ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Cennet Pelin BOYACI GÜNDÜZ

MOLECULAR CHARACTERIZATION OF THE PREDOMINANT LACTIC ACID BACTERIA AND YEASTS IN THE SOURDOUGH AND CHICKPEA FERMENTATIONS AND INVESTIGATION OF SOME LACTIC ACID BACTERIA FOR POTENTIAL STARTER CULTURE USAGE

DEPARTMENT OF FOOD ENGINEERING

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Doctor of Philosophy by the board of jury on 19/07/2018.

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ABSTRACT

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MOLECULAR CHARACTERIZATION OF THE PREDOMINANT LACTIC ACID BACTERIA AND YEASTS IN THE SOURDOUGH AND CHICKPEA FERMENTATIONS AND INVESTIGATION OF SOME LACTIC ACID BACTERIA FOR POTENTIAL STARTER CULTURE USAGE

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In the present study, a total of 20 sourdough, chickpea liquid starter and dough samples were collected from different bakeries at two different times. Sourdough and chickpea fermentations were also conducted under laboratory conditions. Microbiological and chemical properties of collected samples were investigated and lactic acid bacteria and yeasts were isolated and identified by molecular methods. In sourdough fermentations, analysis by 16S rRNA gene sequencing grouped the strains into 18 lactic acid bacteria species and the most frequent isolates were *Lactobacillus sanfranciscensis* (32.7%), *Lactobacillus plantarum* (18.6%) and *Lactobacillus paralimentarious* (15.9%). In chickpea fermentations, 12 lactic acid bacteria species were identified and the most prevalent species were *Weissella confusa* (44.6%), *Enterococcus faecium* (25.6%) and *Weissella cibaria* (11.6%). PCR-RFLP analysis identified *Saccharomyces cerevisiae* in the sourdough (72.5%) and chickpea fermentations (40.7%) as the most frequent yeast species. Other isolated yeast species were *Kazachstania bulderi*, *Pichia membranifaciens*, *Kazachstania servazzii*, *Kazachstania unispora* and *Hanseniaspora valbyensis* for sourdoughs and *Candida parapsilosis*, *Meyerozyma guilliermondii* and *Cryptococcus albidosimilis* for chickpea fermentations. *Pichia fermentans* was isolated from both of the fermentations. According to the technological potential, *Lactobacillus plantarum* XL23 and *Lactobacillus sanfranciscensis* RL976 strains were used as mono- and dual-culture in the production of experimental sourdoughs and *Weissella confusa* RL1139 strain was used as mono-culture in the production of experimental chickpea liquid starters.

Key Words: Sourdough, chickpea liquid starter, LAB, yeasts, PCR

ÖZ

DOKTORA TEZİ

NOHUT MAYASI VE EKŞİ HAMUR FERMANTASYONLARINDAKİ LAKTİK ASİT BAKTERİLERİNİN VE MAYALARIN MOLEKÜLER YÖNTEMLERLE TANIMLANMASI VE BAZI LAKTİK ASİT BAKTERİLERİNİN STARTER KÜLTÜR OLARAK KULLANILMA POTANSİYELLERİNİN ARAŞTIRILMASI

Cennet Pelin BOYACI GÜNDÜZ

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Bu çalışmada, farklı yerlerden iki farklı zamanda 20 adet ekşi hamur, nohut süzüntüsü mayası ve nohut mayası hamuru örneği toplanmıştır. Ayrıca laboratuvar koşullarında da ekşi hamur ve nohut mayası üretimi gerçekleştirilmiştir. Toplanan örneklerin mikrobiyolojik ve kimyasal özellikleri araştırılmıştır. Laktik asit bakterileri ve mayalar izole edilerek moleküler yöntemlerle tanımlanmışlardır. Ekşi hamurlarda 16S rRNA gen sekans analizleri izolatları 18 türe ayırmıştır. *Lactobacillus sanfranciscensis* (%32.7), *Lactobacillus plantarum* (%18.6) ve *Lactobacillus paralimentarious* (%15.9) en sık izole edilen türlerdir. Nohut mayası fermentasyonlarında, *Weissella confusa* (%44.6), *Enterococcus faecium* (%25.6) ve *Weissella cibaria* (%11.6) en sık izole edilenler olmakla birlikte toplamda 12 farklı tür tanımlanmıştır. PCR-RFLP sonuçlarına göre, *Saccharomyces cerevisiae* ekşi hamur (%72.5) ve nohut mayası fermantasyonlarında (%40.7) en çok bulunan maya türüdür. Diğer izole edilen maya türleri ise ekşi hamurlarda *Kazachstania bulderi, Pichia membranifaciens, Kazachstania servazzii, Kazachstania unispora* ve *Hanseniaspora valbyensis,* nohut mayalarında *Candida parapsilosis, Meyerozyma guilliermondii* ve *Cryptococcus albidosimilis* olarak belirlenmiştir. *Pichia fermentans* her iki fermantasyondan da izole edilmiştir. Teknolojik potansiyellerine göre, *Lactobacillus plantarum* XL23 ve *Lactobacillus sanfranciscensis* RL976 saf ve karışık kültür olarak ekşi hamur fermantasyonlarında kullanılırken, nohut mayası fermantasyonlarında *Weissella confusa* RL1139 mono kültür olarak kullanılmıştır.

Anahtar kelimeler: Ekşi hamur, nohut süzüntüsü mayası, LAB, maya, PCR

EXTENDED SUMMARY

In the present study, LAB and yeasts that populate the sourdough and chickpea fermentations were investigated by molecular methods on the samples collected from different locations at two different times. Also chemical and microbiological properties of the collected samples were examined. Some LAB strains were further analysed for their potential to be used as starter culture and selected strains were used in the experimental sourdough and chickpea dough productions.

Totally 20 samples including sourdough (8), chickpea liquid starter (6) and dough (6) samples were collected from different bakeries at two different times. Also sourdough and chickpea fermentations were conducted under laboratory conditions. Microbiological and chemical properties of the collected samples were investigated and a total of 834 lactic acid bacteria and 473 yeast colonies were isolated from samples for molecular identification.

The pH and total acidity levels of the collected sourdough samples were in the range of 3.71-3.96 and 6.78-23.93 mL 0.1 N NaOH /10 g dough, respectively. According to the HPLC analysis, maltose+sucrose, glucose, fructose, ethanol, lactic acid and acetic acid contents were in the range of <LOQ-6.24, 0.81-2.30, 0.78-6.96, 4.39-14.94, 5.15-14.12 and 0.58-2.40 g/kg, respectively. Fermentation quotient of the sourdoughs were in the range of 2.48-5.90. The cell counts of presumptive lactic acid bacteria varied from 4.78 to 11.96 log CFU/g and the highest cell density on mMRS agar were counted as 11.67 log CFU/g in the rye sourdough sample. Presumptive total and non-*Saccharomyces* yeast counts varied from 6.75 to 10.02 log CFU/g on YPD and 2.70 to 8.22 log CFU/g on L-lysine agar media. The highest cell density on YPD agar was counted in the rye sourdough sample. Under laboratory conditions, sourdough was produced at 28°C by propagating over a period of 7 days using the daily back-slopping (refreshment) procedure. At the final refreshment, pH and TTA was determined as 3.60 and

17.56 mL 0.1 N NaOH/10 g dough, respectively. Fermentation quotient of the laboratory produced sourdough was determined as 10.84. Presumptive lactic acid bacteria cell counts on mMRS were 12 log CFU/g at the end of the fermentation.

A total of 439 LAB and 235 yeast isolates were collected from sourdough samples including laboratory scale production. A total of 84 strains representing 178 isolates were confirmed to be members of the lactic acid bacteria. Analysis by 16S rRNA gene sequencing grouped the strains into 18 LAB species, which belonged to six genera: *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* and *Lactococcus. Lactobacillus sanfranciscensis* (32.7%) was the dominant species and followed by *Lactobacillus plantarum* (18.6%) and *Lactobacillus paralimentarious* (15.9%). Also *Lactobacillus paracasei* (7.1%), *Leuconostoc mesenteroides* (4.4%), *Weissella confusa* (3.5%), *Lactobacillus curvatus* (3.5%) and *Lactobacillus brevis* (2.7%) were found as minor species. On the other hand, *Lactobacillus pentosus, Leuconostoc citreum, Lactobacillus paraplantarum, Lactobacillus acidophilus, Enterococcus faecium, Pediococcus inopinatus, Lactobacillus parabrevis, Lactococcus lactis* subsp. *cremoris, Weissella cibaria* and *Pediococcus pentosaceus* were only isolated from 1 or 2 samples.

A total of 153 isolates belonging to 7 yeast species were identified by 26S rRNA gene sequencing. *Saccharomyces cerevisiae* (72.5%) was the dominant yeast species. Other isolated yeast species were *Kazachstania bulderi* (7.2%), *Pichia fermentans* (5.9%), *Pichia membranifaciens* (5.2%), *Kazachstania servazzii* (4.6%), *Kazachstania unispora* (2.6%) and *Hanseniaspora valbyensis* (2%).

The pH and total acidity levels of the collected chickpea liquid starter samples were in the range of 4.82-5.67 and 1.65-3.20 mL 0.1 N NaOH/10 g sample, respectively. The pH and total acidity levels of the collected chickpea dough samples were in the range of 5.12-5.53 and 3.03-5.40 mL 0.1 N NaOH/10 g sample, respectively. According to the HPLC analysis, the content of maltose+sucrose, glucose, fructose, ethanol, lactic acid and acetic acid in the

chickpea liquid starter samples were in the range of 1.25-4.50, 2.59-6.94, 2.18-6.44, 2.49-2.59, <LOQ-0.93 and 0.86-1.23 g/kg, respectively. The content of maltose+sucrose, glucose, fructose, ethanol, lactic acid and acetic acid in the chickpea dough samples were in the range of 20.38-29.38, 5.54-9.80, 4.35-8.44, 2.45-2.81, <LOQ-0.94 and <LOQ-<LOQ g/kg, respectively. Cell counts of presumptive lactic acid bacteria in collected chickpea liquid starters were found to be in the range of 1.60-7.18 log CFU/g on mMRS medium. The mean cell counts of presumptive lactic acid bacteria in collected chickpea dough samples were determined to be in the range of 4.30-6.89 on mMRS medium. Presumptive yeast cells in chickpea liquid and dough samples were in the range of 0-5.85 and <1.00-6.83 log CFU/g on two different media, respectively. According to the microbiological analysis results, the total bacteria counted on NA medium was in the range of 2.20-7.70 and 3.53-7.39 log CFU/g for chickpea liquid starter and dough samples, respectively. The control chickpea liquid starter and dough samples were produced in duplicate under laboratory conditions. Chickpea liquid starter fermentations were conducted at 32 and 37°C for 18 h. At the end of the fermentation, the pH level at 32 and 37°C were 4.91 and 4.75, respectively. Total acidity values were 1.95 and 2.95 mL 0.1 N NaOH/10 g sample for liquid starters fermented at 32 and 37°C, respectively. Following chickpea liquid fermentations, the fermented liquid starter was used in chickpea dough production. At the end of 4 hours of fermentation, the final pH values of both fermentations were close to each other as 4.84 at 32°C and 4.81 at 37°C. Total acidity values were 4.80 and 5.00 mL 0.1 N NaOH/ 10 g dough in the doughs fermented at 32 and 37°C, respectively.

A total of 395 LAB and 238 yeast isolates were collected from chickpea liquid starter and dough samples, including laboratory scale production. A total of 54 strains representing 149 isolates were confirmed to be members of the lactic acid bacteria. Analysis by 16S rRNA gene sequencing grouped the strains into 12 LAB species, which belonged to six genera: *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* and *Streptococcus. Weissella confusa*

(44.6%) was the dominant species, followed by *Enterococcus faecium* (25.6%) and *Weissella cibaria* (11.6%). Furthermore, *Leuconostoc mesenteroides* (5%), *Lactobacillus brevis* (3.3%) and *Streptococcus lutetiensis* (2.5%) were found as minor species. Conversely, *Lactobacillus plantarum, Pediococcus acidilactici*, *Streptococcus salivarius, Enterococcus lactis, Pediococcus pentosaceus* and *Leuconostoc mesenteroides* subsp. *dextranium* were only isolated from 1 or 2 samples.

A total of 59 isolates belonging to 5 species were identified by 26S rRNA gene sequencing. Only one isolate was identified at the genus level as *Wickerhamiella* spp. *Saccharomyces cerevisiae (*40.7%) was the dominant yeast species among all isolated strains. Other isolated yeast species were *Candida parapsilosis* (33.9%), *Meyerozyma guilliermondii* (20.3%), *Pichia fermentans* (3.4%) and *Cryptococcus albidosimilis* (1.7%).

The most frequently isolated lactic acid bacteria species were investigated for technological potential to be used as starter culture in sourdough and chickpea fermentations. According to the technological potential, *Lactobacillus plantarum* XL23 and *Lactobacillus sanfranciscensis* RL976 strains were used as mono- and dual-culture in the production of experimental sourdoughs. Doughs inoculated with mono- or dual-culture of *Lactobacillus plantarum* XL23 reached the pH values less than 4.0 in 12 hours. Dough inoculated with *Lactobacillus sanfranciscensis* RL976 reached pH values less than 4.0 after 24 hours. After 48 hours, the control sourdough exhibited the same patterns with the inoculated sourdoughs and reached pH values below 4.0. Acidity values and LAB counts of the samples confirmed the trend showed by pH. After 24 hours, acidity values of the inoculated sourdoughs were in the range of 15.35-16.03 mL 0.1 N NaOH/10 g dough. At the last refreshment, the highest acidity was determined in the sourdough produced with dual-culture inoculum. As a result of the activities in sourdoughs, some VOC compounds are generated. The SPME-GC-MS chromatographic analysis of the

experimental sourdoughs revealed the presence of 37 VOC compounds belonged to different chemical groups.

Weissella confusa RL1139 strain was used as mono-culture in the production of experimental chickpea liquid starters at 37°C. The final pH and total acidity values of the control and inoculated chickpea liquid starters were 4.92-4.82 and 4.4-4.1 mL 0.1 N NaOH/10 g sample respectively. The final pH values of the control and inoculated chickpea doughs were 4.82 and 4.79, respectively. Final acidity values of the control and inoculated doughs were 5.26 and 5.97 mL 0.1 N NaOH/10 g sample, respectively. The SPME-GC-MS chromatographic analysis revealed the presence of 32 VOC compounds in experimental chickpea fermentations. Butanoic acid was found in all of the fermented chickpea liquid starter and dough samples as the characteristic VOC compound.

In this study, different LAB and yeast species were identified in sourdough, chickpea liquid starter and dough samples and some LAB strains were used in the experimental sourdough and chickpea fermentations as starter culture. Development of starter culture combinations is important to obtain products with same characteristics during the industrial production since by starter culture addition, large scale industrial production of standard sourdough and chickpea breads will be possible everytime at the same quality.

TÜRKÇE GENİŞLETİLMİŞ ÖZET

Bu çalışmada, ekşi hamur ve nohut mayası fermantasyonlarında etkili laktik asit bakterileri ve mayalar farklı yerlerden iki farklı zamanda alınan örneklerden izole edilerek moleküler yöntemlerle tanımlanmışlardır. Ayrıca toplanan örneklerin kimyasal ve mikrobiyolojik özellikleri araştırılmıştır. Bazı laktik asit bakteri suşlarının starter kültür olarak kullanılma potansiyelleri analiz edilmiş ve seçilen suşlar ekşi hamur ve nohut mayası üretimlerinde kullanılmıştır.

Toplamda 20 adet ekşi hamur (8), nohut süzüntüsü mayası (6) ve nohut mayası hamuru (6) örnekleri farklı fırınlardan iki farklı zamanda toplanmıştır. Ayrıca laboratuvar koşullarında da ekşi hamur ve nohut mayası fermantasyonları gerçekleştirilmiştir. Örneklerde mikrobiyolojik ve kimyasal analizler gerçekleştirilmiş ve örneklerden 834 laktik asit bakterisi ve 473 maya izole edilerek moleküler yöntemlerle tanımlanmışlardır.

