of the sensory test, sourdough breads numbered SD14 and SD17 were the most preferred sourdough breads. Sour taste, high volume low moisture loss, good color properties may have caused it to be preferred. It was determined that the sensory values of these sourdough breads were significantly better than the other three sourdough breads ($p < 0.05$).

Table 4.18 Results of sensory analysis of sourdough breads from second combinations

SD bread	Control	SD13	SD14	SD15	SD16	SD17
4h	40±2.33 ^{aA}	44±5.24 ^{aA}	70±6.52 ^{bcA}	45±2.39 ^{aA}	64±5.44 ^{bA}	72±4.59 ^{cA}
24h	36±4.41 ^{aB}	39±3.79 ^{aB}	64±5.05 ^{cB}	40±3.98 ^{aB}	58±3.02 ^{bB}	68±3.43 ^{cB}
7d	33±3.93 ^{aC}	35±4.45 ^{abC}	57±3.32 ^{cdC}	38±2.18 ^{bC}	55±3.15 ^{cC}	60±3.98 ^{dC}

Evaluation was made on 10 panelists, d=day 4th= 4 hour, 24th = 24 hour, 7d= 7 day SD: Sourdough

±: Standard deviation

Means within a rows with different small letters are significantly different ($p<0.05$)

In the column, different capitilized letters represent significant sensory amount differences among sourdough breads.

CHAPTER V

CONCLUSION

In this study; LAB and yeasts isolated from sourdough samples obtained from Konya, Mardin and Gaziantep regions were identified at species level as a result of MALDI-TOF MS and PCR-sequence analysis. Technological properties of these species on bread have been investigated. It was understood that LAB that can produce CO₂ and improve texture properties of sourdough. The importance of isolation of heterofermentative LAB in sourdough bread has been determined since they are important with respect to produce CO₂ and aroma compounds. Homofermentative LAB is important in acid production in sourdough bread. In particular, homofermentative LAB were identified as *P. acidilactici*, *E. faecalis*, *E. hirae* and *W. confusa* (not use in sourdough bread production) in this study. The most dominant LAB species from traditional sourdoughs were *L. brevis*, *L. plantarum* and *P. acidilactici*. The most dominant yeast that isolated from sourdough were *S. cerevisiae*, *P. kudriavzevii* and *K. marxianus*. Combinations of cultures were made in the production of sourdough bread within the scope of the characteristics of the species.

Heterofermentative LAB was produced organic acid, CO₂ and flavoring substances in sourdoughs, while yeasts enable the production of metabolites such as alcohol, ester, carbonyl compounds and CO₂. They affected bread aroma as well as swelling of bread. Sourdough cultures extend the shelf life of bread; they also caused significant increases in nutritional value and physical structure of bread due to hydrolysis proteins and polysaccharides.

During fermentation, the carbohydrates and proteins in the flour undergo biochemical changes with the help of microorganisms and enzymes. These changes affect the

properties of the sourdough and thus the quality of the bread. The pH and softness values of the breads were examined, as a result, it was determined that they provided good quality (SD14 and SD17) with sourdough cultures and especially in breads where produced using heterofermentative and homofermentative LAB and yeasts together. In our study, it was determined that the use of heterofermentative and *S. cerevisiae* together caused; significant differences ($p < 0.05$) low pH and high total acidity values than those used only homofermentative. During fermentation, the pH value of sourdoughs decreased due to acid production by LAB.

Microbial analyzes were performed at the end of the fermentation period. While the numbers of LAB and yeasts increased, the number of MAB decreased due to the decrease in pH. Significant differences ($p < 0.05$) were detected between the sourdough breads and control samples in terms of microbial and chemical properties. L^* , a^* and b^* values in bread interior color analyzes differed compared to control bread and their values increased during storage. The dry matter contents of sourdough breads were determined to be between 68.33 and 72.11% in last combinations on 7th day for second combinations. As a result of fermentation, the starch in the dough partially converts into sugar and then forms "carbon dioxide" and "lactic acid" (Steinkraus, 1997). Therefore, significant differences ($p < 0.05$) were observed in dry matter ratio among sourdoughs.

With the use of LAB, there was a significant improvement in bread quality due to acid production and decrease in pH. Sourdough bread quality improved as a result of competition with selected LAB of selected yeast strains used in the study. Yeasts and LAB worked in harmony with a good fermentative capacity. Significant reductions in undesirable were detected due to organic acids and ethanol formations due to the proliferation of LAB and yeasts.

The specific volume was determined after 4th hours of the breads production. There was no significant difference ($p > 0.05$) between sourdough breads and control bread. The sourdough breads produced were presented to the panelists and they were asked to evaluate the color, homogeneous distribution, pores, taste, yeast, sourness, stickiness, pleasant odor, soft texture, elasticity, odor yeast and chewiness. In the evaluation, a score between 1 and 10 was given. Homofermentative-heterofermentative and yeasts used together scored higher in the evaluation of sourdough breads in terms of color,

homogeneity and pore structure ($p < 0.05$). When the sourdough breads were evaluated by the panelists in terms of taste, it was found difference between the sourdough breads compared to the control. In terms of general acceptability in sourdough breads, differences were determined between bread. *L. plantarum* is effective on low pH. Yeasts and obligate heterofermentative LAB are responsible for the leavening by CO₂ production. As a result of the research, the most overall acceptable sourdough bread cultures (SBC) were made with following combination of LAB and yeasts:

SBC-14: *L. plantarum*+*P. acidilactici*+*E. faecalis*+*C. tropicalis*+*C. glabrata*+*K. unispora*

SBC-17: *L. brevis*+*L. mesenteroides* subsp. *mesenteroides*+*E. hirae*+*L. plantarum*+*P. acidilactici*+*S. cerevisiae*+*K. marxianus*

In the sensory evaluation trials of the second group of sourdough breads, the most popular breads in terms of acceptability were the breads SD14 and SD17. The use of starter culture for bread production emerges as an important phenomenon that needs to be developed and improved in terms of features that can meet the demands of both consumers and producers. It is hoped that the results of this research will contribute to the development of starter culture for sourdough bread production.

REFERENCES

- Akgün, F.B., (2007). Ekşi Maya Tozu Üretimi ve Ekmek Üretiminde Potansiyel Kullanım Alanları. *Y. Lisans Tezi, Pamukkale Üniversitesi Fen Bilimleri Enstitüsü, Denizli.*
- Akyüz, G., (2016). Kluyveromyces lactis' in ekmek mayası olarak kullanılma potansiyelinin incelenmesi. *Y. Lisans Tezi, Ordu Üniversitesi Fen Bilimleri Enstitüsü, Ordu.*
- Altıok, D., (2004). *Kinetic modelling of lactic acid production from whey. Master of Science, Izmir Institute of Technology, Food Engineering, Izmir, Turkey.*
- American Association of Community Colleges (AACC). (2011). American Association of Community Colleges: 2011 fact sheet. Washington, DC: Author. Available from <http://www.aacc.nche.edu/Aboutcc/Documents/FactSheet2011.pdf>.
- Anderson, A. C., Jonas, D., Huber, I., Karygianni, L., Wölber, J., Hellwig, E., Al-Ahmad, A. (2016). Enterococcus faecalis from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. *Frontiers in microbiology*, **6**, 1534.
- Angelakis, E., Million, M., Henry, M., Raoult, D. (2011). Rapid and Accurate Bacterial Identification in Probiotics and Yoghurts by MALDI-TOF Mass Spectrometry. *Journal of Food Science*, **76(8)**.
- Aplevicz, K. S., Mazo, J. Z., Ilha, E. C., Dinon, A. Z., Sant'Anna, E. S. (2014). Isolation and characterization of lactic acid bacteria and yeasts from the Brazilian grape sourdough. *Brazilian Journal of Pharmaceutical Sciences*, **50(2)**, 321–327.

Aplevicz, K. S., Ogliari, P. J., Sant'Anna, E. S. (2013). Influence of fermentation time on characteristics of sourdough bread. *Brazilian Journal of Pharmaceutical Sciences*, **49(2)**, 233–239.

Arendt, E. K., Ryan, L. A., Bello, F. D. (2007). “Impact of sourdough on the texture of bread” *Food Microbiology*, **24(2)**, 165–174.

Ataç, F. (2016). Use of Kefir made from Natural Kefir Grains in Sourdough Bread Making. Master's thesis, Süleyman Demirel University, Graduate School of Natural and Applied Sciences, Food Engineering, Isparta.