Ekşi hamur örneklerinin pH ve toplam asitlik değerleri sırasıyla 3.71-3.96 ve 6.78-23.93 mL 0.1 N NaOH/10 g hamur olarak belirlenmiştir. HPLC analizlerine göre maltoz+sakkaroz, glukoz, fruktoz, etanol, laktik asit ve asetik asit miktarları sırasıyla <Tayin limiti-6.24, 0.81-2.30, 0.78-6.96, 4.39-14.94, 5.15-14.12 ve 0.58-2.40 g/kg olarak hesaplanmıştır. Ekşi hamur örneklerinin fermantasyon katsayısı 2.48-5.90 aralığında belirlenmiştir. Muhtemel laktik asit bakterilerinin sayım sonuçları 4.78-11.96 log KOB/g aralığında bulunmuştur ve mMRS besiyerinde sayılan en fazla koloni çavdar ekşi hamur örneğinde 11.67 log KOB/g olarak tespit edilmiştir. Muhtemel toplam maya ve *Saccharomyces* olmayan maya sayım sonuçları sırasıyla YPD besiyerinde 6.75-10.02 log KOB/g ve L-lysine besiyerinde 2.70-8.22 log KOB/g aralığında belirlenmiştir. YPD besiyerinde sayılan en fazla koloni çavdar ekşi hamur örneğinde tespit edilmiştir. Laboratuvar koşullarında, 28°C sıcaklıkta 7 gün boyunca günlük tazeleme yöntemiyle ekşi hamur üretimi gerçekleştirilmiştir. Son tazelemede pH ve toplam

asitlik değerleri sırasıyla 3.60 ve 17.56 mL 0.1 N NaOH/10 g hamur olarak belirlenmiştir. Laboratuvarda üretilen ekşi hamurun fermantasyon katsayısı 10.84 olarak hesaplanmıştır. Son tazelemedeki muhtemel laktik asit bakteri sayım sonuçları mMRS besiyerinde 12 log KOB/g olarak belirlenmiştir.

Toplamda 439 laktik asit bakterisi ve 235 maya ekşi hamur fermantasyonlarından izole edilmiştir. Toplamda 178 isolatı temsil eden 84 suş laktik asit bakterisi olarak tanımlanmıştır. 16s rRNA sekans sonuçlarına göre izolatlar 6 cinse ait 18 türe ayrılmıştır. Bu türler *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* and *Lactococcus* olarak belirlenmiştir. *Lactobacillus sanfranciscensis* (%32.7), *Lactobacillus plantarum* (%18.6) ve *Lactobacillus paralimentarious* (%15.9) en baskın türler olarak belirlenirken, *Lactobacillus paracasei* (7.1 %), *Leuconostoc mesenteroides* (%4.4), *Weissella confusa* (%3.5), *Lactobacillus curvatus* (%3.5) ve *Lactobacillus brevis* (%2.7) az sayıda örnekte tespit edilen türlerdir. Diğer türler *Lactobacillus pentosus, Leuconostoc citreum, Lactobacillus paraplantarum, Lactobacillus acidophilus, Enterococcus faecium, Pediococcus inopinatus, Lactobacillus parabrevis, Lactococcus lactis* subsp. *cremoris, Weissella cibaria* ve *Pediococcus pentosaceus* sadece 1 veya 2 örnekten izole edilmiştir.

Toplamda 7 türe ait 153 maya izolatı 26S rRNA gen sekans sonuçlarına göre tanımlanmışlardır. *Saccharomyces cerevisiae* (%72.5) ekşi hamur fermantasyonlarındaki baskın tür olarak belirlenirken *Kazachstania bulderi* (%7.2), *Pichia fermentans* (%5.9), *Pichia membranifaciens* (%5.2), *Kazachstania servazzii* (%4.6), *Kazachstania unispora* (%2.6) ve *Hanseniaspora valbyensis* (%2) maya türleri de ekşi hamur fermantasyonlarından izole edilmişlerdir.

Toplanan nohut süzüntü mayası örneklerinin pH ve TTA değerleri sırasıyla 4.82-5.67 ve 1.65-3.20 mL 0.1 N NaOH/10 g örnek olarak bulunurken, nohut mayası hamurlarının pH ve toplam asitlik değerleri sırasıyla 5.12-5.53 ve 3.03-5.40 mL 0.1 N NaOH/10 g örnek olarak hesaplanmıştır. HPLC analizlerine göre nohut mayası süzüntülerinde bulunan maltoz+sakkaroz, glukoz, fruktoz, etanol, laktik asit

ve asetik asit miktarları sırasıyla 1.25-4.50, 2.59-6.94, 2.18-6.44, 2.49-2.59, <Tayin limiti-0.93 ve 0.86-1.23 g/kg olarak belirlenmiştir. Nohut mayası hamurlarındaki maltoz+sakkaroz, glukoz, fruktoz, etanol ve laktik asit miktarları sırasıyla 20.38-29.38, 5.54-9.80, 4.35-8.44, 2.45-2.81, <Tayin limiti-0.94 g/kg ve bütün örneklerde asetik asit miktarları tayin limitinin altında belirlenmiştir. Toplanan nohut süzüntüsü mayası örneklerinin mMRS besiyerinde belirlenen muhtemel laktik asit bakteri sayım sonuçları 1.60-7.18 log KOB/g aralığında bulunmuştur. Toplanan nohut mayası hamurlarının mMRS besiyerinde belirlenen muhtemel laktik asit bakteri sayım sonuçları 4.30-6.89 log KOB/g aralığında bulunmuştur. Nohut süzüntüsü mayası ve hamuru örneklerindeki muhtemel maya sayıları 0-5.85 ve <1.00-6.83 log KOB/g aralığında belirlenmiştir. Mikrobiyolojik analiz sonuçlarına göre, NA besiyerinde sayılan toplam bakteri sayısı sırasıyla nohut süzüntüsü mayası ve hamur örneklerinde 2.20-7.70 and 3.53-7.39 log KOB/g aralığında belirlenmiştir. Laboratuvar koşullarında nohut süzüntüsü fermantasyonları 32 ve 37°C'de 18 saat boyunca iki paralleli olarak yapılmıştır. Fermantasyon sonunda, 32 ve 37°C'de gerçekleştirilen fermantasyonlarda pH değerleri sırasıyla 4.91 ve 4.75 olarak belirlenmiştir. Toplam asitlik değerleri ise 32 ve 37°C'de sırasıyla 1.95 and 2.95 mL 0.1 N NaOH/10 g örnek olarak hesaplanmıştır. Nohut mayası süzüntüleri, nohut mayası hamuru üretiminde kullanılmış ve 4 saatlik hamur fermantasyonu sonucunda, 32°C'de pH değeri 4.84 olarak belirlenirken, 37°C'de 4.81 olarak belirlenmiştir. Toplam asitlik ise 32 ve 37°C'de sırasıyla 4.80 ve 5.00 mL 0.1 N NaOH/10 g örnek olarak belirlenmiştir.

Toplamda 395 laktik asit bakterisi ve 238 maya nohut mayası fermantasyonlarından izole edilmiştir. 149 izolatı temsil eden 54 suş laktik asit bakterisi olarak tanımlanmıştır. 16s rRNA sekans sonuçlarına göre izolatlar 6 cinse ait 12 türe ayrılmıştır. Bu türler *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* ve *Streptococcus* olarak belirlenmiştir. *Weissella confusa* (%44.6) en baskın tür olarak belirlenirken, *Enterococcus faecium* (%25.6) ve *Weissella cibaria* (%11.6) türleri de en sık izole edilen türlerdir. *Leuconostoc*

mesenteroides (%5), *Lactobacillus brevis* (%3.3) and *Streptococcus lutetiensis* (%2.5) az sayıda örnekte tespit edilen türlerdir. Diğer türler *Lactobacillus plantarum, Pediococcus acidilactici, Streptococcus salivarius, Enterococcus lactis, Pediococcus pentosaceus* ve *Leuconostoc mesenteroides* subsp. *dextranium* sadece 1 veya 2 örnekten izole edilmiştir.

Toplamda 5 türe ait 59 maya izolatı 26S rRNA gen sekans sonuçlarına göre tanımlanmışlardır. Sadece bir izolat *Wickerhamiella* spp. olarak cins düzeyinde tanımlanmıştır. *Saccharomyces cerevisiae* (%40.7) nohut mayası fermantasyonlarındaki tüm türler arasında en baskın tür olarak belirlenirken, *Candida parapsilosis* (%33.9), *Meyerozyma guilliermondii* (%20.3), *Pichia fermentans* (%3.4) ve *Cryptococcus albidosimilis* (%1.7) maya türleri de nohut mayası fermantasyonlarından izole edilmişlerdir.

İzole edilen laktik asit bakterilerinin, ekşi hamur ve nohut mayası fermantasyonlarında starter kültür olarak kullanılabilmeleri amacıyla en sık izole edilen laktik asit bakterilerinin teknolojik potansiyelleri araştırılmıştır. Teknolojik potansiyellerine göre, *Lactobacillus plantarum* XL23 ve *Lactobacillus sanfranciscensis* RL976 suşları saf ve karışık kültür olarak laboratuvar koşullarında ekşi hamur üretiminde kullanılmışlardır. *Lactobacillus plantarum* XL23 ile üretilen hamurların pH düzeyleri 12 saat içinde 4.0'ın altına düşmüştür. *Lactobacillus sanfranciscensis* RL976 ile üretilen hamurların pH değerleri 24 saat içinde 4.0'ın altına düşmüştür. 48 saatin sonunda, kontrol ekşi hamuru inokule edilen hamurlarla benzer özellikler göstermiş ve pH değeri 4.0'ün altına inmiştir. Asitlik değerleri ve laktik asit bakteri sayıları da pH ile gözlemlenen benzer durumu yansıtmıştır. 24 saatin sonunda, starter kültürle üretilen ekşi hamurlardaki asitlik değerleri 15.35-16.03 mL 0.1 N NaOH/10 g hamur aralığında belirlenmiştir. En son tazeleme sonunda, en yüksek asitlik dual kültürle üretilen ekşi hamur örneğinde gözlenmiştir. Ekşi hamurdaki mikrobiyal aktiviteler sonucunda bazı uçucu organik bileşikler oluşmaktadır. SPME-GC-MS analiz sonuçlarına göre, laboratuvar

koşullarında üretilen ekşi hamurlarda farklı gruplara ait 37 uçucu organik bileşik tespit edilmiştir.

Laboratuvar koşullarında 37°C'de gerçekleştirilen nohut mayası üretimlerinde *Weissella confusa* RL1139 izolatı saf kültür olarak kullanılmıştır. Kontrol ve kültür inokule edilen süzüntülerdeki son pH ve asitlik değerleri sırasıyla 4.92-4.82 ve 4.4-4.1 mL 0.1 N NaOH/10 g örnek olarak belirlenmiştir. Kontrol ve starter kültür içeren hamur örneklerinde ise son pH değerleri sırasıyla 4.82 ve 4.79 olarak hesaplanmıştır. Son asitlik değerleri ise kontrol ve starter kültür içeren hamur örneklerinde sırasıyla 5.26 ve 5.97 mL 0.1 N NaOH/10 g örnek olarak hesaplanmıştır. SPME-GC-MS analiz sonuçlarına göre 32 uçucu organik bileşik tespit edilmiştir. Nohut mayası fermantasyonlarında tüm örneklerde tespit edilen karakteristik bileşik bütirik asit olmuştur.

Bu çalışmada ekşi hamur, nohut süzüntüsü mayası ve nohut mayası hamuru örneklerinde farklı laktik asit bakterileri ve maya türleri tanımlanmış ve bazı laktik asit bakterileri türleri starter kültür olarak ekşi hamur ve nohut mayası fermantasyonları denemelerinde kullanılmıştır. Starter kültür kombinasyonlarının geliştirilmesi endüstriyel üretimde aynı kalitede son ürün eldesi açısından oldukça önemlidir. Çünkü starter kültür eklenmesi sonucu endüstriyel olarak büyük ölçekli ve her zaman aynı kalitede ekşi hamur ve nohut mayası ekmeklerinin üretimi mümkün olabilecektir.

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LIST OF ABBREVIATIONS

µL	:	Microliter
µm	:	Mikrometer
µM	:	Mikromolar
µmol	:	Micromole
AHC	:	Agglomerative hierarchical clustering
ANOVA	:	Analysis of variance
ATP	:	Adenosine triphosphate
B.	:	*Bacillus*
BLAST	:	Basic local alignment search tool
bp	:	base pair
C.	:	*Candida*
CD	:	Chickpea dough
CFU	:	Colony forming unit
Cl.	:	*Clostridium*
CLS	:	Chickpea liquid starter
CO_2	:	Carbon dioxide
Cr.	:	*Cryptococcus*
d	:	Day
DAP	:	Dihydroxyacetone phosphate
DGGE	:	Denaturing gradient gel electrophoresis
E.	:	*Enterococcus*
EMP	:	The Embden–Meyerhof–Parnas
EPS	:	Exopolysaccharide
ev	:	Electronvolt
FQ	:	Fermentation quotient
g	:	Gram
GAP	:	Glyceraldehyde-3-phosphate
GC-MS	:	Gas chromatography-Mass spectrophotometry
GI	:	Glycaemic index
h	:	Hour
H_2O	:	Water
HPLC	:	High performance lipid chromatography
H'spora.	:	*Hanseniaspora*
I.	:	*Issatchenkia*

ITS	:	Internal transcribed spacer
K.	:	*Kazachstania*
Kg	:	Kilogram
L	:	Litre
LAB	:	Lactic acid bacteria
Lb.	:	*Lactobacillus*
Lc.	:	*Lactococcus*
Leu.	:	*Leuconostoc*
LOD	:	Limit of detection
LOQ	:	Limit of quantification
LST	:	Lauryl Sulfate Tryptose
mg	:	Milligram
min	:	Minute
mL	:	Milliliter
mM	:	Milimolar
mmol	:	Milimole
MPN	:	Most Probable Number
MRS	:	de Man Rogosa Sharpe
NA	:	Nutrient agar
NADH	:	Nicotinamide adenine dinucleotide
NCBI	:	National Center for Biotechnology Information
O_2	:	Oxygen
ºC	:	Centigrade degree
OD	:	Optical density
P.	:	*Pichia*
PCa	:	Principle component analySİ
PCA	:	Plate count agar
PCR	:	Polymerase chain reaction
Pd.	:	*Pediococcus*
R.	:	*Rhodotorula*
RAPD	:	Random Amplified Polymorphic DNA
rflp	:	Restriction Fragment Length Polymorphism
RID	:	Refractive Index Detector
rpm	:	Rotation per minute
s	:	Second
S.	:	*Saccharomyces*

SD	:	Sourdough
SDB	:	Sourdough bacteria medium
SFE	:	Sterile flour extract
SPME	:	Solid phase microextraction
St.	:	*Streptococcus*
T.	:	*Torulaspora*
TBE	:	Tris, boric acid and EDTA
TTA	:	Total titratable acidity
U/g	:	Units per gram
UPMGA	:	Unweighted pair group method with arithmetic average
USDA	:	US Department of Agriculture
UV	:	Ultra violet
VOC	:	Volitile organic compound
vol	:	volume
W.	:	*Weisella*
w/w	:	weight/weight
wt	:	Weight
YPD	:	Yeast Extract Peptone Dextrose

1. INTRODUCTION

Fermentation is one of the oldest known methods of food preservation and production which has been used since ancient times. Fermented foods are produced on a large scale in industry, in addition to traditional small-scale production. Currently, there is an increasing interest into traditional fermented foods as a result of the growing consumer demand for high quality, healthy and natural food products. Nowadays, consumers demand natural food products with a long shelf life. Bread is one of the main staple foods consumed by humans and the trend towards healthy and natural breads with a long shelf life has been increasing.