Aydoğan, S., Akçacık Göçmen, A., Kaya, Y., Koç, H., Nisa, M., Mehmet, G. (2012). Determination of the Relations Between Rheological Properties of Wheat Flour Which Measured with Alveograph, Farinograph and Mixograph. **7(1)**, 74–82.

Aydoğan, S., Akçacık, G. A., Şahin, M., Önmez, H., Demir, B., Yakışır, E. (2013). Determination of physicochemical and rheological properties of bread wheat varieties. *Journal of Field Crops Central Research Institute (Turkey)*.

Bakırcı, F., Köse, E. (2017). Isolation and Identification of Lactic Acid Bacteria and Yeasts from Sourdough. *Akademik Gıda*, **15(2)**, 149–154.

Balgir, P. P., Kaur, B., Kaur, T., Daroch, N., Kaur, G. (2013). In Vitro and In Vivo Survival and Colonic Adhesion of *Pediococcus acidilactici* MTCC5101 in Human Gut. Hindawi Publishing Corporation, BioMed Research International, p:9.

Bartkiene, E., Bartkevics, V., Krungleviciute, V., Pugajeva, I., Zadeike, D., Juodeikiene, G. (2017). Lactic Acid Bacteria Combinations for Wheat Sourdough Preparation and Their Influence on Wheat Bread Quality and Acrylamide Formation. *Journal of Food Science*, vol. 82.

Bhol, S., Bosco, S. J. D. (2014). “Influence of malted finger millet and red kidney bean flour on quality characteristics of developed bread”, *LWT – Food Science and Technology*, 55, 294-300.

Bilgiçli, N., Soylu, S. (2016). “The Evaluation of Wheat and Flour Qualities in Sectoral Perspective”, **5(2)**, 58-67.

Bintsis, T. (2018). Lactic acid bacteria as starter cultures : An update in their metabolism and genetics. *AIMS Microbiology*, **4(4)**, 665–684.

Bircan, D., Güray, C.T, Bostan, K. (2017). “Studies on Sour Dough Bread Making by Different Methods”, *Aydın Gastronomy*, **1(1)**, 1-8.

Biswas, S., Rolain, J. M. (2013). Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. *Journal of Microbiological Methods*, **92(1)**, 14–24.

Bounaix, M. S., Gabriel, V., Robert, H., Morel, S., Remaud-Siméon, M., Gabriel, B., Fontagné-Faucher, C. (2010). Characterization of glucan-producing *Leuconostoc* strains isolated from sourdough. *International journal of food microbiology*, **144(1)**, 1-9.

Caballero, R., Olguín, P., Cruz-Guerrero, A., Gallardo, F., García-Garibay, M., & Gómez-Ruiz, L. (1995). Evaluation of *Kluyveromyces marxianus* as baker's yeast. *Food Research International*, **28(1)**, 37-41.

Chavan, R. S., Chavan, S. R. (2011). Sourdough Technology-A Traditional Way for Wholesome Foods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, **10(3)**, 169–182.

Corsetti, A., Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food research international*, **40(5)**, 539-558.

Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and stalin. *Journal of Food Science*, **63(2)**, 347-351.

Coşkuner, Y., Karababa, E., Ercan, R. (1999). “Technology of Flat Breads Production”, *Gıda* **24(2)**, 89-97.

De Vuyst, L., Neysens, P. (2005). The sourdough microflora: biodiversity and metabolic

interactions. *Trends in Food Science & Technology*, **16(1-3)**, 43-56.

De Vuyst, L., Vancanneyt, M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiology*, **24(2)**, 120-127.

Douglass, A. P., Offei, B., Braun-Galleani, S., Coughlan, A. Y., Martos, A. A., Ortiz-Merino, R. A., ... & Wolfe, K. H. (2018). Population genomics shows no distinction between pathogenic *Candida krusei* and environmental *Pichia kudriavzevii*: one species, four names. *PLoS pathogens*, **14(7)**, e1007138.

Toit, M. D., Huch, M., Cho, G. S., & Franz, C. M. (2014). The genus *Streptococcus*. *Lactic acid bacteria: biodiversity and taxonomy*, 457-505.

Elgün, A., Ertugay, Z. (2002). *Tahıl İşleme Teknolojisi*. Atatürk University, Ziraat Faculty Press, Erzurum.

Elgün, A., Ertugay, Z., Certel, M., & Kotancılar, G. (2002). Analytical quality control in cereals and products–laboratory application guide [Tahıl ve Ürünlerinde Analitik Kalite Kontrolü ve Laboratuvar Uygulama Klavuzu]. *Atatürk University*, Erzurum, Turkey.

Eriksson, E., Koch, K., Tortoe, C., Akonor, T. P., Oduro-Yeboah, C. (2014). Evaluation of the physical and sensory characteristics of bread produced from three varieties of cassava and wheat composite flours. *Food and Public Health*, **4(5)**, 214–222.

Erkmen O. (2021): *Laboratory Practices in Microbiology*. Elsevier Inc., Oxford, 19 April.

Erkmen, O. (2022). *Microbiological analysis of foods and food processing environments*. Academic Press.

Erkmen, O., Bozoglu, T. F. (2016). *Food Microbiology Principles into Practice*. Volume 2: Microorganisms in Food Preservation and Processing. John Wiley and Sons, Ltd., Chichester.

Hendek Ertop, M. (2014). Ekşi hamur formül optimizasyonunun ekmeğin aromatik profili, biyoaktif nitelikleri ve raf ömrü üzerine etkileri. *Y. Lisans Tezi, Erciyes Üniversitesi Fen Bilimleri Enstitüsü, Kayseri.*

Ertop, M. H., & Hayta, M. (2016). The effect of sourdough fermentation on bread bioactive compounds and their bioavailability. *GIDA-Journal of Food*, **41(2)**, 115-122.

Ertürk, A., Arslantaş, N., Sarıca, D., & Demircan, V. (2015). Families' bread consumption and waste in urban areas of Isparta city, Turkey. *Akademik Gıda*, **13(4)**, 291-298.

Evren, M., Apan, M., Tutkun, E., Evren, S. (2011). Lactic acid bacteria found in traditional fermented foods. *Electronic Journal of Microbiology*, TR, **9(1)**, 11-17. (in Turkish)

Fairfax, M. R., Lephart, P., R., Salimnia, H. (2014). *Weissella confusa*: problems with identification of an opportunistic pathogen that has been found in fermented foods and proposed as a probiotic. *Frontiers in Microbiology*, 5, 254.

Felsenstein, J. (1985). *Confidence limits on phylogenies: an approach using the bootstrap*. **39(4)**, 783–791.

Feyereisen, M., Mahony, J., Kelleher, P., Roberts, R. J., O’Sullivan, T., Geertman, J. M. A., & van Sinderen, D. (2019). Comparative genome analysis of the *Lactobacillus brevis* species. *BMC genomics*, **20(1)**, 1-15.

Fitzsimons, A., O’Connell, M. (1994). Comparative analysis of amylolytic lactobacilli and *Lactobacillus plantarum* as potential silage inoculants. *FEMS microbiology letters*, **116(2)**, 137-145.

Fonseca, G. G., Heinzle, E., Wittmann, C., & Gombert, A. K. (2008). The yeast *Kluyveromyces marxianus* and its biotechnological potential. *Applied microbiology and biotechnology*, **79**, 339-354.

Franz, C. M., Stiles, M. E., Schleifer, K. H., Holzapfel, W. H. (2003). Enterococci in foods—a conundrum for food safety. *International journal of food microbiology*, **88**(2-3), 105-122.

Gobbetti, M., & Gänzle, M. (Eds.). (2012). *Handbook on sourdough biotechnology*. Springer Science & Business Media.

Gänzle, M. G., Loponen, J., Gobbetti, M. (2008). Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. *Trends in food science & technology*, **19**(10), 513-521.

Gerçekaslan, K. E., Kotancılar, H. G., Karaoğlu, M. M. (2007). “Bread Staling and Methods for Measuring Degree of Staling-I”, *Gıda*, **32**(6), 305-315.

Giraffa, G. (2002). Enterococci from foods. *FEMS microbiology reviews*, **26**(2), 163-171.

Güre, S., (2009). Determination Of Proteolytic Effects Of Lactic Acid Bacteria Isolated From Sourdoughs On Gluten. Master’s thesis, Süleyman Demirel University, Graduate School of Natural and Applied Sciences, Isparta, 73s. (in Turkish)

Thakur, M., Deshpande, H. W., Bhate, M. A. (2017). Isolation and Identification of Lactic Acid Bacteria and their Exploration in Non-Dairy Probiotic Drink. *International Journal of Current Microbiology and Applied Sciences*, **6**(4), 1023–1030.

Huang, W., Kim, Y., Li, X., Rayas-Duarte, P. (2008). Rheofermentometer parameters and bread specific volume of frozen sweet dough influenced by ingredients and dough mixing temperature. *Journal of Cereal Science*, **48**(3), 639-646.

İpek, Ö., (2017). Technological Properties of Some Baker’s Yeast Isolated from Turkish Sourdough. Master’s thesis, Ankara University, Graduate School of Natural and Applied Sciences, Food Engineering, Ankara. (in Turkish)

Jurinke, C., Oeth, P., Van Den Boom, D. (2004). MALDI-TOF mass spectrometry: A

versatile tool for high-performance DNA analysis. *Applied Biochemistry and Biotechnology - Part B Molecular Biotechnology*, **26(2)**, 147–163.

Kahya, S., Buyukcangaz, E., Carli, T., Reaction, P. C. (2014). Optimization of Polymerase Chain Reaction (PCR). *Uludag Univ. J. Fac. Vet. Med.*, **32(1)**, 31–38.

Katina, K., Arendt, E., Liukkonen, K. H., Autio, K., Flander, L., Poutanen, K. (2005). Potential of sourdough for healthier cereal products. *Trends in Food Science and Technology*, **16(1–3)**, 104–112.

Khalid, K. H., Ohm, J. B., & Simsek, S. (2017). Whole wheat bread: Effect of bran fractions on dough and end-product quality. *Journal of Cereal Science*, **78**, 48–56.

Kotancılar, H. G., Karaoğlu, M. M., Gerçekaslan, K. E., (2006). Ekşi Hamur Katkısının Beyaz Tava Ekmeğinin Bayatlaması Üzerine Etkisi, *Atatürk Üniversitesi, Ziraat Fakültesi Dergisi*, **37(1)**, 103–110.

Küçükçuban, A. (2012). Küçükçuban, A. (2012). Sıvı ekşi hamur sistemi için uygun laktik asit bakteri kombinasyonunun belirlenmesi, *Master's thesis, Pamukkale Üniversitesi Fen Bilimleri Enstitüsü*.

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, **33(7)**, 1870–1874.

Lahtinen, S., Ouwehand, A. C., Salminen, S., & von Wright, A. (Eds.). (2011). *Lactic acid bacteria: microbiological and functional aspects*. Crc Press.

Lane, M. M., Morrissey, J. P. (2010). *Kluyveromyces marxianus* : A yeast emerging from its sister's shadow. *Fungal Biology Reviews*, **24(1–2)**, 17–26.

Lane, M. M., Burke, N., Karreman, R., Wolfe, K. H., O'Byrne, C. P., & Morrissey, J. P. (2011). Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*. *Antonie Van Leeuwenhoek*, **100**, 507–519.

Lau, S. W., Chong, A. Q., Chin, N. L., Talib, R. A., & Basha, R. K. (2021). Sourdough

microbiome comparison and benefits. *Microorganisms*, **9(7)**, 1355.

Lee, P. Y., Costumbrado, J., Hsu, C. Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *JoVE (Journal of Visualized Experiments)*, (62), e3923.

Liu, T., Li, Y., Sadiq, F. A., Yang, H., Gu, J., Yuan, L. (2018). Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread. *Food Chemistry*, 242(May 2017), 404–411.

Ly, B. C. K., Dyer, E. B., Feig, J. L., Chien, A. L., Del Bino, S. (2020). Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement. *Journal of Investigative Dermatology*, **140(1)**, 3-12.e1.

Maddocks, S., & Jenkins, R. (2016). *Understanding PCR: A practical bench-top guide*. Academic Press.

Manini, F., Casiraghi, M. C., Poutanen, K., Brasca, M., Erba, D., Plumed-Ferrer, C. (2016). Characterization of lactic acid bacteria isolated from wheat bran sourdough. *LWT-food Science and Technology*, **66**, 275-283.

Martínez-Anaya, M. A. (2003). Associations and interactions of microorganisms in dough fermentations: effects on dough and bread characteristics. In *Handbook of dough fermentations* (pp. 87-120). CRC Press.

Matejčková, Z., Spodniaková, S., Dujmić, E. (2019). *Modelling growth of Lactobacillus plantarum as a function of temperature : Effects of media*. **58(2)**, 125–134.

Menderis, M., Atlı, A., Köten, M., & Kılıç, H. (2008). Gluten indeks değeri ve yaş gluten/protein oranı ile ekmeklik buğday kalite değerlendirmesi. *Haran Üniversitesi Ziraat Fakültesi Dergisi*, **12(3)**, 57-64.

Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., Gobbetti, M. (2012). Lactic Acid Bacterium and Yeast Microbiotas of 19 Sourdoughs Used for Traditional/Typical Italian Breads: Interactions between

Ingredients and Microbial Species Diversity. *Applied and Environmental Microbiology*, **78(4)**, 1251–1264.

Nacef, M., Chevalier, M., Chollet, S., Drider, D., & Flahaut, C. (2017). MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. *International journal of food microbiology*, **247**, 2-8.

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press, USA.

Oberoi, H. S., Babbar, N., Sandhu, S. K., Dhaliwal, S. S., Kaur, U., Chadha, B. S., & Bhargav, V. K. (2012). Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant *Pichia kudriavzevii* HOP-1. *Journal of Industrial Microbiology and Biotechnology*, **39(4)**, 557-566.

Olano, A., Chua, J., Schroeder, S., Minari, A., La Salvia, M., Hall, G. (2001). *Weissella confusa* (basonym: *Lactobacillus confusus*) bacteremia: a case report. *Journal of clinical microbiology*, **39(4)**, 1604-1607.

Park, J., Seo, J. S., Kim, S. A., Shin, S. Y., Park, J. H., Han, N. S. (2017). Microbial diversity of commercial makgeolli and its influence on the organoleptic characteristics of Korean rice sourdough, Jeung-Pyun. *Journal of Microbiology and Biotechnology*, **27(10)**, 1736–1743.

Park, Y. W., Bell, L. N. (2004). Determination of moisture and Ash contents of foods. In L. M. L. Nollet (Ed.). *Handbook of food analysis* (pp. 55–82). (2nd ed.). New York: Marcel Dekker.

Parniakov, O., Bals, O., Barba, F. J., Mykhailik, V., Lebovka, N., Vorobiev, E. (2018). Application of differential scanning calorimetry to estimate quality and nutritional properties of food products, *Critical Reviews in Food Science and Nutrition*, **58(3)**, 362-385.

Plessas, S., Alexopoulos, A., Bekatorou, A., Mantzourani, I., Koutinas, A.A., Bezirtzoglou, E., (2011). “Examination of freshness degradation of sourdough bread made with kefir through monitoring the aroma volatile composition during storage”, *Food Chemistry*, 124, 627-633.

Polat, Y. (2007). “In Addition Pumpkin Powder to Wheat Flour Effect on Bread Quality of Flour. Master’s thesis, İstanbul Teknik University, Graduate School of Natural and Applied Sciences, Food Engineering, İstanbul, 51s.

Rehman, S., Paterson, A., Piggott, J. R. (2006). Flavour in sourdough breads: A review. *Trends in Food Science and Technology*, **17(10)**, 557-566.

Reiner, K. (2012). Carbohydrate fermentation protocol. *Energy*, 11, 12.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, **4(4)**, 406-425.

Salvetti, E., Torriani, S., & Felis, G. E. (2012). The genus *Lactobacillus*: a taxonomic update. *Probiotics and antimicrobial proteins*, **4**, 217-226.

Sevgili, A., Erkmen, O., Koçaslan, S. (2021). Identification of lactic acid bacteria and yeasts from traditional sourdoughs and sourdough production by enrichment. *Czech Journal of Food Sciences*, **39(4)**, 312–318.

Sgaramella, V. (1996). YAC protocols. Methods in molecular biology, vol. 54. *FEBS Letters*, **398(2–3)**, 338–338.