In recent years, traditional sourdough bread production has gained importance due to increasing demand by consumers for more organic and healthy foods (Arendt et al., 2007; Mariotti et al., 2014; Torrieri et al., 2014; Behera and Ray, 2015). Actually, the use of the sourdough process for the production of sourdough bread has a long tradition but recently there has been an increasing demand for sourdough bread both globally and in Turkey. Sourdough is a mixture of flour (mainly wheat or rye) and water that is fermented with lactic acid bacteria (LAB) and yeasts (Gobbetti, 1998; Vogel et al., 1999; De Vuyst and Neysens, 2005). The use of the sourdough process as a form of leavening during the production of sourdough bread is one of the oldest biotechnological processes in food production and has been used for thousands of years and is "generally regarded as safe". Via the sourdough process, sensorial properties, nutritional values and the shelf life of bread are improved in a natural way. Chickpea bread is a traditional bread produced using a chickpea dough, which is made by fermenting chickpeas in hot water for 16-18 hours. Chickpea bread has a long history in this country and is well-known in the Aegean Region, Thrace Region and also some parts of the Middle Anatolia and Mediterranean Regions of Turkey; it is produced in limited bakeries and, in addition to this country, it is also produced in Greece

and Macedonia (Hatzikamari et al., 2007a). In the literature, research on chickpea fermentation is limited and studies mainly focus on the production of bakery products using chickpea liquid starter and dough. Studies identifying the microorganisms of chickpea fermentations are very scarce. In order to protect such traditional foods, scientific research is essential.

Sensorial properties and the shelf life of breads are improved by fermentation with different microorganisms. Chickpea dough is defined as a "sweet dough" in some regions because of its taste. Sourdough has a lower pH and sour taste compared with chickpea dough. In fact, sourdough and chickpea dough contrast each other but they have a common feature, i.e., exhibiting differing aroma profiles caused by different microorganisms. The above-mentioned bread types are different from each other in many aspects but both are fermented traditional products with great potential. They offer many advantages over bread produced using baker's yeast, i.e., sensorial properties and nutritional value are improved with fermentation, and shelf life is increased. During the production of these breads no additives or commercial yeasts are used and fermentation is conducted spontaneously; therefore, microorganisms play an important role during the fermentation of these foods. In particular, LAB and yeasts are the main microorganisms that are responsible for this type of fermentation. The compounds generated by LAB inhibit the growth of other microorganisms and contribute to the taste and flavor of the final product. Identification of the microorganisms responsible for this fermentation is important for the quality of the final product. As a result of identifying these microorganisms, by the use of highly sensitive methods, starter culture combinations can be developed and then industrial products can be produced to achieve consistent quality of the final product. For this purpose, microorganisms that are responsible for this fermentation must be isolated and then identified using certain techniques. Nowadays, successful identification is performed using genotypic methods and allows investigation of the microbial diversity as a result of the sensitive and fast identification to species and strain

level. The interactions between LAB and yeasts that populate the sourdough and chickpea doughs should be understood, as only then will it be possible to produce these bread types industrially to reach consumers everywhere.

The objectives of the present study are:

- Isolation of LAB and yeasts from sourdough samples produced using traditional method without adding any commercial yeasts collected from three different bakeries in Mersin, Antalya and Ankara at two different times.
- Isolation of the LAB and yeasts from the chickpea liquid starter and dough samples collected from three different bakeries in Aydın, İzmir and Nevşehir at two different times.
- Identification of isolated LAB and yeasts using molecular methods.
- Investigation of the technological properties of the identified LAB to develop starter culture combinations for sourdough and chickpea fermentations.

2. LITERATURE OVERVIEW

2.1. Fermentation

Fermentation is one of the oldest food processing and preservation technique which can be traced back thousands of years. Organoleptic properties are improved and shelf life is extended by fermentation (Smid and Hugenholtz, 2010; Ray and Joshi, 2015). In fermented foods, microbial stability and safety is improved even at ambient temperatures and sensorial properties such as taste, flavor and aroma are developed. The common microorganisms involved in food fermentations are bacteria, mainly LAB, yeasts and molds. LAB, in particular, and then yeasts are the most commonly found microorganisms in fermented foods (Ray and Montet, 2015).

Fermentation plays different roles in food processing as given below: (Hutkins, 2006; Ray and Joshi, 2015).

1. Foods are preserved by fermentation as a result of the formation of inhibitory compounds such as organic acids (lactic acid, acetic acid, formic acid, and propionic acid), ethanol, carbon dioxide, diacetyl, reutrin, bacteriocins, etc., and sometimes in combination with a decrease of water activity by drying or using salt.
2. Food safety is improved by fermentation as a result of the inhibition of pathogens and removal of toxic compounds (Adams and Nicolaides, 1997; Gaggia et al., 2011).
3. Nutritional value is increased (Poutanen et al., 2009).
4. Shelf life is extended (Van Boekel et al., 2010).
5. Functionality and sensory properties of end products are enhanced.
6. Technological aspects and overall quality of the food is developed.

A diverse range of fermented foods are found worldwide and a number of them are globally distributed and produced on a large scale in industry, in addition to small scale production at home (Smid and Hugenholtz, 2010). On the other hand, some fermented foods are produced in specific regions or many regions with different cultural practices. Global fermented foods include plant based fermented foods such as table olives, pickles, sauerkraut, vinegar, cereal base fermented foods such as bread, sourdough, alcoholic beverages such as wine, beer and fermented meat and dairy products such as cheese, yoghurt etc., which are produced worldwide on a large scale. Local fermented foods can be derived from different sources such as cereals, fish, meat, milk, dairy products, vegetables and plants, and are produced in many different parts of the world. Tarhana, şalgam, boza, hardaliye, turşu (Turkey); kefir, koumiss (Caucasian, Central Asia); kimchi (Korea); tempeh (Indonesia); brovada (Italy); gundruk (Indiana, nepal); Pak-Gard-Dong (Thailand); khalpi (Nepal) and fufu (Nigeria) are a few examples of regional fermented foods of the world (Erten and Tangüler, 2014; Erten et al., 2016; Erten et al., 2017; Oguntoyinbo and Franz, 2017; Patra et al., 2017; Swain and Ananadharaj, 2017; Wiander, 2017).

2.1.1. Biochemistry of the Fermentation Process

The word fermentation derives from the Latin verb *fervere* meaning "to boil" as a result of the impression of boiling observed at the beginning of wine fermentation, with the continous release of gas bubbles to the surface (Okafor, 2007). In the middle of the 19th century, Louis Pasteur established the role of microorganisms during fermentation and showed that fermentation is a microbial process; he also demonstrated that it occurs without oxygen (O_2) with the words “life without air” (*la vie sans l’air*) (Prajapati and Nair, 2008; Dufour et al., 2011; Mckay et al., 2011; Madigan et al., 2012).

The word "fermentation" has different meanings (Okafor, 2007). From a microbiological point of view, the term fermentation can be used for any process

produces a microbial product by the mass culture of microorganisms (Stanbury, 2000). However, biochemically, fermentation is defined as a metabolic process involving a carbon source in which organic compounds act as both electron donors and acceptors and energy is generated under anaerobic conditions (Madigan et al., 2012; Erten et al., 2016). In industrial microbiology, the term "fermentation" is any process in which microorganisms are grown on a large scale, even if the final electron acceptor is not an organic compound (i.e., even if growth is carried out under aerobic conditions) (Okafor, 2007).

Every cell needs energy for growth and maintenance and that energy is generated by energetic pathways via metabolic processes (Dufour et al., 2011; Erten et al., 2016). The released energy is conserved in cells by the simultaneous synthesis of energy-rich compounds to drive future energy-requiring cell functions. In living organisms, energy is primarily conserved in phosphorylated compounds and the most important energy-rich phosphate compound in cells is adenosine triphosphate (ATP).

Fermentation and cellular respiration are the two series of reactions that are linked to energy conservation. Fermentation is a form of anaerobic catabolism in which an organic compound functions as both an electron acceptor and an electron donor. During fermentation, ATP is produced by substrate-level phosphorylation directly from energy-rich intermediates via the steps of carbohydrate catabolism (Madigan et al., 2012). The fermentable substrate in fermentation is both the electron donor and electron acceptor and not all compounds can be fermented; however sugars, especially hexoses are excellent fermentable substrates which are preferred by many microorganisms and there are only a few organisms that cannot utilize it (Waites et al., 2001). When the final acceptor is an inorganic compound, the process is called respiration and respiration is referred to as aerobic if the final acceptor is O_2 or anaerobic if the final acceptor is an O_2 substitute, i.e., some other inorganic compound e.g., sulphate or nitrate (Okafor, 2007).

Fermentation and respiration are alternative metabolic choices available to some microorganisms. For example, yeasts are organisms that can both ferment and respire and fermentation is necessary under low level O_2 conditions and when terminal electron acceptors are absent. If sufficient O_2 is available, respiration can take place. Considerably more ATP is produced during respiration, compared with fermentation, and is the preferred choice. However, in many microbial habitats that lack O_2 or other electron acceptors that can be a substitute for O_2, fermentation is the only option for energy conservation (Madigan et al., 2012).

In glycolysis, one molecule of glucose is converted into two molecules of pyruvate and energy and reducing power is conserved in the form of ATP and NADH, respectively. If O_2 is present, glycolysis leads to aerobic respiration; however, in the absence of O_2 it leads to fermentation (Hardin et al., 2012). Under anaerobic conditions, the electrons that are removed from NADH are transferred to pyruvate and then pyruvate is reduced to various fermentation end products, which vary depending on the microorganism (Tortora et al., 2010). The most common end products of pyruvate reduction are lactic acid by LAB, and ethanol and carbon dioxide by yeasts and some other microorganisms (Hardin et al., 2012). During ethyl alcohol fermentation, pyruvate is reduced to acetaldehyde and then acetaldehyde is converted to ethanol by yeasts and some other microorganisms. In lactic acid fermentation, pyruvate is converted to lactic acid as the major end product by LAB.

2.1.2. Carbohydrate Catabolism

Carbohydrate catabolism is the breakdown of carbohydrate molecules to produce energy. In particular, the six carbon sugar, glucose ($C_6H_{12}O_6$), is very important for catabolism. In many vertebrates, including humans, glucose is the main sugar in the blood and also the main energy source for most of the cells in the body. In plants, glucose is the monosaccharide that is produced upon the breakdown of starch. Also, it is one-half of the disaccharide sucrose (glucose+

fructose), the major sugar in the vascular system of most plants (Hardin et al., 2012). As it can be seen, glucose is very important for metabolism and many energy-rich substances are converted into the intermediates of the pathway for glucose catabolism in plants, animals and microorganisms.

Energy is produced from carbohydrates via different pathways such as Embden–Meyerhof–Parnas (Glycolysis), Pentose Phosphate, Entner–Doudoroff and Phosphoketolase (Okafor, 2007; Tortora et al., 2010). Glycolysis, which is also known as the Embden–Meyerhof–Parnas (EMP) pathway, named after its major discoverers, is the most common route and is found in all major groups of organisms, including filamentous fungi, yeasts and many bacteria (Waites et al., 2001).

EMP pathway or Glycolysis is the first stage of carbohydrate catabolism and it occurs in most living cells. The result of this pathway is the breakdown of glucose into pyruvate to generate ATP. Whether glucose is fermented or respired, it travels through this pathway. During glycolysis, two molecules of ATP are consumed and four molecules of ATP are generated and the net energy yield in glycolysis is two molecules of ATP per molecule of glucose (Tortora et al., 2010; Dufour et al., 2011; Madigan et al., 2012).

During the first stage of glycolysis, glucose is phosphorylated and becomes glucose-6-phosphate, is isomerized to fructose-6-phosphate and then 1,6-bisphosphate is produced via phosphorylation. These stages of glucose breakdown consume two molecules of ATP. Fructose 1,6-bisphosphate is then split into two 3-carbon phosphates, glyceraldehyde-3-phosphate (GAP) and its isomer dihydroxyacetone phosphate (DAP) (Wang et al., 2001; Dufour et al., 2011). DAP is isomerized to GAP as only GAP is directly processed through the pathway. Subsequently, a molecule of inorganic phosphate is added to GAP to generate 1,3-bisphosphoglycerate along with the reduction of NAD^+ to NADH (Nicotinamide adenine dinucleotide) by glyceraldehyde-3-phosphate dehydrogenase (Madigan et al., 2012). This redox reaction occurs twice as two molecules of GAP are produced

from glucose. Oxidation of the resultant one glucose molecule to two pyruvate molecules as the end product generates energy in the form of four ATP molecules. However, the net gain in glycolysis is two molecules of ATP per molecule of glucose due to its consumption in the earlier reactions as shown in *Equation 1* (Waites et al., 2001).

$$Glucose\,(C_6) + 2\,ADP + 2P_i + 2\,NAD^+ \rightarrow 2\,pyruvate\,(C_3) + 2\,ATP + 2\,NADH + 2\,H^+$$

Eq. 1

The phosphoketolase (PK) pathway is characteristically observed in heterolactic bacteria, *Leuconostoc* and some *Lactobacillus* species. In this pathway, phosphoketolase is the key enzyme and coverts 5-carbon pentoses such as xylulose, into a 2-carbon acetyl phosphate and 3-carbon glyceraldehyde 3-phosphate (Butler et al., 2010). In this pathway, glucose fermentation yields lactic acid, ethanol and CO_2. It produces only half the yield of ATP compared with the EMP pathway but also allows pentose formation from hexose sugars for nucleic acid synthesis and the catabolism of pentoses (Waites et al., 2001; Okafor, 2007).

2.1.3. Lactic Acid Bacteria (LAB)

LAB produce lactic acid as the major end product of fermentation. LAB comprises a diverse group of microorganisms that have a common metabolic property, i.e., the production of lactic acid from the fermentation of carbohydrates as the major end product (Mayo et al., 2010). The produced lactic acid may be in the form of L (+) or D (-) or a mixture of both (Caplice and Fitzgerald, 1999).

The NAD^+ used during glycolysis must be regenerated for the continuation of glycolysis (Hames and Hooper, 2000). In lactic acid fermentation, NAD^+ is regenerated by the conversion of pyruvate to lactic acid as the end product via

lactate dehydrogenase under anaerobic conditions, as shown in *Equation 2* on a per-glucose basis:

$$2\,pyruvate\,(C_3) + 2\,NADH + 2\,H^+ \rightleftharpoons 2\,lactic\,acid + 2\,NAD^+ \quad Eq.\ 2$$

LAB are one of the most industrially important groups of bacteria used in many processes including food production, health improvement, and the production of macromolecules, enzymes and metabolites. They are found in many foods including milk and dairy products, plant based foods, cereals, and also meat and meat products. LAB are very important in food fermentations and have a very long history of use in the production of many fermented food products such as yoghurt, cheese, pickles, olives, sourdough etc. Many species are used for the production and preservation of fermented foods; in addition, enzymatic activities of LAB contribute to the final organoleptic, rheological and nutritional properties of fermented products (Leroy and De Vuyst, 2004; Mayo et al., 2010).

LAB are Gram-positive, catalase-negative, facultatively anaerobic, usually non-motile, non-respiring and non-spore-forming rods or cocci (Hammes and Hertel, 2009). These unicellular prokaryotes are grouped in the order *Lactobacillales* under the class *Bacilli* of the phylum *Firmicutes*. The order includes 6 families as follows: *Aerococcaceae*, *Carnobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostoccaceae* and *Streptococcaceae* (Garrity and Holt, 2001; Holzapfel and Wood, 2014). LAB are a rapidly expanding group of bacteria with 6 families and 40 genera in their broad physiological definition (Holzapfel and Wood, 2014), with *Carnobacterium*, *Enterococcus (E.)*, *Lactobacillus (Lb.)*, *Aerococcus*, *Lactococcus (Lc.)*, *Leuconostoc (Leu.)*, *Oenococcus*, *Pediococcus (Pd.)*, *Streptococcus (St.)*, *Tetragenococcus*, *Vagococcus* and *Weissella (W.)* generally considered to be the principal LAB genera from a food technology point of view (Axelsson, 2004). Also, the genus *Bifidobacterium*

is often considered to be the genuine LAB due to sharing some typical features but it is phylogenetically unrelated and has a unique mode of sugar fermentation. The genus belongs to the phylum *Actinobacteria* since the members of the genera are phylogenetically more related to the *Actinomycetaceae* group of bacteria (Axelsson, 2004). *Bifidobacterium* metabolizes glucose via the 'Bifidus pathway' (Scardovi, 1986) to yield lactic acid and acetic acid and this is a special pathway, unique to the genus, which clearly separates them from LAB (Hammes and Hertel, 2009; Endo and Dicks, 2014).