Sogawa, K., Watanabe, M., Sato, K., Segawa, S., Ishii, C., Miyabe, A., Murata, S., Saito, T., Nomura, F. (2011). Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms. *Analytical and Bioanalytical Chemistry*, **400(7)**, 1905–1911.

Elez-Martinez, P., Escola-Hernandez, J., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high-intensity pulsed electric fields. *Food Microbiology*, **22(4)**, 311-319.

Soro-Yao, A. A., Schumann, P., Thonart, P., Djè, K. M., & Pukall, R. (2014). The Use of MALDI-TOF Mass Spectrometry, Ribotyping and Phenotypic Tests to Identify Lactic Acid Bacteria from Fermented Cereal Foods in Abidjan (Côte d'Ivoire). *The Open Microbiology Journal*, **8(1)**, 78–86.

Steinkraus, K. H. (1997). Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control*, **8(5-6)**, 311-317.

TGK. (2013). Türk Gıda Kodeksi Buğday Unu Tebliği. Tebliğ No: 2013/9, Sayı 28606.

Todorov, S., Gotcheva, B., Dousset, X., Onno, B., & Ivanova, I. (2000). Influence of growth medium on bacteriocin production in *Lactobacillus plantarum* ST31. *Biotechnology & Biotechnological Equipment*, **14(1)**, 50-55.

Uysul, A., Kurt, Ş., Soylu, S., Soylu, E. M., & Kara, M. (2019). Identification of microorganism species in leafy vegetables using MALDI-TOF MS (matrix assisted laser desorption/ionization mass spectrometry) technique. *Yüzüncü Yıl Üniversitesi Journal of Agricultural Sciences*, **29(4)**, 595-601.

Valan, M., Al-dhabi, N. A., Ilavenil, S. (2016). In vitro importance of probiotic *Lactobacillus plantarum* related to medical field. *Saudi Journal of Biological Sciences*, **23(1)**, S6–S10.

Valjakka, T., Kerojoki, H. (2003). Sourdough bread in Finland and Eastern Europe. (Ed: Karel Kulp and Klaus Lorenz). Handbook of dough fermentations. New York: Marcel Dekker, Inc., p: 277-333.

Vuyst, Luc De, Comasio, A., Kerrebroeck, S. Van. (2021). Sourdough production : fermentation strategies, microbial ecology, and use of non-flour ingredients. *Critical Reviews in Food Science and Nutrition*, 1–33.

Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobбен, G. J. (2002). Mannitol production by lactic acid bacteria: a review. *International Dairy Journal*, **12(2-3)**, 151-161.

Yağmur, G., Tanguler, H., Leventdurur, S., Elmaci, S. B., Turhan, E. Ü., Francesca, N.,

Settanni, L., Moschetti, G., Erten, H. (2016). Identification of Predominant Lactic Acid Bacteria and Yeasts of Turkish Sourdoughs and Selection of Starter Cultures for Liquid Sourdough Production Using Different Flours and Dough Yields. *Polish Journal of Food and Nutrition Sciences*, **66(2)**, 99–107.

Yılmaz, S., Duyan, S., Artuk, C., & Diktaş, H. (2014). Mikrobiyolojik Tanımlamada MALDI-TOF MS Uygulamaları. *TAF Preventive Medicine Bulletin*, **13(5)**.

Yörük, G. & Güner, A. Laktik asit bakterilerinin sınıflandırılması ve Weissella türlerinin gıda mikrobiyolojisinde önemi. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, **6(2)**, 163-176.

APPENDICES

APPENDIX A-MEDIA

Table A.1 Peptone broth

Ingredients	Amount (g)
Peptone	10.0
Sodium Chloride	5.0

Ingredients dissolved in 1.0 L of distilled water by stirring. Then, 5 ml of peptone broth dispensed to sterilized test tubes and then, it is sterilized at 121°C for 15 minutes in an autoclave (Erkmen, 2021).

Table A.2 MRS (De Man, Rogosa and Sharpe) agar/broth

Ingredients	Amount (g)
Proteose peptone No. 3	10.0
Beef extract	10.0
Yeast extract	5.0
Glucose	20.0
K ₂ HPO ₄	1.0
Sodium acetate trihydrate	5.0
Manganese sulfate. H ₂ O	0.2
Manganese sulfate. 4H ₂ O	0.5
Tri-ammonium citrate	2.0
Agar	15.0

Ingredients were dissolved in 1.0 L of distilled water by steaming and pH adjusted to 6.4 then, it is sterilized at 121°C for 15 minutes in an autoclave. After that it is cooled to about 47°C and poured into sterile petri plates under aseptic conditions with 18-20 mL then, petri plates are stored at 4°C. MRS broth is prepared without the use of agar (Erkmen, 2021).

Table A.3 Potato Dextrose (PD) agar/broth

Ingredients	Amount (g)
Potato, infusion from	200.0
Glucose	20.0
Agar	15.0

200g of peeled potatoes for 1 hour in 1.0 L of distilled water Then, it is filtered and filtered to one liter and glucose and agar added and mixed, pH adjusted to 6.4 and then, it is sterilized at 121°C for 15 minutes on autoclave. After that it is cooled to about 57°C and poured into sterile petri plates under aseptic conditions with 18-20 mL then, petri plates are stored at 4°C (Erkmen, 2021).

Table A.4 Potato Dextrose (PD) broth

Ingredients	Amount (g)
Potato, infusion from	200.0
Dextrose	20.0

200 g of peeled potatoes for 1 hour in 1.0 L of distilled water Then, it is filtered and filtered to one liter and glucose. Heated to dissolve the medium completely. Sterilized by autoclaving at 121°C for 15 minutes. It is cooled to about 57°C and poured into sterile petri plates under aseptic conditions with 18-20 mL then, petri plates are stored at 4°C (Erkmen, 2021).

Table A.5 Oxidation Fermentation (O-F) broth

Ingredients	Amount g/L
Peptone	2.0
Sodium chloride	5.0
Dipotassium phosphate	0.3
Bromcresol purple	0.008

These ingredients dissolved in the 1.0 L of distilled water and 5.0 g glucose was added to this broth and pH adjusted to 7.0. Then, 8 ml dispensed to sterilized tubes containing inverted Durham tubes and then, it is sterilized at 121°C for 15 minutes in an autoclave (Erkmen, 2021).

Table A.6 Preparation of Liquid Broth for Carbohydrate (Glucose) fermentation test

Ingredients	Amount g
Bovine extract	10.0
Neopeptone	10.0
Yeast extract	5.0
Tween 80	1.0
K ₂ HPO ₄	2.0
CH ₃ COONa x 3H ₂ O	5.0
Diamonium citrate	2.0
MgSO ₄	0.2
MnSO ₄	0.05
Bromcresol purple	0.17
Glucose	1%

These ingredients are dissolved in 1.0 L distilled water. After, 10 ml dispensed to sterilized tubes containing inverted Durham tubes and then heated to dissolve the medium completely. Sterilized by autoclaving at 121°C (Erkmen, 2021).

Table A.7 Preparation of liquid broth for carbohydrate (Sucrose, Maltose, Lactose) Fermentation Test

Ingredients	Amount g
Malt extract	12
Sucrose/ Lactose/ Maltose	10
Yeast extract	3

12 g malt extract, 5 g maltose, and 3 g yeast extract are dissolved in 1.0 L distilled water. Sucrose and lactose mixture are prepared with the same amount and heated to dissolve. Then, Durham tubes are put each test tubes and 10 ml mixture are dispensed to sterilized test tubes. Finally, all test tubes are sterilized by autoclaving at 121°C (Erkmen, 2021).

Table A.9 APT agar

Ingredients	Amount of per liter (g)
Tryton	12.5
Yeast extract	7.5
K ₂ HPO ₄	5.0
NaCl	5.0
Sodium citrate	5.0
Sodium carbonate	1.25
Glucose	10.0
Tween 80	0.2
Magnesium sulfite	0.8
Magnesium chloride	0.14
Ferrous sulfate	0.04
Agar	15.0

Ingredients (except agar) are dissolved in 1.0 L distilled water by steaming. pH is adjusted 6.7 and is added agar. It is boiled and heated until dissolving the agar. Then, it is sterilized at 121°C for 15 min. After sterilization, it is cooled 45°C and poured into sterile Petri dishes (Erkmen, 2021).