From a biochemical perspective, LAB can be grouped according to their ability to ferment glucose as homofermentative (homolactic) or heterofermentative (heterolactic). Homofermentative bacteria produce lactic acid as the main fermentation end product (Von Wright and Axelsson, 2012), whereas heterofermentatives produce a variety of fermentation end products such as ethanol, CO_2, acetic acid, formic acid, acetaldehyde, diacetyl and acetoin, in addition to lactic acid (Kleerebezem and Hugenholtz, 2003). Fermentation metabolism of some LAB are shown in Table 2.1.

Table 2.1. Fermentation metabolism of some LAB

Species	Obligately homofermentative	Facultatively heterofermentative	Obligately heterofermentative
Lactococcus	+		
Pediococcus	+		
Streptococcus	+		
Enterococcus	+		
Weisella			+
Leuconostoc			+
Oenococcus			+
Lactobacillus	*Lb. acidophilus*	*Lb. paralimentarius*	*Lb. brevis*
	Lb. amylovorus	*Lb. paracasei*	*Lb. buchneri*
	Lb. farciminis	*Lb. pentosus*	*Lb. fermentum*
	Lb. helveticus	*Lb. plantarum*	*Lb. reuteri*
	Lb. mindensis	*Lb. curvatus*	*Lb. sanfranciscensis*
	Lb. salivarius	*Lb. casei*	*Lb. hammesii*
	Lb. amylolyticus	*Lb. sakei*	*Lb. spicheri*
	Lb. manihotivorans	*Lb. alimentarius*	*Lb. kefir*
	Lb. suntoryeus	*Lb. rhamnosus*	*Lb. acidifarinae*
	Lb. satsumensis	*Lb. kimchii*	*Lb. panis*
	Lb. kefiranofaciens	*Lb. graminis*	*Lb. pontis*
	Lb. amylophilus	*Lb. coryniformis*	*Lb. reuteri*
	Lb. delbrueckii	*Lb. cypricasei*	*Lb. suebicus*
	subsp. *delbrueckii*	*Lb. versmoldensis*	*Lb. zymae*
	Lb. delbrueckii	*Lb. zeae*	*Lb. rossii*
	subsp. *bulgaricus*	*Lb. acidipiscis*	*Lb. parakefiri*
	Lb. delbrueckii		*Lb. paracollinoides*
	subsp. *lactis*		*Lb. frumenti*
	Lb. delbrueckii		*Lb. ferintoshensis*
	subsp. *indicus*		*Lb. durianis*
			Lb. diolivorans

2.1.3.1. Homofermentative LAB

In homofermentatives, hexoses are fermented via the EPM as shown in Figure 2.1. The families using this pathway are *Enterococcaceae*, *Lactobacillaceae* and *Streptococcaceae*, except for one group in the genus *Lactobacillus*. The members of these families use the glycolytic pathway and in this pathway, glucose is converted into lactic acid as the end product. In theglycolytic pathway, fructose-1,6-diphosphatase is the key enzyme and theoretically 2 moles of ATP are generated per mole of glucose consumed (Endo and Dicks, 2014).

Homofermentative LAB include *Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus* and also *Vagococcus.* In addition, some species of *Lactobacillus* such as *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis*, *Lb. delbrueckii* subsp. *indicus*, *Lb. acidophilus*, *Lb. helveticus*, *Lb. salivarius* and some others such as *Lb. amylolyticus, Lb. mindensis, Lb. manihotivorans, Lb. suntoryeus, Lb. satsumensis, Lb. kefiranofaciens, Lb. farciminis, Lb. amylophilus* and *Lb. amylovorus* use this pathway (Schleifer and Ludwig, 1995; Von Wright and Axelsson, 2012).

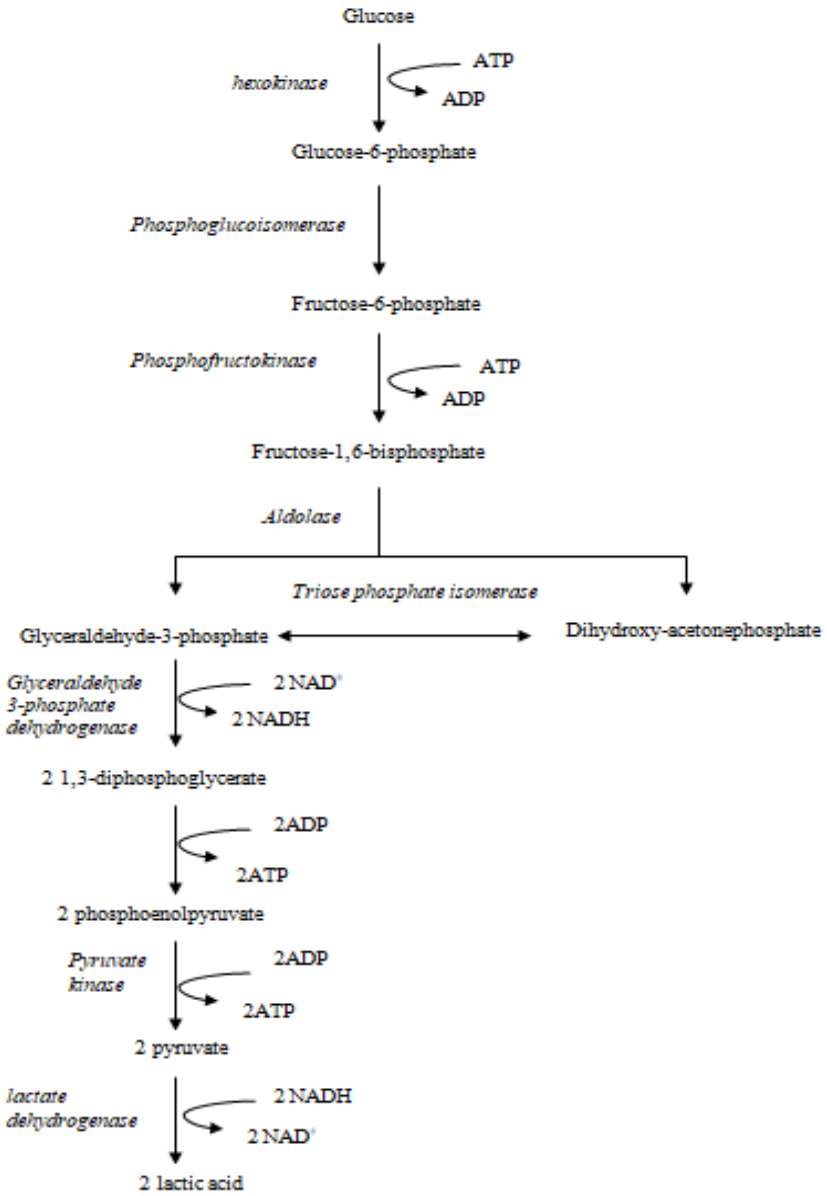

Figure 2.1. Fermentation of glucose in homofermentatives adapted from Endo and Dicks (2014) and Hames and Hooper (2000)

2.1.3.2. Heterofermentative LAB

During heterofermentative or heterolactic fermentation, hexoses are metabolized through the pentose phosphoketolase pathway. Heterofermentatives produce some other compounds such as CO_2 and ethanol or acetic acid besides lactic acid as shown in Figure 2.2 (Endo and Dicks, 2014). Heterofermentative LAB include members of the *Leuconostocaceae* family including the genera *Leuconostoc, Oenococcus* and *Weissella*. Also several species in the genus *Lactobacillus* use heterolactic fermentation. Obligate heterofermentative lactobacilli include the mainly isolated species *Lb. brevis, Lb. buchneri, Lb. fermentum, Lb. reuteri, Lb. sanfranciscensis, Lb. hammesii, Lb. spicheri, Lb. kefir, Lb. panis, Lb. pontis, Lb. reuteri, Lb. suebicus, Lb. zymae Lb. rossii, Lb. parakefiri, Lb. paracollinoides, Lb. frumenti, Lb. ferintoshensis, Lb. durianis, Lb. diolivorans* and *Lb. acidifarinae* (Schleifer and Ludwig, 1995; Von Wright and Axelsson, 2012).

Heterofermentative LAB cannot metabolize hexoses via the EMP pathway due to the lack of the glycolytic enzyme fructose 1,6 bisphosphate aldolase; therefore, they cannot break down fructose 1,6-bisphosphate into triose phosphate. However, they oxidize glucose 6-phosphate to 6-phosphogluconate and then 6-phosphogluconate is decarboxylated to pentose phosphate together with 1 mole of CO_2. This decarboxylation steps leads to CO_2 gas production (Madigan et al., 2012). Following decarboxylation step, the three carbon metabolite GAP and acetyl phosphate are produced. The first metabolite, GAP, is then converted to lactic acid. On the other hand, the second metabolite, acetyl phosphate, is converted into ethanol or acetate (Mayo et al., 2010). The energetic yield of this pathway is lower than homolactic fermentation and yields only 1 mole of ATP per mole of consumed hexose. During the conversion of acetyl phosphate to acetic acid in the presence of alternative electron acceptors, an extra ATP is generated (Sobczak and Lolkema, 2005; Pessione, 2012). As a result of following a different pathway,

heterofermentative LAB produce more flavor and aroma compounds, such as acetaldehyde and diacetyl, compared with homofermentatives (Jay et al., 2005).

Facultative heterofermentatives use both pathways and ferment hexoses and also pentoses (Hammes et al., 1991). This group includes some species of lactobacilli as follows *Lb. casei*, *Lb. curvatus*, *Lb. plantarum*, *Lb. sakei* and also *Lb. graminis*, *Lb. alimentarius*, *Lb. coryniformis*, *Lb. paracasei*, *Lb. pentosus*, *Lb. rhamnosus*, *Lb. kimchii*, *Lb. cypricasei*, *Lb. versmoldensis*, *Lb. zeae*, *Lb. paralimentarius* and *Lb. acidipiscis* (Schleifer and Ludwig, 1995; Von Wright and Axelsson, 2012).

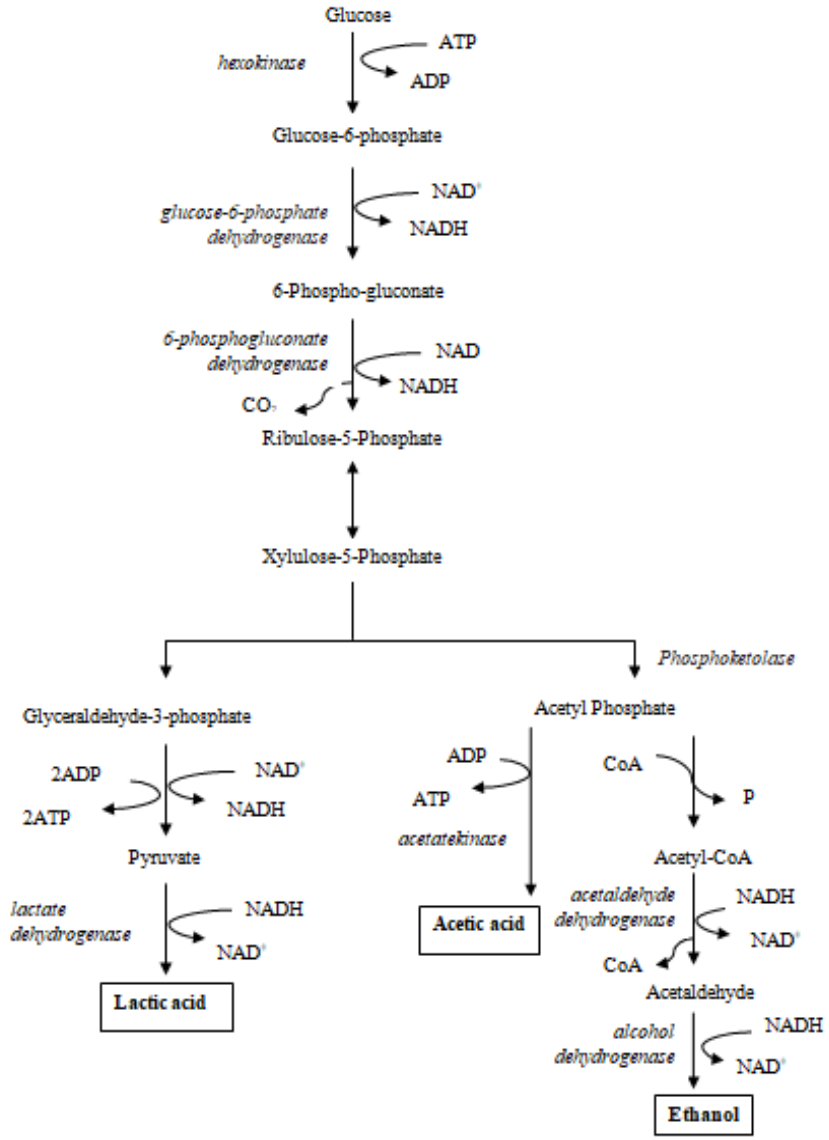

Figure 2.2. Heterofermentative metabolism adapted from Butler et al. (2010) and (Endo and Dicks, 2014)

2.1.4. Yeasts

Under anaerobic conditions, NAD^+ is regenerated via alcoholic fermentation, which is mainly conducted by yeasts. During alcoholic fermentation,

pyruvic acid produced via glycolysis is converted to acetaldehyde and CO_2 by *pyruvate decarboxylase*, as shown in *Equation 3*. Following that step, acetaldehyde is reduced to produce ethanol, the alcohol for which the process is named, by *alcohol dehydrogenase* and at the same time NADH is oxidized to NAD^+, as shown in *Equation 4* (Hames and Hooper, 2000). It is a low-energy-yield process because most of the energy contained in the initial glucose molecule remains in the end product, ethanol (Tortora et al., 2010; Hardin et al., 2012). Equations of the process are shown in Equations 5 and 6:

$$pyruvate + H^+ \rightarrow acetaldehyde + CO_2 \quad \textit{Eq. 3}$$

$$acetaldehyde + NADH + H^+ \rightarrow ethanol + NAD^+ \quad \textit{Eq. 4}$$

$$2\,pyruvate + 2\,NADH + 4H^+ \rightarrow 2\,ethanol + 2\,CO_2 + 2\,NAD^+ \quad \textit{Eq. 5}$$

$$glucose + 2\,ADP + 2\,P_i + 2\,H^+ \rightarrow 2\,ethanol + 2\,CO_2 + 2\,ATP \quad \textit{Eq. 6}$$

Yeasts are an important group of eukaryotic microorganisms (Tamang and Fleet, 2009). They are classically defined as unicellular fungi that belong to different taxonomic groups and among these, *Saccharomyces (S.)* sensu stricto yeast species are widely known all over the world, especially in the production of fermented beverages and foods (Sicard and Legras, 2011).

Alcoholic fermentation by yeast cells is a key process in the baking, brewing and winemaking industries (Hardin et al., 2012). In dough fermentation, yeast cells break down glucose to ethanol and CO_2. Ethanol is driven off during baking and CO_2 is trapped in the dough and causes the bread dough to rise. The CO_2 produced is responsible for the bubbles in beer and in sparkling wines and ethanol made by yeasts is the alcohol in alcoholic beverages. In brewing, both ethanol and CO_2 are essential as beer is an alcoholic carbonated beverage (Tortora et al., 2010; Hardin et al., 2012).