Table A.10 HHD medium

Ingredients	Amounts of per liter (g)
Fructose	2.5
KH ₂ PO ₄	2.5
Typticase peptone	10.0
Phytone peptone	1.5
Casamino acids	3.0
Yeast extract	1.0
Tween 80	1.0
Bromcresol green (1% aq. solution)	20.0
Agar	20.0

Ingredients (except agar) are dissolved in 1.0 L distilled water by steaming. pH is adjusted 7.0 and is added agar. It is boiled and heated until dissolving the agar. Then, it is sterilized at 121°C for 15 min. After sterilization, it is cooled 45°C and poured into sterile Petri dishes (Erkmen, 2021).

APPENDIX B- TABLES OF ANALYSIS RESULTS

Table B.1 Sourdough microorganisms values as LAB, MAB and yeasts

Sourdough	LAB log (cfu)/g	MAB log (cfu)/g	Yeasts log (cfu)/g
Control	2.45±0.16 ^a	6.48±0.10 ^a	3.42±0.03 ^a
SD1	6.71±0.16 ^{de}	5.51±0.07 ^k	5.27±0.06 ^b
SD2	6.51±0.06 ^c	4.95±0.08 ^j	5.35±0.07 ^{bc}
SD3	7.20±0.08 ^g	4.54±0.06 ^g	5.87±0.10 ^e
SD4	7.64±0.24 ⁱ	4.27±0.05 ^e	6.53±0.14 ^g
SD5	6.58±0.19 ^{cd}	5.03±0.07 ^j	5.54±0.08 ^d
SD6	6.81±0.07 ^e	4.71±0.08 ^h	5.78±0.11 ^e
SD7	7.01±0.10 ^f	4.45±0.06 ^f	6.18±0.15 ^f
SD8	7.48±0.08 ^h	4.15±0.02 ^d	6.78±0.17 ^h
SD9	6.32±0.11 ^b	4.80±0.08 ⁱ	5.48±0.08 ^{cd}
SD10	6.53±0.06 ^c	4.63±0.06 ^h	5.62±0.09 ^d
SD11	7.30±0.08 ^g	4.05±0.04 ^c	6.83±0.16 ^h
SD12	8.02±0.12 ^j	3.91±0.03 ^b	6.98±0.18 ⁱ

Values are the mean ± SD. In the columns, different small letters represent significant differences

Table B.2 Sourdough pH and titratable acidity results

Sourdough	pH	TTA (%)
Control	5.12±0.03 ^a	2.88±0.08 ^a
SD1	4.72±0.05 ^h	3.11±0.03 ^{bc}
SD2	4.66±0.02 ^f	3.17±0.07 ^c
SD3	4.60±0.03 ^e	3.21±0.14 ^c
SD4	4.08±0.01 ^{bc}	3.45±0.18 ^{de}
SD5	4.70±0.05 ^{gh}	3.10±0.02 ^{abc}
SD6	4.64±0.02 ^{ef}	3.18±0.03 ^c
SD7	4.65±0.02 ^f	3.20±0.10 ^c
SD8	4.10±0.04 ^c	3.40±0.09 ^{de}
SD9	4.80±0.06 ⁱ	3.00±0.03 ^{ab}
SD10	4.78±0.05 ⁱ	3.09±0.02 ^{abc}
SD11	4.24±0.01 ^d	3.34±0.12 ^d
SD12	4.05±0.02 ^b	3.50±0.07 ^e

Values are the mean ± SD. In the columns, different small letters represent significant differences

Table B.3 Sourdough volume results

Sourdough	Volume (%)
Control	156.30±10.1 ^a
SD1	169.40±7.2 ^b
SD2	165.10±6.8 ^{ab}
SD3	169.98±7.1 ^b
SD4	169.00±7.0 ^b
SD5	169.10±8.4 ^b
SD6	168.60±6.9 ^b
SD7	168.50±6.7 ^b
SD8	169.48±7.2 ^b
SD9	170.30±7.5 ^b
SD10	168.12±6.9 ^b
SD11	167.98±6.3 ^b
SD12	171.65±7.8 ^b

Values are the mean ± SD. In the columns, different small letters represent significant differences

Sourdough bread	pH	TTA
Control	5.55±0.12 ^a	2.32±0.01 ^a
SD1	5.18±0.10 ^g	2.36±0.02 ^a
SD2	4.66±0.08 ^e	2.72±0.03 ^d
SD3	4.70±0.08 ^e	2.70±0.03 ^d
SD4	4.21±0.07 ^d	2.84±0.04 ^e
SD5	5.18±0.10 ^g	2.35±0.02 ^a
SD6	5.05±0.11 ^{fg}	2.45±0.03 ^b
SD7	4.39±0.13 ^{cd}	2.92±0.03 ^f
SD8	4.37±0.05 ^b	3.10±0.04 ^h
SD9	5.18±0.14 ^g	2.35±0.02 ^a
SD10	4.98±0.09 ^f	2.56±0.02 ^c
SD11	4.40±0.05 ^{cd}	2.80±0.04 ^e
SD12	4.28±0.08 ^{bc}	2.98±0.06 ^g

Table B.4 Sourdough breads pH and titratable acidity

Values are the mean ± SD. In the columns, different small letters represent significant differences

Table B.5 Moisture of sourdough breads results

Sourdough bread	0. day	3. day	7. day
Control	30.15±1.62 ^{abA}	25.10±1.25 ^{aB}	20.15±1.10 ^{aC}
SD1	32.23±1.84 ^{bcdA}	22.98±1.05 ^{bB}	21.95±1.22 ^{bC}
SD2	33.16±1.75 ^{cdA}	27.84±1.34 ^{cdB}	26.32±1.35 ^{dC}
SD3	32.14±1.82 ^{bcdA}	30.76±1.28 ^{efgB}	26.84±1.37 ^{deC}
SD4	34.18±2.34 ^{cdA}	32.55±1.45 ^{ghB}	28.63±1.38 ^{efC}
SD5	29.17±1.26 ^{aA}	26.65±1.30 ^{acB}	24.32±1.24 ^{cC}
SD6	33.56±1.67 ^{cdA}	31.12±1.32 ^{fgB}	26.87±1.36 ^{deC}
SD7	31.64±1.01 ^{bcA}	29.92±1.26 ^{efB}	28.41±1.40 ^{efC}

SD8	34.55±2.24 ^{dA}	33.17±1.48 ^{hB}	29.74±1.42 ^{fC}
SD9	32.56±1.73 ^{bcdA}	28.94±1.37 ^{deB}	25.32±1.25 ^{cdC}
SD10	32.45±1.56 ^{bcdA}	29.97±1.32 ^{efB}	27.14±1.36 ^{deC}
SD11	32.87±1.83 ^{cdA}	29.92±1.34 ^{efB}	27.08±1.36 ^{deC}
SD12	34.78±2.45 ^{dA}	32.17±1.50 ^{ghB}	30.05±1.46 ^{fC}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitalized letters represent significant dry matter amount differences among sourdough breads.

Table B.6 Dry matter of sourdough bread results

Sourdough bread	0. day	3. day	7. day
Control	69.85±3.21 ^{abA}	74.90±4.12 ^{adB}	79.85±4.17 ^{acC}
SD1	67.77±3.18 ^{abA}	77.02±4.45 ^{aB}	78.05±4.15 ^{acC}
SD2	66.84±3.05 ^{abA}	72.16±4.14 ^{abcdB}	73.68±4.05 ^{bcC}
SD3	67.86±3.12 ^{abA}	69.24±4.18 ^{bcdB}	73.16±4.08 ^{bcC}
SD4	65.82±3.14 ^{bA}	67.45±4.20 ^{bcB}	71.37±4.10 ^{bC}
SD5	70.83±3.34 ^{aA}	73.35±4.34 ^{acdB}	75.68±4.24 ^{abC}
SD6	66.44±3.56 ^{abA}	68.88±4.12 ^{bcB}	73.13±4.25 ^{bcC}
SD7	68.36±3.26 ^{abA}	70.08±3.98 ^{bcdB}	71.59±3.97 ^{bC}
SD8	65.45±3.45 ^{bA}	66.83±3.78 ^{hB}	70.26±3.87 ^{bC}
SD9	67.44±3.38 ^{abA}	71.06±4.05 ^{bcdB}	74.68±3.75 ^{abcC}
SD10	67.55±3.40 ^{abA}	70.03±3.90 ^{bcdB}	72.86±3.78 ^{bcC}
SD11	67.13±3.41 ^{abA}	70.08±4.00 ^{bcdB}	72.92±4.10 ^{bcC}
SD12	65.22±3.16 ^{bA}	67.83±3.42 ^{bcB}	69.95±3.85 ^{bC}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitilized letters represent significant dry matter amount differences among sourdugh breads.

Table B.7 Crust and crumb color of sourdough breads

Sourdough bread	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Control	38.43±2.8 ^a	12.48±0.2 ^a	12.45±0.6 ^a	54.34±1.9 ^{ab}	6.32±0.1 ^a	18.41±0.7 ^a
SD1	40.03±2.8 ^a	12.40±0.4 ^b	17.78±0.9 ^{de}	52.14±2.6 ^{ad}	6.48±0.2 ^a	18.57±0.8 ^{ae}
SD2	30.56±2.3 ^b	13.92±0.1 ^f	21.42±0.6 ⁱ	47.26±2.1 ^c	5.13±0.7 ^c	17.23±0.7 ^{cd}
SD3	32.97±4.2 ^{bc}	11.23±0.1 ^c	17.12±0.9 ^{cd}	51.18±2.6 ^d	6.21±0.3 ^{ab}	18.64±0.6 ^{ae}
SD4	39.65±2.8 ^a	12.38±0.3 ^{ab}	19.67±0.2 ^g	54.04±1.9 ^{abd}	5.69±0.6 ^b	19.70±0.8 ^f
SD5	36.56±3.4 ^{ac}	11.56±0.5 ^c	18.98±0.4 ^{fg}	57.24±1.5 ^e	4.38±0.3 ^{de}	17.95±0.5 ^{ad}
SD6	47.23±2.5 ^d	12.87±0.4 ^{de}	12.45±0.6 ^a	57.76±1.6 ^{ef}	4.93±0.6 ^{cd}	19.97±0.4 ^f
SD7	38.64±2.0 ^a	12.71±0.2 ^d	16.68±0.9 ^{bc}	53.19±1.6 ^{abd}	3.45±0.5 ^f	17.16±0.4 ^{cd}
SD8	36.25±3.4 ^{ac}	12.42±0.3 ^d	16.21±0.7 ^b	58.45±2.4 ^f	3.98±0.4 ^{ef}	18.52±0.7 ^{ae}
SD9	40.05±2.2 ^a	13.32±0.7 ^e	19.34±0.4 ^g	56.13±1.9 ^{be}	3.54±0.1 ^f	17.21±0.2 ^{cd}
SD10	37.41±3.5 ^a	11.26±0.6 ^c	16.05±0.3 ^b	60.36±2.4 ^{ef}	4.68±0.3 ^{cd}	19.26±0.6 ^{ef}
SD11	31.14±2.3 ^b	13.43±0.4 ^{ef}	19.52±0.6 ^g	52.83±2.6 ^{ad}	3.98±0.6 ^{ef}	16.93±0.3 ^c

SD12	32.18±2.8 ^b	12.34±0.8 ^d	18.28±0.3 ^{ef}	52.21±2.4 ^{ad}	3.64±0.2 ^f	15.95±0.7 ^b
-------------	------------------------	------------------------	-------------------------	-------------------------	-----------------------	------------------------

Values are the mean ± SD. In the columns, different small letters represent significant differences.



Table B.8 Texture results

Sourdough bread	4 hours					24 hours				
	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mj)	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mj)
Control	2452.50±21.5 ^{aA}	0.81±0.05 ^{abA}	0.85±0.06 ^{abcA}	1986.53±24.6 ^{aA}	1688.55±21.5 ^{aA}	3879.10±30.2 ^{aB}	0.77±0.04 ^{aB}	0.80±0.06 ^{aB}	2986.91±30.2 ^{aB}	2389.53±33.5 ^{aB}
SD1	1142.30±20.7 ^{aA}	0.83±0.05 ^{baA}	0.85±0.04 ^{abcA}	948.11±21.3 ^{baA}	805.89±22.3 ^{baA}	2698.30±31.4 ^{hB}	0.69±0.03 ^{bcB}	0.80±0.07 ^{aB}	1861.83±28.7 ^{kB}	1489.46±23.7 ^{kB}
SD2	2130.40±21.3 ^{iA}	0.79±0.03 ^{abA}	0.84±0.04 ^{abcA}	1683.02±18.7 ^{kaA}	1408.68±18.7 ^{jaA}	3864.60±30.8 ^{aB}	0.73±0.02 ^{abB}	0.76±0.05 ^{abB}	2821.16±27.6 ^{lB}	2144.08±13.2 ^{lB}
SD3	2098.50±22.1 ^{hA}	0.76±0.04 ^{aA}	0.87±0.05 ^{caA}	1594.86±17.5 ^{jaA}	1393.91±17.6 ^{jaA}	3904.00±25.6 ^{fbB}	0.67±0.03 ^{cbB}	0.82±0.06 ^{aB}	2615.68±23.4 ^{hbB}	2144.85±13.8 ^{hbB}
SD4	1100.40±18.6 ^{dA}	0.78±0.06 ^{abA}	0.84±0.03 ^{abcA}	858.31±16.1 ^{faA}	722.70±9.4 ^{eaA}	2780.30±26.8 ^{dB}	0.68±0.05 ^{bcB}	0.78±0.07 ^{abB}	1890.60±18.7 ^{gB}	1474.67±14.7 ^{gB}
SD5	2120.70±21.3 ^{hiA}	0.62±0.02 ^{caA}	0.78±0.02 ^{aA}	1314.83±17.7 ^{iaA}	1024.26±16.5 ^{iaA}	3851.90±28.7 ^{gB}	0.61±0.04 ^{deB}	0.70±0.04 ^{bB}	2349.66±24.5 ^{iB}	1644.76±15.6 ^{iB}
SD6	1500.40±19.5 ^{gA}	0.61±0.03 ^{caA}	0.90±0.06 ^{caA}	915.24±16.3 ^{gaA}	820.06±14.7 ^{gaA}	2847.40±25.4 ^{eB}	0.56±0.03 ^{efB}	0.80±0.03 ^{aB}	1594.54±21.4 ^{eB}	1275.64±13.2 ^{fbB}
SD7	1230.50±16.7 ^{eA}	0.64±0.02 ^{cdA}	0.83±0.05 ^{abcA}	787.52±17.5 ^{eaA}	650.49±14.2 ^{daA}	2780.60±26.5 ^{dB}	0.53±0.03 ^{fbB}	0.80±0.05 ^{aB}	1473.72±22.5 ^{dB}	1178.97±14.1 ^{ebB}
SD8	1177.20±21.4 ^{baA}	0.69±0.05 ^{daA}	0.86±0.07 ^{bcA}	812.27±16.8 ^{daA}	698.55±15.1 ^{daA}	2179.20±25.4 ^{cbB}	0.65±0.06 ^{cdB}	0.81±0.06 ^{aB}	1416.48±26.7 ^{cbB}	1147.35±12.7 ^{dbB}
SD9	1080.80±15.6 ^{cdA}	0.75±0.07 ^{aA}	0.79±0.04 ^{abA}	810.60±21.5 ^{laA}	640.37±16.7 ^{kaA}	2790.80±31.2 ^{ibB}	0.58±0.05 ^{efB}	0.78±0.08 ^{abB}	1618.66±28.6 ^{jbB}	1262.55±18.2 ^{jbB}
SD10	1428.70±20.1 ^{faA}	0.64±0.04 ^{cdA}	0.83±0.05 ^{abcA}	914.37±20.8 ^{gaA}	754.35±9.6 ^{faA}	2880.10±25.7 ^{efB}	0.57±0.06 ^{efB}	0.78±0.09 ^{abB}	1641.66±18.7 ^{fbB}	1280.49±17.5 ^{fbB}
SD11	1480.60±16.5 ^{gA}	0.78±0.06 ^{abA}	0.80±0.03 ^{abA}	1154.87±23.4 ^{haA}	923.89±10.4 ^{haA}	2780.70±26.9 ^{dB}	0.54±0.05 ^{fbB}	0.70±0.05 ^{bbB}	1501.58±16.8 ^{dbB}	1051.11±13.8 ^{cbB}
SD12	1070.40±15.4 ^{caA}	0.62±0.04 ^{caA}	0.83±0.07 ^{abcA}	663.65±17.3 ^{caA}	550.83±14.8 ^{caA}	2080.50±24.3 ^{bbB}	0.57±0.04 ^{efB}	0.80±0.07 ^{abB}	1185.89±17.6 ^{bbB}	948.71±15.6 ^{bbB}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitalized letters represent significant texture results

amount differences among sourdough breads.