Under anaerobic conditions, yeasts perform alcoholic fermentation and produce ethanol and CO_2 from each mole of glucose. On the other hand, under

highly aerobic conditions and at a low concentrations of sugar, yeast consume glucose by the aerobic oxidative pathway to produce biomass. This is known as Pasteur effect. However, under high glucose concentrations, yeasts do not shift the metabolism from fermentative to a complete oxidative mode. Instead, the yeasts continue to ferment the glucose and perform an aerobic fermentation. This phenomenon is referred as the Crabtree effect (Reed and Nagodawithana, 1990). The yeasts exhibiting this performance are referred to as Crabtree-positive yeasts (Pronk et al., 1996; Johnston, 1999; Sicard and Legras, 2011).

2.2. Sourdough

The use of the sourdough process as a means of leavening is one of the oldest biotechnological processes in cereal food production. Bread can be made with either baker's yeast or sourdough for dough leavening, and sourdough bread is leavened with a sourdough starter (Catzeddu, 2011). The sourdough starter is a mixture of flour and water that are spontaneously fermented with LAB and yeasts. Sourdough microflora determine the bread characteristicsin terms of acid production, aroma and leavening (Hammes and Ganzle, 1998; Vogel et al., 1999; Moroni et al., 2009). Back-slopping, the addition of new flour and water to the dough, allows a composite ecosystem of LAB and yeast to take place inside the dough. The yeast is mainly responsible for the production of CO_2, and LAB, mainly heterofermantative, are responsible for the production of lactic and/or acetic acid; both microorganisms are responsible for the production of aromatic precursors of the bread (Catzeddu, 2011). During the production of sourdough, the produced lactic and acetic acids in the flour and water mixture causes a typical sour-tasting end product (Chavan and Chavan, 2011).

The use of sourdough in baking is an ancient craft that is currently undergoing a revival of interest. Bread production has relied on the use of sourdough as a leavening agent for most of human history as the primary form of bread leavening, whereas the use of baker's yeast as a leavening agent dates back

less than 150 years (Ganzle, 2014b). Nowadays, traditional sourdough bread is mostly produced in retail and artisan bakeries, but has also started to be used in industrial baking instead of baker's yeast as the leavening agent (Catzeddu, 2011).

2.2.1. History of Sourdough

Bread is considered to be the oldest processed food as its history goes back to ancient times. It is known that cereals were first cultivated in the Middle East 10,000 years ago. In ancient times, bread was unleavened since as there were no raising agents (Fob, 2011). In its earliest form, bread would probably be equivalent to today's modern flat breads, i.e., the Indian "chapatti", Mexican "tortilla" and Middle East's "pita"(Cauvain, 2001; Fob, 2011).

One of the oldest sourdough breads dates from 3,700 BC and was excavated in Switzerland, but the start of the use of sourdough in bread leavening can be traced back several thousand years earlier to the origin of agriculture in ancient Egypt (Fob, 2011; Ganzle, 2014b). In early Egypt, as well as in the Roman Empire, bread was produced on a large scale and it is known that back-slopping, dough acidification and yeast from winemaking were used for sourdough fermentations. It is believed that from there sourdough spread gradually to Europe, throughout ancient Greece and the Roman Empire into the present. The Romans learned this baking process due to their connection with the Greek civilization. In the first century, some methods for dough leavening, such as sourdough that was air-dried after 3 days of fermentation, the use of dried grapes as a starter culture and the use of back-slopping of dough, were reported by the Romans (Catzeddu, 2011). In Europe, sourdough fermentation was the main process for dough leavening, before the use of excess brewer's yeast became common in the 15^{th} and 16^{th} centuries (Ganzle, 2014b). In France, sourdough was used alone to ensure fermentation of the dough and also wine, vinegar or rennet was added in some French regions until the discovery of the use of brewer's yeast for bread making (Cappelle et al., 2013). After the use of brewer's yeast became common, brewing

and baking were carried out in the same facility. In Germany, bakers and brewers were often organised in the same place and in many cities bakers had the right to brew (Krauß, 1994; Brandt, 2005; Cappelle et al., 2013).

In the United States, sourdough bread is usually associated with San Francisco as sourdough bread from this city is the most famous type currently produced in the United States. Sourdough was introduced to the San Francisco area after the California gold rush and Canada after the Klondike gold rush in the 19th century (Cappelle et al., 2013). The sourdough starter was relatively easy to preserve for using as a leavening agent for baking by pioneers or gold prospectors travelling in slow-moving wagon parties. If sourdough failed, another starter could be prepared from flour and water during the journey (Catzeddu, 2011; Ganzle, 2014b).

Artisanal bread production relied on the use of sourdough as the main leavening agent until the 20th century. However, in the second half of 19th century, baker's yeast started to be used as the leavening agent instead of sourdough. Baker's yeast was a rapid and simple leavening process that was suitable for the adaptation to mechanized bread production in modern baking processes (Catzeddu, 2011). However, sourdough bread still continued to play a significant role in bread production in some parts of Europe, particularly in countries where rye bread is common, including Scandinavia, the Baltic States, Germany, Eastern Europe and the former Soviet Union, as well as in parts of the Middle East (Ganzle, 2014b).

In recent years, traditional sourdough breads have again started to attract consumers due to the high nutritional value, healthy properties, pronounced flavor, prolonged shelf life and natural production, i.e., without the use of any additives.

2.2.2. Sourdough Production

Sourdough is defined as the mixture of wheat or rye flour and water, fermented spontaneously by LAB and yeasts (Vogel et al., 1999; Corsetti, 2013). The acidifying and leavening capacity of the dough is optimized by consecutive

refreshments, also known as re-buildings, replenishments, back-slopping etc. (Corsetti and Settanni, 2007; Corsetti, 2013). For back-slopping, a flour and water mixture is fermented for a certain time at a defined temperature and it is then added as an inoculum to start the fermentation of a new mixture of flour and water (Corsetti, 2013).

Back-slopping, the addition of a new flour and water to the dough, allows a composite ecosystem of yeast and LAB to populate inside the dough, giving it its typical sour taste. The technological performance of the dough and flavor, the nutritional value, shelf life and overall quality of the bread are affected by the metabolic activity of the sourdough microorganisms (Catzeddu, 2011).

Sourdough bread offers many advantages over bread produced by baker's yeast such as leavening of dough without using any commercial yeast, improving the dough properties, as well as enhancing the flavor and taste of the bread. In addition, the nutritional value of sourdough bread is improved due to the higher bioavailability of minerals and lower glycaemic index (GI). Furthermore, the shelf life is extended as a result of the longer mold-free period, anti-staling effect and prevention of rope formation in bread (Hansen, 2012).

2.2.2.1. Flour in Sourdough Production

The history of sourdough started with the beginning of agriculture and sourdough bread has been produced on at artisanal scale in different parts of the world throughout the centuries, resulting in different types of knowledge from agricultural practices and technological processes through to cultural heritage (Cappelle et al., 2013). Flour and water are the raw materials in the production of sourdough. In rye-growing areas, rye sourdough is very common and rye flour is used for sourdough production. Besides rye flour, whole-meal wheat and white flour are commonly used in sourdough production in many countries. Moreover, due to gluten-free bread production, other flours are being started to be used in sourdough production such as corn, sorghum or rice flours (Ganzle, 2014b).

2.2.2.1.(1). Wheat Flour

In bread making, flour is the most important ingredient as it impacts the development of the specific characteristics of bakery products. It consists of protein, starch and other carbohydrates, ash, fibres, lipids, water and small amounts of vitamins, minerals and enzymes (Chavan and Chavan, 2011). Wheat flour is the most common flour used and wheat-based products are a major source of nutrients in many regions of the world (Fincher and Stone, 1986; Hoseney et al., 1988). Therefore, wheat is one of the world's most important grains and is primarily used for human consumption, with almost 15% used for animal feed (Manley, 2000; Morris and Bryce, 2000). In Turkey, climate and ecological properties are suitable for agricultural activities and wheat is one of the most commonly produced cereal crops. The total area of land that is suitable for agriculture is around 11.3 million hectares and wheat surpasses other cereals in terms of the number of hectares dedicated to its cultivation accounting for 67% in this country (Anonymous, 2013). Wheat is used as the raw material for bread, bulgur, biscuits, pasta and breakfast cereals, and provides bulk and structure to bakery products as it has the ability to form dough after mixing with water (Cotton and Ponte, 1973). Glutenin and gliadin are two proteins that form gluten in wheat flour. Gluten is very important in leavened doughs for the development of dough strength, as the formation of gluten creates an elastic and extensible matrix. In addition, the gas-holding capacity of wheat dough is dependent upon gluten since as it entraps huge amounts of the gas (CO_2) produced by yeasts during fermentation or by chemical leavening, thus yielding a leavened product (Hoseney, 1994; Lai and Lin, 2006; Salovaara and Gänzle, 2012).

For the production of sourdough, wheat (*Triticum durum* or *Triticum aestivum*) flour is commonly used. Wheat kernels are naturally contaminated by microorganisms and the cereal based products produced from wheat, and wheat flour, can be a source of viable LAB (Corsetti et al., 2007a; Alfonzo et al., 2013). Besides the flour, LAB can originate from the equipment used in the flour milling

and then these microflora can contribute to the sourdough fermentation and/or production process (Berghofer et al., 2003; Alfonzo et al., 2013).

2.2.2.1.(2). Rye Flour

Besides wheat products, sourdough fermentation is a traditional process employed in rye (*Secale cereale*) baking (Gänzle et al., 2008). After baker's yeast began to be used as the leavening agent in modern baking processes, the use of sourdough for bread production was reduced (Catzeddu, 2011). However, sourdough bread still continued to play a significant role in bread production in rye-growing areas, where rye bread has a major share of the bread market. Rye sourdoughs have been characterized from Germany (Böcker et al., 1995), Scandinavian countries including Finland (Salovaara and Katunpaa, 1984), Sweden (Spicher and Lonner, 1985), Denmark (Knudsen, 1924; Rosenquist and Hansen, 2000) and also Poland, the Czech Republic, Austria, Portugal and the Baltic States in Europe and Russia (Hansen, 2012; Ganzle, 2014b). The continued use of sourdough in those countries relates to the use of rye flour in bread production as sourdough was used for the acidification of rye dough to reach optimal rye bread quality (Cappelle et al., 2013). After the introduction of baker's yeast as a leavening agent, the aim of using sourdough fermentation in rye baking shifted to its use as an acidifying agent. Free sugars and amylolytic enzymes are higher in rye than in other cereals and endogenous enzymes in rye can be used to break down the starch to simple fermentable sugars (Salovaara and Gänzle, 2012). Rye flour contains high levels of pentosan compared with wheat flour, and the pentosans inhibit the formation of the gluten network in rye doughs and in the structure forming process of a rye dough the proteins play a lesser role than in wheat doughs (Cauvain, 1998). Sourdough fermentation promotes the solubilisation of rye pentosans at the dough stage and enhances water binding and gas retention of the dough resulting in conversion of the starch to gel which form a matrix during baking (Martinez-Anaya and Devesa, 2000; Brandt, 2007; Catzeddu, 2011). The

solubility and swelling behaviour of pentosans increase at the low pH values characteristic of sourdoughs (Hammes and Ganzle, 1998; Arendt et al., 2007). Gluten is lacking in rye and the gas-holding capacity of rye dough is dependent upon polymeric arabinoxylans (Salovaara and Gänzle, 2012). Acidification is also very important in rye bread production for inhibiting the flour α-amylase thus preventing excessive starch degradation (Catzeddu, 2011). Acidification is very important in both flours, wheat and rye, since cereal phytases are activated due to the acidificiation and more nutrients become available (Fretzdorff and Brummer, 1992; De Vuyst and Neysens, 2005). The acidification of rye doughs improves their physical properties by making them more elastic and extensible (Arendt et al., 2007).

2.2.2.1.(3). Other Flour Types

Other cereal and also legume flours can be used in sourdough production. Studies have been conducted on sourdoughs produced with other cereal and legume flours such as maize flour in combination with rye flour (Rocha and Malcata, 2016), faba bean (*Vicia faba* L.) flour obtained from different cultivars (Coda et al., 2017), quinoa flour (Ruiz Rodriguez et al., 2016a), chickpea, lentil and bean flour combinations with wheat flour (Rizzello et al., 2014), hull-less barley (*Hordeum vulgare*) flour with wheat flour (Mariotti et al., 2014), sorghum flour (Schober et al., 2007), Teff (*Eragrostis tef*) together with wheat flour, rice sourdough (Meroth et al., 2004), amaranth flour (Sterr et al., 2009; Ruiz Rodriguez et al., 2016b) etc.

2.2.2.2. Classification of Sourdough Production Methods

Sourdoughs are grouped as shown below on the basis of the production technology applied (Figure 2.3):

-Type I traditional sourdoughs

-Type II semi fluid sourdoughs

-Type III dried sourdoughs

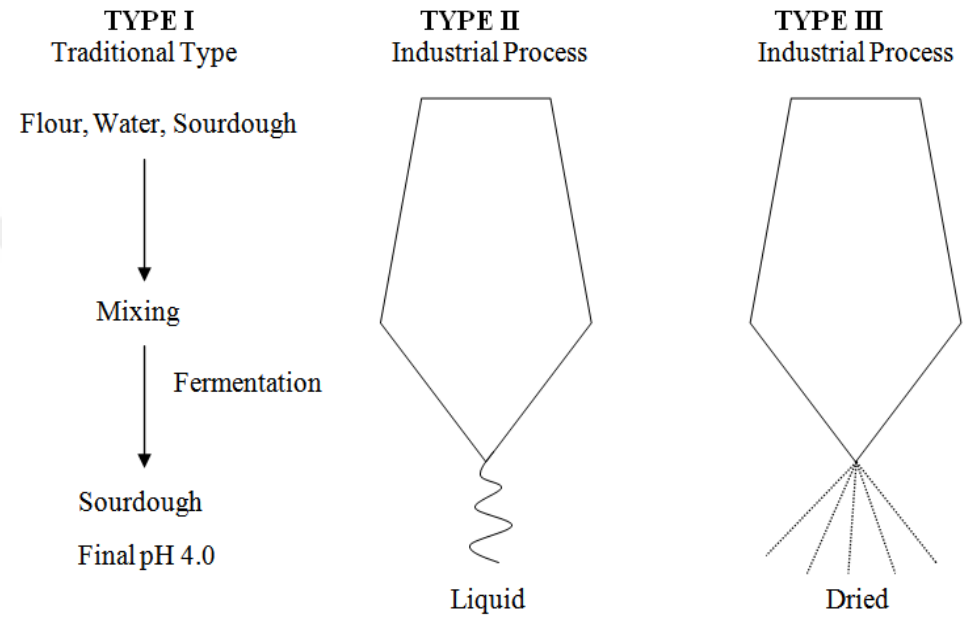

Figure 2.3. Sourdough types adapted from Corsetti and Settanni (2007)

Type I-traditional sourdoughs are produced by using a sourdough that was part of the previous fermentation (Chavan and Chavan, 2011). These types of sourdoughs are characterized by continuous and daily refreshment/back-slopping to maintain the microorganisms in an active state. Dough leavening is achieved without the addition of baker's yeast (Corsetti, 2013). Fermentation is performed spontaneously at an ambient temperature of around 20–30°C and the pH is approximately 4.0 (De Vuyst and Neysens, 2005). At the beginning, a sourdough starter is prepared by mixing flour and water and the mixture is fermented in a warm place. After 12-24 hours visible fermentation occurs accompanied by sour, alcoholic odor. A portion of fermented sourdough is used to inoculate the next

batch and the inoculation of each new batch with sourdough containing active fermenting yeasts and LAB results in more rapid fermentation. After a few refreshments characterized with different dough yield, temperature and time, a natural starter culture sourdough with a stable fermentation microbiota consisting of heterofermentative LAB and yeasts is established (Hammes, 1991). In this type of sourdoughs, sourdoughs are regularly refreshed for very long periods of time with stable fermentation microbiota documented over a period of more than 20 years (Ganzle, 2014b).