Table B.9 Enthalpy results for sourdough breads that produced LAB and yeasts combinations

Sourdough bread	Days	Entalphy (j/g)
Control	0.	105.4±7.8 ^{aA}
	3.	135.7±5.6 ^{aB}
	7.	260.3±8.2 ^{aC}
SD1	0.	103.7±5.4 ^{abA}
	3.	118.4±4.2 ^{cB}
	7.	210.3±7.6 ^{cC}
SD2	0.	93.1±3.2 ^{cdA}
	3.	100.6±4.1 ^{efB}
	7.	187.3±6.4 ^{dC}
SD3	0.	102.5±3.8 ^{abA}
	3.	121.5±5.8 ^{gbcB}
	7.	212.6±8.1 ^{cC}
SD4	0.	89.7±4.0 ^{deA}
	3.	95.1±4.8 ^{fgB}
	7.	178.9±6.7 ^{dC}
SD5	0.	103.4±4.3 ^{abA}
	3.	125.6±4.8 ^{dB}
	7.	230.3±6.1 ^{bC}
SD6	0.	90.5±3.3 ^{deA}
	3.	115.5±3.6 ^{cdB}
	7.	187.4±6.3 ^{dC}
SD7	0.	101.7±4.3 ^{abA}
	3.	120.5±5.7 ^{bcB}
	7.	210.8±5.9 ^{cC}
SD8	0.	85.7±4.2 ^{efA}
	3.	93.6±3.6 ^{gB}
	7.	167.4±6.8 ^{eC}
SD9	0.	95.6±4.8 ^{cdA}
	3.	110.3±4.7 ^{dB}
	7.	205.4±6.0 ^{cC}
SD10	0.	98.7±4.1 ^{bcA}
	3.	115.2±4.9 ^{cdB}
	7.	210.7±5.7 ^{cC}
SD11	0.	92.1±3.4 ^{dA}
	3.	102.6±4.3 ^{eB}
	7.	208.7±5.9 ^{cC}
SD12	0.	80.5±4.6 ^{fA}
	3.	89.7±4.8 ^{gB}
	7.	165.7±6.5 ^{eC}

Values are the mean $\pm$ SD. In the rows, different small letters represent significant differences. In the columns, different capitalized letters represent significant enthalpy amount differences among sourdough breads.

Table B.10 Microbiological results for second combinations

Sourdough	LAB log (kob)/g	MAB log (kob)/g	Yeasts log (kob)/g
Control	2.98 $\pm$ 0.09 ^a	5.88 $\pm$ 0.03 ^a	7.97 $\pm$ 0.02 ^a
SD13	6.86 $\pm$ 0.12 ^b	4.68 $\pm$ 0.09 ^b	5.89 $\pm$ 0.06 ^b
SD14	8.64 $\pm$ 0.09 ^c	4.14 $\pm$ 0.02 ^c	7.88 $\pm$ 0.06 ^c
SD15	6.58 $\pm$ 0.03 ^b	5.16 $\pm$ 0.12 ^d	6.80 $\pm$ 0.06 ^d
SD16	7.18 $\pm$ 0.08 ^b	4.61 $\pm$ 0.09 ^b	6.12 $\pm$ 0.03 ^b
SD17	9.12 $\pm$ 0.01 ^c	4.10 $\pm$ 0.12 ^c	6.01 $\pm$ 0.03 ^b

Values are the mean $\pm$ SD. In the columns, different small letters represent significant differences.

Table B.11 Sourdough pH and TTA results for second combinations

Sourdough	pH	TTA (%)
Control	5.62 $\pm$ 0.01 ^a	3.34 $\pm$ 0.08 ^a
SD13	3.62 $\pm$ 0.08 ^b	3.36 $\pm$ 0.06 ^b
SD14	3.43 $\pm$ 0.03 ^c	3.90 $\pm$ 0.06 ^c
SD15	4.15 $\pm$ 0.01 ^d	3.25 $\pm$ 0.10 ^b
SD16	3.60 $\pm$ 0.15 ^b	3.67 $\pm$ 0.04 ^b
SD17	3.45 $\pm$ 0.03 ^c	3.95 $\pm$ 0.02 ^d

Values are the mean $\pm$ SD. In the columns, different small letters represent significant differences.

Table B.12 Sourdough volume results for second combinations

Sourdough	Volume (%)
Control	140.87 $\pm$ 12.4 ^a
SD13	156.56 $\pm$ 10.1 ^b
SD14	180.87 $\pm$ 10.1 ^c
SD15	153.54 $\pm$ 10.3 ^b
SD16	162.60 $\pm$ 10.3 ^b
SD17	189.12 $\pm$ 10.3 ^d

Values are the mean $\pm$ SD. In the columns, different small letters represent significant differences.

Table B.13 Sourdough bread pH and TTA results for second combinations

Sourdough bread	pH	TTA (%)
Control	5.92 $\pm$ 0.04 ^a	2.30 $\pm$ 0.03 ^a
SD13	3.90 $\pm$ 0.03 ^e	2.42 $\pm$ 0.04 ^b
SD14	3.75 $\pm$ 0.02 ^c	3.10 $\pm$ 0.05 ^d
SD15	4.32 $\pm$ 0.05 ^f	2.34 $\pm$ 0.02 ^a
SD16	3.85 $\pm$ 0.03 ^d	2.78 $\pm$ 0.04 ^c

SD17	3.70±0.02 ^b	3.21±0.05 ^e
------	------------------------	------------------------

Values are the mean ± SD. In the columns, different small letters represent significant differences.

Table B.14 Sourdough bread moisture results for second combinations

Sourdough bread	0. day	3. day	7. day
Control	30.10±1.28 ^{aA}	28.14±1.30 ^{aB}	26.54±1.23 ^{aC}
SD13	32.16±1.34 ^{bA}	30.12±1.42 ^{bB}	28.12±1.27 ^{aC}
SD14	34.80±1.42 ^{cA}	32.13±1.45 ^{cB}	30.17±1.35 ^{bC}
SD15	31.45±1.15 ^{abA}	29.02±1.32 ^{abB}	27.89±1.31 ^{aC}
SD16	34.20±1.16 ^{cA}	32.17±1.40 ^{cB}	30.13±1.45 ^{bC}
SD17	35.20±1.56 ^{cA}	33.23±1.50 ^{cB}	31.67±1.43 ^{bC}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitalized letters represent significant dry matter amount differences among sourdough breads.

Table B.15 Sourdough bread dry matter results for second combinations

Sourdough bread	0. day	3. day	7. day
Control	69.90±3.45 ^{aA}	71.86±3.68 ^{aB}	73.46±4.12 ^{aC}
SD13	67.84±3.47 ^{abA}	69.88±3.56 ^{aB}	71.88±4.10 ^{aC}
SD14	65.20±3.32 ^{abA}	67.87±3.48 ^{aB}	69.83±3.68 ^{aC}
SD15	68.55±3.50 ^{abA}	70.98±3.65 ^{aB}	72.11±4.03 ^{aC}
SD16	65.80±3.42 ^{abA}	67.83±3.71 ^{aB}	69.87±3.70 ^{aC}
SD17	64.80±3.51 ^{bA}	66.77±3.74 ^{aB}	68.33±3.72 ^{aC}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitalized letters represent significant dry matter amount differences among sourdough breads.

Table B.16 Crust and crumb color of sourdough breads for second combinations

Sourdough bread	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Control	43.50±2.3 ^a	13.12±1.5 ^a	15.62±1.5 ^a	61.10±4.2 ^a	5.72±0.3 ^a	17.41±1.8 ^a
SD13	38.10±2.6 ^a	11.37±1.2 ^a	17.88±1.8 ^b	55.70±4.2 ^a	5.43±0.4 ^a	20.93±2.1 ^b
SD14	37.50±3.1 ^a	14.94±1.5 ^a	17.93±1.3 ^{bc}	52.60±5.1 ^c	4.05±0.2 ^a	15.41±1.7 ^c
SD15	42.40±2.7 ^a	11.31±1.3 ^a	14.86±1.7 ^b	52.80±4.8 ^c	3.58±0.1 ^a	18.23±1.8 ^b
SD16	38.80±3.1 ^a	11.16±1.7 ^a	16.36±1.5 ^b	58.30±4.7 ^a	4.90±0.5 ^a	18.80±1.9 ^b
SD17	36.40±3.2 ^b	13.25±1.3 ^a	18.94±1.8 ^c	54.50±5.2 ^b	4.52±0.4 ^a	19.34±1.7 ^b

Values are the mean ± SD. In the columns, different small letters represent significant differences.

Table B.17 Texture results

Sourdough bread	4 hours					24 hours				
	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mj)	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mj)
Control	2315.6±22.6 ^{aA}	0.80±0.06 ^{aA}	0.85±0.04 ^{aA}	1852.48±24.1 ^{aA}	1574.61±16.7 ^{aA}	4780.4±32.4 ^{aB}	0.76±0.05 ^{aB}	0.80±0.05 ^{abB}	3633.10±28.7 ^{aB}	2906.48±23.8 ^{aB}
SD13	1245.3±21.3 ^{dA}	0.77±0.05 ^{abA}	0.80±0.06 ^{abcA}	958.88±18.7 ^{dA}	767.10±11.2 ^{dA}	2663.3±25.6 ^{eB}	0.70±0.04 ^{abB}	0.81±0.04 ^{aB}	1864.31±21.3 ^{eB}	1510.09±21.3 ^{eB}
SD14	1180.4±19.7 ^{cA}	0.72±0.05 ^{bcA}	0.77±0.03 ^{bcdA}	849.89±16.5 ^{cA}	654.41±13.4 ^{cA}	2080.7±24.8 ^{cB}	0.65±0.07 ^{bcB}	0.76±0.06 ^{abcB}	1352.46±17.8 ^{cB}	1027.87±17.6 ^{cB}
SD15	1360.7±18.6 ^{cA}	0.78±0.07 ^{abA}	0.72±0.05 ^{dA}	1061.35±20.7 ^{eA}	766.29±13.7 ^{dA}	2789.5±27.4 ^{fB}	0.73±0.08 ^{abB}	0.74±0.03 ^{bcB}	2036.34±20.9 ^{fB}	1506.89±14.8 ^{eB}
SD16	1185.2±20.6 ^{cA}	0.74±0.04 ^{abA}	0.75±0.04 ^{cdA}	874.68±17.5 ^{cA}	656.01±12.6 ^{cA}	2160.4±19.7 ^{dB}	0.68±0.06 ^{abcB}	0.73±0.04 ^{cB}	1469.07±15.3 ^{dB}	1072.42±14.3 ^{dB}
SD17	1120.5±19.7 ^{bA}	0.65±0.05 ^{cA}	0.83±0.07 ^{abA}	728.33±16.8 ^{bA}	604.51±12.8 ^{bA}	1980.7±23.4 ^{bB}	0.60±0.04 ^{cB}	0.80±0.05 ^{abB}	1188.42±12.4 ^{bB}	950.74±16.7 ^{bB}

Values are the mean ± SD. In the columns, different small letters represent significant differences. In the rows, different capitalized letters represent significant texture results amount differences among sourdough breads.

Table B.18 Enthalpy results for second combinations

Days	Control	SD13	SD14	SD15	SD16	SD17
0.	110.3±8.7 ^{aA}	109.7±7.4 ^{aA}	90.7±4.3 ^{bA}	108.4±5.4 ^{aA}	98.4±6.1 ^{bA}	75.6±5.2 ^{cA}
3.	116.7±7.6 ^{aB}	112.3±5.4 ^{abB}	105.3±6.7 ^{bB}	113.6±4.7 ^{aB}	110.3±5.3 ^{abB}	87.5±4.1 ^{cB}
7.	245.9±6.8 ^{aC}	240.6±4.5 ^{aC}	238.9±5.3 ^{aC}	240.7±3.4 ^{aC}	243.1±4.7 ^{aC}	145.8±4.2 ^{cC}

Values are the mean ± SD. In the rows, different small letters represent significant differences. In the columns, different capitalized letters represent significant enthalpy amount differences among sourdough breads.

APPENDIX C- SENSORY ANALYSIS FORM AND PICTURES

Table C.1 Sensory characteristics of sourdough bread samples

External features				Internal features									
Volume	Crust color	Shape	Crust features	Color	Taste	Wetness	Dryness	Swallowability	Acidity	Tissue softness	Pore size	Chewability	General acceptability
4. h													
24. h													
7. d													

Table C.2 Sourdough breads during baking



Table C.3 Crumb of bread after baking



CURRICULUM VITAE (CV)

AYŞE SEVGİLİ

PERSONEL INFORMATION:

Name Surname: Ayşe SEVGİLİ

EDUCATION:

Degree	Department	University	Graduation Year
Bachelor Degree	Food Engineering	AKDENİZ University	06.03.2012
Master Degree	Food Engineering	GAZİANTEP University	29.02.2016
Doctorate Degree	Food Engineering	GAZİANTEP University	2016-

Master Thesis Study: Improving Lycopene Production by *Blakeslea trispora* using Ingredients.

Doctorate Thesis Study: Identification of Lactic Acid Bacteria and Yeasts of Turkish Sourdoughs and Selection of Starter Cultures for Sourdough Products.

Doctorate Project: Genetic Identification of Lactic Acid Bacteria and Yeasts Isolated from Sourdoughs and their Usability in Sourdough Bread Production, 1002 TUBITAK Project (2020-2021).

Education Mobility: Erasmus program in Warsaw University of Life Sciences for 5 months in 2009.

WORK EXPERIENCE:

Lecturer	Naci Topçuoğlu Vocational School-Food Quality Control and Analysis Program	2019-
----------	--	-------

Administrative Duties:

Food Quality Control and Analysis Program Coordinator

PUBLICATIONS:

A. Articles published in international peer-reviewed journals:

A1. Improved Lycopene Production from Different Substrates by Mated Fermentation of *Blakeslea Trispora*, [Foods](#). 2019 Apr; 8(4): 120.

A2. Identification of lactic acid bacteria and yeasts from traditional sourdoughs and sourdough production by enrichment, Czech Journal of Food Sciences, 2021 39: 312–318.

B. Papers presented at international scientific meetings and published in the proceedings book:

B1. Bindak R, Erkmen O, Sevgili A. Statistical Evaluation of the Effect of Glucose, Sucrose and Natural Fat Additives on Lycopene Production. III. International Vocational and Technical Sciences Congress. 21 June 2018, Gaziantep-Turkey.

B2. Sevgili A, Erkmen O. Mardin Ekşi Hamurlarından Laktik Asit Bakterileri ve Maya İzolasyonu. Eurasia 3rd International Multidisciplinary Studies Congress.

B3. Sevgili A, Erkmen O. Ekşi Hamurdaki Laktik Asit Bakterileri ve Mayaların Metabolizması. Eurasia 3rd International Multidisciplinary Studies Congress.

B4. Koçaslan S, Sevgili A, Erkmen O. Morphological and Technological Characteristics of Isolated Yeast and Lactic Acid Bacteria Species from Sourdough Samples. EJONS 12th International Congress on Mathematic, Engineering and Natural Sciences, 9-11 July 2021, Paris-France.

B5. Büyük K, Sevgili A, Erkmen O. The Effects of Sourdough on the of Morphological Structure of Bread. EJONS 12th International Congress on Mathematic, Engineering and Natural Sciences, 9-11 July 2021, Paris-France.

B6. Sevgili A, Erkmen O. Effect of Different Combination Lactic Acid Bacteria and Yeasts on Physicochemical, Texture and Calorimetric Properties of Sourdough Bread. EJONS 12th International Congress on Mathematic, Engineering and Natural Sciences, 9-11 July 2021, Paris-France.

B7. Sevgili A, Erkmen O. Physicochemical and Microbiological Characteristics of Sourdough. EJONS 12th International Congress on Mathematic, Engineering and Natural Sciences, 60-66, 9-11 July 2021. Paris-France.

B8. Sevgili A, Erkmen O. The Microscopic Life in Human Body: Microbiota. 3rd International Modern Scientific Research Congress, Istanbul-Turkey.

- E. Papers presented at national scientific meetings and published in proceedings books:

E1. Sevgili A, Erkmen O. Fermentasyonda Glikoz ve Sıvı Yağlar Kullanarak *Blakeslea trispora* ile Likopen Üretimi, 18th National Congress of Biotechnology, poster statement. Konya-Turkey.