Type I sourdoughs can be further classified into Type Ia, Ib and Ic (De Vuyst and Neysens, 2005). Type Ia sourdoughs can be pure cultures from natural sourdoughs of different origin, for example, San Francisco French bread (Gobbetti and Corsetti, 1997; Tucker, 2016). Type Ib sourdoughs are spontaneously developed, mixed culture sourdoughs prepared from wheat and rye, or their mixtures, through multiple-stage fermentation processes. Traditional rye sourdough is an example of that type. When the fermentation is completed, the fully developed starter, mother dough, is used as the inoculum for future batches of bread dough. Consecutive back-sloppings of a new batch from a previous batch ensure the continuity of the microflora (De Vuyst and Neysens, 2005; Tucker, 2016), which has a very important role in the acidification and leavening of the dough as well as aroma formation. According to environmental conditions, different species of LAB dominate the fermentation. Type Ic sourdoughs, for example, African sorghum sourdoughs, are fermented at high temperatures (>35°C) in tropical regions (Stolz, 1999; De Vuyst and Neysens, 2005; Paramithiotis and Drosinos, 2017).

Large-scale sourdough fermentation processes resulted in the development of type II sourdoughs (De Vuyst and Neysens, 2005). Type II sourdoughs are an industrial type of sourdough in the form of semi-fluid or liquid preperations so it is easily pumpable in an industrial bakery (Chavan and Chavan, 2011). Adapted strains are used to start the fermentation, which mainly serve as dough acidifiers

(Paramithiotis and Drosinos, 2017). These types of processes continue for 2-5 days and are often performed at increased fermentation temperature (>30 °C) to speed-up the process (Böcker et al., 1995; Hammes and Ganzle, 1998). The resulting sourdough has a high acid content at a pH of 3.5 after 24 hours of fermentation. The high dough yield (DY) values of Type II sourdoughs enable pumping of the dough (De Vuyst and Neysens, 2005).

Type III sourdoughs are dried doughs in powder form and is often used by industrial bakeries as the quality constant (Chavan and Chavan, 2011). They are initiated by defined starter cultures and used as acidifier supplements and aroma carriers during bread making. Drying leads to an increased shelf life of the sourdough and also provides a stock product until further use. Spray or drum drying processes are the most commonly used techniques and dried sourdoughs are simple to be used in dough processing and also result in standardized end products. This type of sourdoughs can be distinguished by color, aroma and acid content (Stolz and Bocker, 1996; De Vuyst and Neysens, 2005).

In contrast to Type I preparations, doughs of Types II and III require the addition of baker's yeast (*S. cerevisiae*) for leavening (De Vuyst and Neysens, 2005).

2.2.3. Sourdough Microflora

From a microbiological point of view, the effect of sourdough fermentation is related to the metabolic activities of two groups of microorganisms: yeasts and LAB. Nevertheless, LAB are mainly responsible for all the nutritional and functional advantages of sourdough fermentation, whereas yeasts are mostly related to leavening and aroma formation (Rizzello et al. 2017). LAB and yeasts isolated from some sourdough samples is shown in Table 2.2.

Fermented sourdoughs include Gram-negative aerobes (e.g., *Pseudomonas*) and facultative anaerobes (*Enterobacteriaceae*), as well as Gram-positive LAB including mainly *Lactobacillus* species and also some *Enterococcus*,

Lactococcus, Pediococcus, Leuconostoc and *Weissella* species. Sourdough is rich in fermentable carbohydrates and therefore allows the spontaneous development of characteristic LAB species, as previously mentioned. These LAB can originate from the cereals or flours and depend on the flour preparation and sourdough production technology applied (De Vuyst and Neysens, 2005).

Alfonzo et al. (2013) investigated the wheat flour microflora used to produce sourdough bread in Southern Italy using phenotypic characteristics and genetic analysis and it was found that flours harbor LAB of high technological potential with respect to sourdough bread production. Analysis by 16S rRNA gene sequencing grouped the strains into 11 LAB species, which belonged to six genera: *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Weissella*. It was determined that *W. cibaria, Lb. plantarum, Leu. pseudomesenteroides* and *Leu. citreum* were the most prevalent species

In the production of different types of sourdoughs, different LAB species dominate the fermentation. Type Ia sourdoughs contain well-adapted microflora with a stable composition, especially obligate heterofermentative *Lb. sanfranciscensis.* Actually majority of type I sourdoughs contain *Lb. sanfranciscensis*. *Lb. sanfranciscensis* (previously named *Lb. sanfrancisco* or *Lb. brevis* subsp. *lindneri*), which is an obligate heterofermentative *Lactobacillus* species that was first isolated from German rye sourdoughs and from the San Francisco French bread processes (Sugihara et al., 1970; Kline and Sugihara, 1971; Salovaara and Gänzle, 2012). For example, San Francisco French bread is produced by using a Type Ia starter and *Lb. sanfranciscensis* is responsible for the souring activity by producing large amounts of lactic and acetic acids, resulting in a strong sour taste, and also helps dough leavening due to gas production (Gobbetti and Corsetti, 1997; Tucker, 2016). Type Ib sourdoughs consist of obligate heterofermentative strains of *Lb. sanfranciscensis* and also, depending on the fermentation conditions, other species such as *Lb. brevis* and also *Lb. buchneri, Lb. fermentum, Lb. fructivorans, Lb. pontis, Lb. reuteri, W. cibaria, Lb. alimentarius,*

Lb. casei, Lb. paralimentarius and *Lb. plantarum, Lb. acidophilus, Lb. delbrueckii, Lb. farciminis* and *Lb. mindensis* occur in relevant cell counts (Hammes and Ganzle, 1998; Vogel et al., 1999). *Lb. sanfranciscensis* is replaced by species that are more adapted to higher temperatures in sourdoughs fermented at increased temperatures (Meroth et al., 2003).Type Ic sourdoughs contain *Lb. fermentum, Lb. pontis* and *Lb. reuteri* species, as well as *Lb. amylovorus* (Hamad et al., 1992). Different process parameters of type II sourdough fermentations result in a different LAB flora including *Lb. acidophilus, Lb. delbrueckii, Lb. amylovorus, Lb. farciminis, Lb. johnsonii, Lb. brevis, Lb. fermentum, Lb. frumenti, Lb. pontis, Lb. panis, Lb. reuteri* and *W.confusa* species (Vogel et al., 1999; Müller et al., 2001). Type III sourdoughs predominantly contain LAB those are resistant to drying and are able to survive in that form, for example, *Lb. brevis, Pd. pentosaceus* and *Lb. plantarum* strains (Böcker et al., 1995; De Vuyst and Neysens, 2005).

The production of lactic acid and CO_2 is the most prominent metabolic activity of LAB in sourdough; whereas, gas production and aroma formation is achieved by yeasts. Type Ia and Ib sourdoughs can contain *Candida (C.) humilis* (*Torulaspora (T.) holmii, C. milleri*) and also *S. exiguus*. Type Ic sourdoughs can contain *Issatchenkia (I.) orientalis* (*C. krusei*) (De Vuyst and Neysens, 2005). Types II and III can contain *S. cerevisiae* as baker's yeast if added, since as normally this type does not contain yeasts at important levels. In addition, *Kazachstania (K.) exigua* (formerly *S. exiguus*) can be found in the sourdough environment which is tolerant to more acidic environments (Cappelle et al., 2013).

The microflora of sourdough varies according to the environment, type and fermentation conditions of produced sourdoughs, as reported in many studies. LAB and yeasts have been identified in sourdoughs, collected from different countries, using phenotypic and molecular methods.

Corsetti et al. (2001) characterized the microflora of 25 wheat sourdoughs from Italy. The number of LAB and yeasts ranged from 7.5 to 9.3 log CFU/g and from 5.5 to 8.4 log CFU/g, respectively. Isolated LAB and yeasts were identified

by conventional physiological and biochemical tests and also confirmed with molecular techniques 16S rDNA and 16S/23S rRNA spacer region PCR. The microflora distribution was heterogeneous. *Lb. sanfranciscensis* (30%) and then *Lb. alimentarius* (20%) were the most isolated species. Other isolated species include *Lb. brevis, Leu. citreum, Lb. plantarum, Lc. lactis* subsp. *lactis, Lb. fermentum, Lb. acidophilus, W. confusa* and *Lb. delbrueckii* subsp. *delbrueckii*. *S. cerevisiae* was largely found in sourdoughs, and some of the sourdoughs also contain *S. exiguus* and *C. krusei.*

Ricciardi et al. (2005) determined the composition of the LAB community of sourdoughs produced in Southern Italy using a set of 29 phenotypic tests. Counts of LAB ranged between 10^7–10^8 cfu/g and a total of 111 LAB strains were randomly isolated. Most strains were identified as *Lb. plantarum, Lb. paracasei, Lb. casei, Lb. brevis* and *Leu. mesenteroides.*

In another study conducted in Italy, yeasts were identified in collected homemade sourdough samples. Results showed that *S. cerevisiae* was the dominant species, followed by *C. milleri, C. humilis, S. exiguus* and *I. orientalis* (Pulvirenti et al., 2004).

Randazzo et al. (2005) evaluated the LAB in 9 artisanal wheat sourdough samples, that were collected in different areas of Sicily, using physiological and biochemical methods along with molecular techniques. Restriction fragment length polymorphism and 16s ribosomal DNA gene sequencing showed a variety of species with the dominance of *Lb. sanfranciscensis* and *Lb. pentosus* in all tested sourdoughs. In addition, *Lb. casei, Lb. kimchii/Lb. alimentarius* and *Lb. plantarum* were identified.

Valmorri et al. (2006) characterized the lactobacilli community of 20 sourdoughs, collected from different cities in central Italy, using a novel polyphasic approach consisting of a two-step multiplex PCR system, 16S rRNA gene sequence analysis and physiological features. Yeast and LAB counts were in the range of 5.03-8.61 and 7.55-9.45 log CFU/g, respectively. Identified species included *Lb.*

plantarum, Lb. alimentarius, Lb. paralimentarius, Lb. sanfranciscensis, Lb. brevis, Lb. fermentum, Lb. rossiae, W. cibaria and *Lb. graminis/Lb.sakei/Lb. curvatus*. In another study, Valmorri et al. (2010) identified yeasts from different sourdough samples using PCR-RFLP analysis and isolates were identified as mainly *S. cerevisiae*, with the other dominant species being *C. milleri, C. krusei* and *T. delbrueckii.*

In another study conducted in Italy, molecular techniqes, 16Sr DNA sequencing and RAPD-PCR were used for the identification and typing of LAB isolated from 25 samples of sourdoughs. Twelve different species of LAB were identified, and most isolates were classified as facultative heterofermentative lactobacilli. *Lb. pentosus* dominated the lactic microflora of many samples and other frequently isolated species were *Lb. plantarum, Lb. brevis, W. confusa* and *Lb. sanfranciscensis.* Other species were identified as *Lb. casei, Lb. zeae, Pd. pentosaceus, Lb. sakei, Lb. alimentarius, Lb. farciminis* and *Leu. citreum* (Catzeddu et al., 2006).

Minervini et al. (2012a) reported the microbiota of 19 Italian sourdoughs used for the manufacture of traditional/typical breads through a culture-dependent method and pyrosequencing. The most frequent LAB isolates were *Lb. sanfranciscensis, Lb. plantarum* and *Lb. paralimentarius*. *S. cerevisiae* was identified in many of the sourdoughs and along with *C. humilis*, *K. barnettii* and *K. exigua* yeasts.

Gaglio et al. (2017) investigated the microbial community of Italian sourdoughs using culture-dependent and culture-independent approaches. LAB and yeast counts were approximately 10^9 CFU/g and 10^6 CFU/g, respectively. Identified LAB species present in Sicilian sourdough were *Lb. sanfranciscensis*, *Lb. paralimentarius*, *Lb. brevis* and *Lb. coryniformis* and yeasts were *S. cerevisiae*, *Pichia (P.) guiliermondii*, *P. segobiensis*, *Rhodotorula (R.) acuta* and R. *mucilaginosa.*

Another study conducted in Italy identified the LAB and yeast species *Lb. sanfranciscensis, C. milleri* and *S. cerevisiae* as the microbiota characterizing the sourdough of Italian PDO Tuscan bread (Palla et al., 2017).

Lattanzi et al. (2013) used a similar method, pyrosequencing and culture-dependent methods, in the investigation of 18 sourdoughs used for the manufacture of traditional/typical Italian breads and the results of pyrosequencing were in agreement with the results of the culture-dependent method. *Lb. sanfranciscensis* was identified in almost all the sourdoughs. *Lb. plantarum* and *Leu. citreum* were also isolated with a relatively high frequency. *S. cerevisiae* was identified in many samples and *C. humilis* were also identified in some of the sourdoughs, along with *Lc. lactis, Lb. brevis, Lb. casei* and also *Lb. curvatus, Lb. fermentum, Leu. mesenteroides, Pd. acidilacticii* and *W. cibaria* species.

From French wheat sourdough samples, a total of 20 morphologically different strains were chosen and identified as *Lb. plantarum, Lb. paralimentarius, Lb. sanfranciscensis, Lb. spicher, Lb. sakei* and also, two isolates belonging to a novel *Lactobacillus* species, proposed in that work as *Lb. hammesii* (Valcheva et al., 2005)

In another study involving 16 sourdoughs used for the manufacture of traditional French breads, *Lb. sanfranciscensis* was determined as the dominant species in French sourdoughs according to the results of genotypic analyses. The median values of cell density of LAB was 9.2 log CFU/g and the ratio between LAB and yeasts ranged from 10,000:1 to 10:1. Other species frequently encountered were *Lb. parabrevis/Lb. hammesii, Lb. plantarum* and *Leu. mesenteroides* and for the first time *Lb. xiangfangensis* and *Lb. diolivorans* were found in sourdough. The yeast microbiota of French sourdoughs was dominated by *S. cerevisiae* and also *K. servazzii* (formerly *S. servazzii*) was found as the dominant or co-dominant yeast species in two samples (Lhomme et al., 2015).

Scheirlinck et al. (2009) investigated the predominant sourdough LAB species during the production of two Belgian artisan sourdough by using molecular

methods, and sourdoughs were found to be mainly dominated by *Lb. spicheri, Lb. plantarum* and *Lb. sanfranciscensis.*

Viiard et al. (2012) analysed the lyophilized starter and industrial sourdough in Estonia and reported *Lb. helveticus* as the dominant LAB species in the analysed samples. Furthermore, *Lb. panis* and *Lb. pontis* and also some other species, *Lb. vaginalis, Lb. reuteri, Lb casei/paracasei, Lb. fermentum* and *Lb. paralimentarius* were identified in the study.

Saeed et al. (2009) investigated the wheat sourdough samples that were collected from different bakeries in Pakistan and isolates were identified as phenotpic methods. LAB isolates were identified as *Lb. brevis, Lb. fermentum* and *Lb. plantarum. S. cerevisiae* was also identified in the samples which is certainly related to the addition of baker's yeast to the doughs

Zhang et al. (2015) investigated Chinese traditional sourdoughs, collected from different areas of China, using culture-dependent and DGGE methods. The culture dependent method results showed that *S. cerevisiae* and *Lb. plantarum* were the predominant species among the yeasts and LAB microflora. According to the PCR-DGGE approach, *S. cerevisiae* was predominant, while the yeast *C. tropicalis* represented the subdominant species of the yeast community. Among the LAB community, *Lb. sanfranciscensis* was the predominant species, while *Lc. qarvieae, E. faecium, Lb. delbrueckii* and *E. cecorum* were among the less dominant species.

In addition, some studies have investigated Turkish sourdoughs (Menteş et al., 2004; Gül et al., 2005; Şimşek et al., 2006; Dertli et al., 2016; Yagmur et al., 2016). Menteş et al. (2004) collected 20 sourdough samples from Ankara, Bursa and Trabzon, and investigated LAB flora using phenotypic methods. The study reported the dominant species as *Lb. alimentarius* (31 of 150 isolates) and *Lb. plantarum* (21 of 150 isolates). Other species were *Lb. sake, Lb. acidophilus, Lb. fermentum, Lb. curvatus, Lb. delbrueckii* subsp. *delbrueckii, Lb. farciminis, Lb. casei* subsp. *casei, Lb. helveticus, Lb. collinoides, Lb. buchneri, Lb. brevis, Lb.*

amylophilus, Lb. reuteri, Lb. divergens, Lb. viridescens, Lb. amylovorus and *Lb. agilis*

Gül et al. (2005) collected 14 sourdough samples from Isparta and reported the LAB as *Lb. divergens* (6.1%), *Lb. brevis* (15.1%), *Lb. amylophilus* (6.1%), *Lb. sake* (6.1%), *Lb. acetotolerans* (6.1%), *Lb. plantarum* (3%), *Pd. pentosaceus* (6.1%) and *Pd. acidilactici* (6.1%) species and yeasts were *S. cerevisiae* (27%), *T. delbrueckii* (2.7%), *T. holmii* (10.8%) and *T. a unisporus* (2.7%). LAB and yeast counts were in the range of 5.28-9.66 and 6.33-9.96 log CFU/g, respectively.

Şimşek et al. (2006) analysed the LAB microflora in sourdough samples collected from Usak and reported that *Lb. brevis* spp. *lindneri, Lb. viridenscens, Pediococcus* sp. and *Lb. delbrueckii* are the strains with best potential as sourdough starters.

Dertli et al. (2016) investigated the Turkish wheat sourdoughs from the Eastern Black Sea region of Turkey and reported the presence of 47 distinct LAB strains belonging to 11 different including: *Lb. plantarum, Lb. paraplantarum, Lb. curvatus, Lb. rossiae, Lb. sanfranciscensis, Lb. brevis, Lb. paralimentarius, W. paramesenteroides, Leu. mesenteroides, Leu. pseudomesenteroides* and *W. cibaria.* The pH of the sourdoughs ranged from 3.37 to 3.95. The LAB and yeast counts of these samples ranged between 8.35 and 8.91 log CFU/g and 6.70 and 6.96 CFU/g, respectively.

Another study conducted in our country investigated the microbial flora in different sourdough samples collected from Ankara, Trabzon, Kütahya, Isparta and Adana. The main LAB species identified were *Lb. sanfranciscensis, Pd. pentosaceus, Lb. plantarum, Lb. namurencis, Lb. rossiae, Leu. mesenteroides* and *Lb. zymae*. *Lb. spicheri, Lb. paralimentarius, Lb. mindensis, Lb. farciminis, Lb. acetotolerans, Lb. casei, E. faecium* and *E. durans* were also found in sourdoughs at subdominant levels. Among yeasts, mainly *S. cerevisiae* and also *P.*

guiliermondii and *T. delbrueckii* were detected as the predominant yeast species in sourdoughs (Yagmur et al., 2016).

Another study, conducted on the isolated bacteria and yeasts from rye, maize flours and sourdoughs, reported the most frequently isolated yeasts as *S. cerevisiae* and *C. pelliculosa*. The most frequently isolated LAB in their study were *Lb. brevis, Lb. curvatus,* and *Lb. lactis* spp. *lactis; Lc. lactis* spp. *lactis, E. casseliflavus, E. durans, E. faecium, S. constellantus* and *S. equinus* (Rocha and Malcata, 1999).

Table 2.2. LAB and yeasts isolated from various sourdoughs

Place	**LAB**	**Yeast**	**Reference**
Southern Italy	*Lb. sanfranciscensis* *Lb. alimentarius* *Lb. brevis* *Leu. citreum* *Lb. plantarum* *Lc. lactis* subsp. *lactis* *Lb. fermentum* *Lb. acidophilus* *W. confusa* *Lb. delbrueckii* subsp. *delbrueckii*	*S. cerevisiae* *S. exiguus* *C. krusei*	(Corsetti et al., 2001)
Italy		*S. cerevisiae* *C. milleri* *C. humilis* *S. exiguus* *I. orientalis*	(Pulvirenti et al., 2004)
Italy	*Lb. plantarum* *Lb. paracasei* *Lb. casei* *Lb. brevis* *Leu. mesenteroides*		(Ricciardi et al., 2005)
Italy	*Lb. sanfranciscensis* *Lb. pentosus* *Lb. casei* *Lb. kimchii* *L. alimentarius* *Lb. plantarum*		(Randazzo et al., 2005)

Table 2.2. Continued

Italy	*Lb. plantarum* *Lb.alimentarius* *Lb.paralimentarius* *Lb. sanfranciscensis* *Lb. brevis* *Lb. fermentum* *Lb. rossiae* *W. cibaria* *Lb. graminis/ Lb.sakei/Lb. curvatus*		(Valmorri et al., 2006)
Italy		*S. cerevisiae* *C. milleri* *C. krusei* *T. delbrueckii*	(Valmorri et al., 2010)
France	*Lb. plantarum* *Lb. paralimentarius* *Lb. sanfranciscensis* *Lb. spicher* *Lb. sakei* *Lb. hammesii*		(Valcheva et al., 2005)
Italy	*Lb. pentosus* *Lb. plantarum* *Lb. brevis* *W. confusa* *Lb. sanfranciscensis* *Lb. casei* *Lb. zeae* *Pd. pentosaceus* *Lb. sakei* *Lb. alimentarius* *Lb. farciminis* *Leu. citreum*		(Catzeddu et al., 2006)
Pakistan	*Lb. brevis* *L. fermentum* *L. plantarum*	S. *cerevisiae*	(Saeed et al., 2009)
Belgium	*Lb. spicheri* *Lb. plantarum* *Lb. sanfranciscensis*		(Scheirlinck et al., 2009)
Italy	*Lb. sanfranciscensis* *Lb.plantarum* *Lb. paralimentarius*	*S.cerevisiae* *C. humilis* *K. barnettii* *K. exigua*	(Minervini et al., 2012a)

Table 2.2. Continued

France	*Lb. sanfranciscensis* *Lb. parabrevis/* *Lb. hammesii* *Lb. plantarum* *Leu. mesenteroides* *Lb. xiangfangensis* *Lb. diolivorans*	*S. cerevisiae* *K. servazzii*	(Lhomme et al., 2015)
Turkey	*Lb. brevis* spp. *lindneri* *Lb. viridenscens* *Pd.* spp. *Lb. delbrueckii*		(Şimşek et al., 2006)
Italy	*Lb. sanfranciscensis* *Lb. plantarum* *Leu. citreum* *Lc. lactis,* *Lb. brevis,* *Lb. casei* *Lb. curvatus,* *Lb. fermentum,* *Leu. mesenteroides,* *Pd. acidilacticii* *W. cibaria*	*S. cerevisiae* *C. humilis*	(Lattanzi et al., 2013)
Estonia	*Lb. helveticus* *Lb. pontis* *Lb. vaginalis* *Lb. reuteri* *Lb casei/paracasei* *Lb. fermentum* *Lb. paralimentarius*		(Viiard et al., 2012)
China	*Lb. plantarum* *Lb. sanfranciscensis* *Lc. qarvieae* *E. faecium* *Lb. delbrueckii* *E.cecorum*	*S. cerevisiae* *C.tropicalis*	(Zhang et al., 2015)
Turkey	*Lb. divergens* *Lb. brevis* *Lb. amylophilus* *Lb. sake* *Lb. acetotolerans* *Lb. plantarum* *Pd. pentosaceus* *Pd. acidilactici*	*S.cerevisiae* *T.delbrueckii* *T. holmii* *T.unisporus*	(Gül et al., 2005)

Table 2.2 Continued

Turkey	*Lb. plantarum* *Lb. paraplantarum* *Lb. curvatus* *Lb. rossiae* *Lb. sanfranciscensis* *Lb. brevis* *Lb. paralimentarius* *W. paramesenteroides* *Leu. mesenteroides* *Leu. pseudomesenteroides* *W. cibaria.*		(Dertli et al., 2016)
Turkey	*Lb. sanfranciscensis* *Pd. pentosaceus,* *Lb. plantarum,* *Lb. namurencis,* *Lb. rossiae,* *Leu. mesenteroides* *Lb. zymae.* *Lb. spicheri,* *Lb. paralimentarius,* *Lb. mindensis,* *Lb. farciminis,* *Lb. acetotolerans,* *Lb. casei,* *E. faecium* *E. durans*	*S. cerevisiae* *P. guiliermondii* *T. delbrueckii*	(Yagmur et al., 2016)

2.2.4. Beneficial Effects and Functional Aspects of Sourdough Technology

Sourdough fermentation is a traditional process for the production of wheat and rye breads. At present it is also used for the production of other bread types with different flours and for the manufacture of various cereal-based products such as breads, cakes and crackers. The use of sourdough technology improves the quality of cereal products which is characterized by its flavor, nutritional value texture and shelf life (Arendt et al., 2007). In addition, it confers a natural image to the product from a consumer perspective (Salovaara, 1998). The typical characteristic of sourdough mainly relies on the metabolic activities of its active microflora, basically represented by LAB and yeasts. As a result of the metabolic activities of LAB and yeasts, biochemical changes occur during sourdough fermentation and the quality of the dough and bread are affected (Galle, 2013).

Various components in flours (carbohydrates, nitrogen sources, minerals, lipids and free fatty acids and enzyme activity) and process parameters of the dough (temperature, dough yield, O_2, fermentation time, and number of sourdough propagation steps) influence the microflora of sourdough and the propeties of the leavened product, as during fermentation, the action of microbial and indigenous enzymes causes the biochemical changes in the components of the flour (Hammes and Ganzle, 1998; Chavan and Chavan, 2011). The rate and extent of these changes greatly influence the properties of the sourdough and the quality of the resulting baked product (Arendt et al., 2007). Organic acid production, volatile compound synthesis, proteolytic and amylolytic activities, improvement of the texture and sensorial properties, delaying microbial spoilage and EPS production occur during sourdough fermentation (Hammes and Ganzle, 1998; Gobbetti et al., 1999).

The microflora involved in fermented foods contribute to the improvement of the organoleptic properties, the shelf life of the final products and their nutritional profile (Kotzekidou and Tsakalidou, 2006), due to the metabolic activity of the microorganisms which is governed by the interaction with the grain constituents. LAB produce lactic and acetic acids and the pH is typically decreased below pH 5 with acidification contributing to the activation of certain enzymes such as proteases, amylases, hemicellulases and phytases. It has been reported that the activity of enzymes and microbial metabolites affect the nutritional quality of bread, including proteins, starches, lipids, dietary fibres, vitamins, minerals and phenolics, via different mechanisms (Poutanen et al., 2009). Sourdough fermentation can influence the nutritional quality by decreasing or increasing level of these compounds and enhancing or retarding the bioavailability of nutrients. In particular, enhanced mineral bioavailability, fibre solubilisation, the production of bioactive peptides and reduction of starch digestibility are important potential mechanisms in sourdough fermentation (Poutanen et al., 2009).

2.2.4.1. Organic Acid Production

During sourdough fermentation, LAB produce lactic and acetic acids and pH is typically reduced to below 5. Organic acid production enhances flavor, improves texture and also inhibits pathogenic and spoilage organisms as a result of the low pH levels and inhibitory effect of some organic acids (Salovaara and Gänzle, 2012). During the sourdough process, lactic acid and acetic acid in particular exert an inhibitory effect (Hansen, 2012). The inhibitory effect of organic acids are related to impacting cell homeostatic systems since, as the decreasing pH acidifies the cell and then the cell consumes a great amount of energy to maintain intracellular pH homeostasis (Kang et al., 2003; Hassan et al., 2015; Erkmen and Bozoglu, 2016). Organic acids dissociate depending on their dissociation constants (pKa), temperature and certain other factors, and produce protons. However, the cytoplasmic membrane is impermeable to protons. On the other hand, organic acids are mainly *lipophilic* and undissociated molecules of organic acids easily enter through the cell membrane. In the cell, a higher internal pH than pKa causes organic acids to dissociate when entering the cytoplasm, which decreases the intracellular pH by releasing the proton. Protons acidify the cytoplasm and cells try to overcome this problem by pumping out the protons to the external environment. The cell uses the main part of its energy content to remove newly formed protons, which results in slower growth kinetics (León Peláez et al., 2012; Hassan et al., 2015). At very low pH levels (4.5 or below), it is difficult to remove all the protons from the cell and it cannot retain its internal pH. Bacteria maintain internal pH near neutrality to prevent denaturation of structural proteins, enzymes, nucleic acids and phospholipids. Organic acids can also affect membrane permeability. A low pH and high proton concentration in the cell denature proteins, reduces the membrane proton gradient, neutralizizing the proton-motive force. The exposure of cellular compounds to protons can affect the ionic bonds of macromolecules and structure. A low pH may damage cellular

macromolecules in the cell wall, cell membrane, metabolic enzymes, protein synthesis and genetic material (Erkmen and Bozoglu, 2016).

Furthermore, the acetic acid formed is of major importance for the development of flavor and contribute to the aroma of bread dough. Therefore, the content of lactic and acetic acids in sourdoughs is very important for the taste and flavor of sourdough bread (Hansen and Hansen, 1996). The molar ratio between lactic and acetic acid is defined as the fermentation quotient (FQ) and the FQ should be around 4 in sourdoughs to obtain a balanced bread taste (Hansen, 2012); with the optimum considered to be in the range of 2.0-2.7 (Hammes and Ganzle, 1998). Regarding rye sourdough bread, the optimal FQ was reported to be in the range of 1.5-4.0 (Spicher, 1983).

A drop of pH causes some modifications by enhancing the performance of certain enzymes, such as amylases, proteases, hemicellulases and phytases. Differences in pH and enzyme activity affect the nutritional quality of the structure-forming components; i.e., protein, starch, lipid, dietary fibre, vitamins, minerals, sterols and phenolics, via different mechanisms (Gänzle et al., 2008; Poutanen et al., 2009).

In rye sourdoughs, acidification is also important as the acidification of rye doughs improves the physical properties of the dough by making them more elastic and extensible and confers the acid flavor notes so characteristic of rye breads (Cauvain, 1998).

2.2.4.2. Antibacterial and Antifungal Activities

During sourdough fermentation, the rapid consumption of fermentable carbohydrates by LAB and the formation of lactic acid, accompanied by a reduction of the pH, have an inhibitory effect on other microorganisms. Besides lowering the pH, a wide range of antimicrobial compounds, for example, diacetyl, hydrogen peroxide, acetic acid and other short chain fatty acids, are synthesised by

LAB and some of them effectively for the inhibite pathogen bacteria and fungi (Gänzle and Gobbetti, 2013).

Larsen et al. (1993) screened 335 LAB strains isolated from sourdoughs and 18 isolates that belonged to three different *Lactobacillus* species, *Lb. sakei* (formerly *Lb. bavaricus*), *Lb. curvatus* and *Lb. plantarum* showed antimicrobial activity indicated by a proteinaceous compound. Pepe et al. (2003) reported ropiness development in breads was inhibited for more than 15 days with LAB and inhibition of rope symptoms increased at a low pH (3.7 to 4.3). *Lb. plantarum* E5 and *Leu. mesenteroides* A27 showed the most effective antirope activity in their study. Corsetti et al. (1996) reported antimicrobial activity by *Lactobacilli* isolated from wheat sourdoughs belonging to the species *Lb. sanfranciscensis, Lb. brevis, Lb. fructivorans, Lb. fermentum, Lb. plantarum, Lb. farciminis, Lb. acidophilus, Lb. alimentarius* and *Lb. hilgardii. Lb. sanfranciscensis* and *Lb. plantarum* strains showed the largest spectrum of inhibition among the strains. On the other hand, *Lb. fermentum* and *Lb. alimentarius* strains had the narrowest inhibition spectrum. Furthermore, a bacteriocin-like inhibitory substance from *Lb.sanfranciscensis* C57 has been characterized. Antimicrobial activity of *Lb. reuteri* LTH2584, LTH3566 and *Lb. sanfranciscensis* LTH2594 isolated from wheat and rye sourdoughs was reported previously and the antimicrobial compound produced by *Lb. reuteri* LTH2584 exhibited the broadest inhibitory spectrum (Ganzle, 1998; Messens and De, 2002).

Inhibitory activity of certain antimicrobial compounds produced under bread-making conditions can be changed and the assessment of certain compounds under sourdough bread production conditions is necessary to elucidate any antimicrobial effects (Coda et al., 2011). A total of 437 *Lactobacillus* strains isolated from sourdoughs were screened for their antimicrobial compound production against four indicator strains (*Lb. farciminis* CC10, *Lb. sakei* LMG 2313, *Lb. delbrueckii* spp. *bulgaricus* B397 and *Listeria innocua* 4202) and 85 strains produced an inhibition zone against one or more indicators. It was reported

that a bacteriocin-like inhibitory substance was produced by *Lb. pentosus* isolated from sourdough, which was also active under sourdough conditions (Corsetti et al., 2004). In another study, *in situ* bacteriocinogenic activity of lacticin 3147-like bacteriocin from *Lc. lactis* M30 was reported (Settanni et al., 2005). In another study, prevention of visual rope generation caused by *B. subtilis* and *B. licheniformis* in sourdoughs at low pH values was reported. However, higher pH values were not effective in preventing rope since bacteriocins produced by *Lactobacillus* strains have optimal activities at pH 3.0-4.0 (Menteş et al., 2005; Menteş et al., 2007; Settanni and Corsetti, 2008). Corsetti et al. (1998) reported the antimold activity of some *Lactobacillus* spp. Among the species, *Lb. sanfranciscensis* had the largest spectrum and inhibited molds related to bread spoilage such as *Fusarium*, *Penicillium*, *Aspergillus* and *Monilia*. Caproic acid and also acetic, formic, propionic, butyric and *n*-valeric acids, in particular, were responsible for the antimold activity. Another study investigated the antifungal activity of several sourdough LAB. *Lb. plantarum* 21B showed a very broad spectrum of activity and inhibited many fungal species belonging to *Eurotium*, *Penicillium*, *Endomyces*, *Aspergillus*, *Monilia* and *Fusarium* that are most commonly isolated from contaminated baked goods (Lavermicocca et al., 2000).

2.2.4.3. Phytase Activity

Whole meal cereals are good sources of minerals but the bioavailability of minerals may be limited due to the presence of phytate, the salt form of phytic acid. Phytic acid (myoinositol hexakisphosphate) is a compound found in most cereal grains, legumes and nuts and it strongly binds minerals like iron and zinc (Lopez et al., 2002; Rizzello et al., 2017). By forming insoluble complexes with dietary cations, it impairs mineral absorption in humans (Poutanen et al., 2009; Hansen, 2012). The low pH values associated with sourdough fermentation lead to the solubilisation of the phytic acid complex as a result of the phytase activity of grain raw materials, LAB and yeasts; therefore, mineral bioavailability is increased

(Chavan and Chavan, 2011) as phytase activity is accelerated in the acidic environment of sourdough fermentation (Leenhardt et al., 2005). Acid production and lowering the pH is the major mechanism for LAB to improve mineral bioavailability (Poutanen et al., 2009). A wide variation in phytase activity has been detected in some yeasts and LAB isolated from sourdoughs (Chaoui et al., 2003; De Angelis et al., 2003; Reale et al., 2004).

2.2.4.4. Starch Digestibility

Sourdough has also been shown to be useful in the production of breads with slow starch digestibility and hence low glyceamic responses (Chavan and Chavan, 2011). Dietary carbohydrate represents a major source of plasma glucose and an increase in the amount of rapidly digestible carbohydrate in the diet increases blood glucose levels (GI). The level of starch digestibility is generally characterized by the rate and duration of the glyceamic response (Singh et al., 2010), and the GI is an important indicator of starch digestibility. Various physiological factors such as binding of α-amylase to substrates, gastric emptying, enzyme inhibitors, properties of digestive enzymes and viscosity within the digestive tract affect starch digestibility (Zhang et al., 2008; Zhang and Hamaker, 2009). The use of sourdough fermentation technology, especially in low pH levels, leads to a significant reduction in the glyceamic response (GI about 50) in comparison with usual yeast leavened white bread (white-wheat flour; GI 100) (Adam et al., 2003; Ostman, 2003; Fardet et al., 2006; De Angelis et al., 2007; Maioli et al., 2008; De Angelis et al., 2009). The effect of the sourdough process on the starch digestibility can be related to the formation of organic acids. It was reported that response of glucose and insulin are reduced in subjects who consumed sourdough compared with whole-meal bread alone and concluded that lactic acid lowers the rate of starch digestion in bread (Liljeberg et al., 1995). In addition, the chemical in sourdough fermentation can affect starch gelatinisation and promote the formation of resistant starch that is less digestible (Ostman, 2003;

Gobbetti et al., 2014). Moreover, pH-dependent proteolysis produces significant amount of peptides and amino acids (Nilsson et al., 2007; Gänzle et al., 2008), and increased levels of free phenolic compounds in fermented cereals may have a role in regulating glucose metabolism to decrease the GI (Katina et al., 2007; Solomon and Blannin, 2007; Poutanen et al., 2009).

2.2.4.5. Protease Activity

Proteolytic enzymes, proteases, are grouped into proteinases and peptidases. Proteinases catalyse protein degradation into smaller peptide fractions and peptidases hydrolyse specific peptide bonds or completely breakdown peptides to amino acids (Gänzle et al., 2008). The gluten protein network in wheat doughs determines dough rheology, gas retention and thus bread volume and texture. The viscoelastic properties of the gluten network allow the entrapment of CO_2 released during fermentation and result in breads with a light, porous crumb structure. Gluten is divided into two fractions according to the solubility in alcohol-water solutions namely, the soluble monomeric gliadins (50-60%) and insoluble polymeric glutenins (40-50%) (Osborne, 1907; Payne et al., 1984). They are regarded as gluten proteins and show different structure and functionality. The viscous properties of doughs are associated with the gliadins and low molecular weight glutenins. On the other hand, high molecular weight glutenins provide strength and elasticity to dough (Loponen, 2006; Gänzle et al., 2008). Their most important function is the formation of the gluten network during the preparation of doughs (Wang et al., 2015).

In sourdough production, acidification causes increased gluten solubility and endogenous cereal proteinase activity. Primary proteolysis, the break down of proteins to peptides, is mainly attributable to endogenous cereal proteases in wheat and rye sourdoughs (Thiele et al., 2002; Loponen et al., 2004; Tuukkanen et al., 2005). The flour endogenous proteinases of flour have an optimum pH at 3.0 to 4.0 and they are considered to be important for proteolysis in sourdough fermentations.

Besides primary proteolysis by cereal proteases, strain-specific proteolytic activity of LAB also contributes to proteolysis. Microbial reduction of disulfide bonds in gluten proteins by heterofermentative lactobacilli increase the solubility of gluten proteins and make them more susceptible to proteolytic degradation. The degradation of wheat and rye proteins is very important for bread flavor, volume and texture (Gänzle et al., 2008).

The partial hydrolysis of glutenins during sourdough fermentation results in disruption of the gluten network and increases the solubility of gluten proteins (Gänzle et al., 2008). The increasing solubility of gluten proteins promotes swelling and increased water uptake (Schober et al., 2003). Besides gluten, the partial acid hydrolysis of starch also leads to increased water binding capacity (Galle, 2013). Degradation of gluten protein structures in sourdoughs affects the viscoelastic properties of the final dough depending on the extent of the protein degradation. The rheological consequence of gluten degradation is the reduction of elasticity and firmness of the sourdough and subsequent bread dough. A weaker gluten network increases the expansion of dough, but also decreases gas retention. Therefore, the acidity level of sourdough and subsequent bread dough must be carefully controlled to attain increased volume (Galle, 2013). It is generally observed that a limited degree of proteolysis during sourdough fermentation is beneficial and improves the bread flavor without adverse effects on texture and volume (Thiele et al., 2002; Gänzle et al., 2008).

The gluten proteins of wheat and secalins of rye belong to prolamins, which are responsible for coeliac disease (gluten-sensitive enteropathy), a chronic gastrointestinal tract disorder where the ingestion of gluten from wheat, rye and barley and their crossbred varieties, leads to damage of the small intestinal mucosa by an autoimmune mechanism in genetically susceptible individuals (Green and Cellier 2007; Tye-Din and Anderson, 2008). The current treatment for coeliac disease is a life-long gluten-free diet. However, controlled proteolysis in wheat and rye sourdoughs may be used as a tool to reduce gluten levels to such an extent that

the products can be tolerated by coeliac patients (Rizzello et al., 2007; Katina and Poutanen, 2013).

Proteolytic degradation during fermentation provides the substrates for microbial growth and conversion of amino acids to flavor precursor compounds (Thiele et al., 2002) and antifungal metabolites (Lavermicocca et al., 2000). Therefore, protein hydrolysis and amino acid metabolism affect the flavor of sourdough and contribute to the beneficial effects of sourdough fermentation on bread quality; as proteins are degraded to free amino acids that may be converted to flavor compounds; i.e. the liberated amino acids act as flavour precursors during sourdough fermentation. The hydrolysis of peptides (secondary proteolysis) by sourdough lactobacilli leads to the accumulation of aminoacids in the dough in a strain dependent manner. On the other hand, yeasts decrease amino acids levels in dough (Gänzle et al., 2008). As a result, both the sourdough yeasts and LAB may facilitate the flavour formation, either directly via metabolizing amino acids to flavor compounds or indirectly by transforming them into secondary compounds that can serve as new precursors for further conversions (Loponen, 2006).

Different strains of LAB exhibit proteolytic activity during sourdough fermentation. Among LAB, *Lb. sanfranciscensis* has been shown to be particularly capable of degrading proteins or peptides, and proteinase, dipeptidase and aminopeptidase are the main enzymes that characterize the proteolytic system of this bacterium (Gobbetti et al., 1994; Gobbetti et al., 1996a). Gobbetti et al. (1996b) reported high proteolytic activity on gluten and especially high peptidase activities of *Lb. brevis* subsp. *lindneri*, *Lb. plantarum* and *Lb. farciminis* strains during sourdough fermentation. In particular, aminopeptidase, dipeptidase, tripeptidase and iminopeptidase activities were the highest in *Lb. brevis* subsp. *lindneri* CBI and A79 strains. In another study, gluten breakdown activities of *Lactobacilli* and *Pediococci* strains isolated from sourdough were investigated and besides *Lactobacillus* species, *Pd. pentosaceus* showed high proteolytic acitivity on gluten (Gerez et al., 2006). In addition, the presence of proteolytic *Lb. casei* strains

with the capacity to individually metabolize the coeliac-disease-related 33-mer peptide in sourdough was reported (Alvarez-Sieiro et al., 2016). Rollán et al. (2005) reported that *Lb. plantarum* CRL 759 and CRL 778 have an active proteolytic system, which is responsible for the high amino acid release during sourdough fermentation and the hydrolysis of the 31–43 α-gliadin-like fragment.

2.2.4.6. Wheat Germ Stability

Wheat germ is particularly rich in vitamins, lipids, high quality proteins and contains a significant amount of dietary fibre. However, the use of wheat germ in bread making is still moderate because of its poor shelf life stability; high lipase and lipoxygenase activities lead to the release of free fatty acids resulting in rancidity of baked goods(Gobbetti et al., 2014). Acidification with sourdough fermentation technology can affect wheat germ stability, chemical and nutritional characteristics, and also the texture and sensory characteristics of the white bread and it was reported that sourdough fermented wheat germ is an ingredient able to enhance the nutritional, texture and sensory properties of bread (Rizzello et al., 2010). It was also reported that sourdough fermentation partially inhibits the endogenous lipase activity of wheat germ and also increases the shelf life (Minervini et al., 2010).

2.2.4.7. Exopolysaccharide Production

Microbial exopolysaccharides (EPS), long chain sugar polymers, are metabolites produced by bacteria, microalgae and to a lesser extent, yeasts and fungi (Sutherland, 1972; De Vuyst and Degeest, 1999). Extracellular polysaccharides are secreted into the extracellular environment in the form of slime or associated with the cell surface in the form of capsules. Many food-grade microorganisms produce EPS especially LAB, propionibacteria and bifidobacteria (Cerning, 1990; Abbad Andaloussi et al., 1995; Cerning, 1995). Some LAB species are a good source of EPS which are also recognized for their contribution to the

texture, mouth feel, taste perception and stability of the final food product (Jolly et al., 2002; Gonzalez, 2006). Most of the EPS-producing LAB strains studied were isolated from dairy products but it is known that some LAB species produce EPS and links between specific metabolic activities of sourdough cultures and product quality are well-described for traditional sourdoughs (Galle et al., 2010). Tieking et al. (2003) analysed a total of 111 LAB and found EPS production by sourdough origining LAB such as *Lb. sanfranciscensis*, *Lb. frumenti*, *Lb. pontis*, *Lb. reuteri*, *Lb. panis* and *W. confusa*. They reported the production of EPS from sucrose as a metabolic activity which is common among sourdough LAB. In another study, production of linear dextrans from sucrose by *W. confusa* and *W. cibaria* isolated from wheat sourdoughs was reported (Amari et al., 2013). In another study, a strain of *W. confusa* produced dextrans and isomalto-oligosaccharides in sourdoughs without strong acidification. It was reported that the dextran significantly increased the viscosity of the sourdoughs (Katina et al., 2009). The application of dextran-enriched sourdoughs in bread baking has been reported to provide mildly acidic wheat bread with improved volume (up to 10%) and crumb softness (25–40%) (Di Cagno et al., 2006; Lacaze et al., 2007; Katina et al., 2009). Di Cagno et al. (2006) reported EPS synthesized from sucrose by sourdough *W. cibaria, Lb. plantarum* and *Pd. pentosaceus* strains. Another study reported, EPS production by *Lb. sanfranciscensis* during sourdough fermentation (Korakli et al., 2001).

2.3. Chickpea Fermentation

2.3.1. Chickpea (*Cicer arietinum* L.)

Chickpea is a legume of the family Fabaceae, in the Plantae Kingdom. It belongs to the Faboideae subfamily and *Cicer* genus. Cultivated chickpea (*Cicer arietinum* L.) is reported to be one of the first grain legumes domesticated in the world and ranks as the third most important legume worldwide (Hannan et al.,

2001). It is also a very important legume in Turkey and has been grown for nearly 7,400 years (Bhardwaj et al., 1999).

The history of chickpea dates back to around 7,000 BC and has been found in prehistoric sites in the east Mediterranean area. Helbaek (1970) reported the oldest known occurrence of chickpea in Hacilar near Burdur in Turkey, dated to about 5,450 BC (Helbaek, 1970). Chickpea has its origin in southeastern Turkey, and after its domestication in the Middle East, reached the Mediterranean region, India and Ethiopia (Ladizinsky, 1975; Varshney et al., 2017).

The mean annual production of chickpea was reported to be 10.16 million tons from 2004 to 2013. Chickpeas are produced in over 50 countries with India having the largest production and accounting for over 70% of total global production. Pakistan, Australia and Turkey are the next most important producers. Turkey accounts for 4 % of the world's production (Muehlbauer and Sarker, 2017). In Turkey, the mean yields of chickpea, harvest area and total production were reported to be 1191 (kg/ha), 437472 ha and 520935 tonnes production, respectively (Muehlbauer and Sarker, 2017).

The chickpea is the most cultivated legume in Turkey with 45% of the total among 8 legumes (Anonymous, 2013). It is an important source of protein and carbohydrates and the quality of protein is considered to be better than other pulses. Moreover, it contains significant amounts of all the essential amino acids except sulphur-containing amino acids, minerals (Ca, Mg, P and K), vitamins (riboflavin, niacin, thiamin, folate and the vitamin A precursor β-caroten) and dieatary fibre (Jukanti et al., 2012; Bidyarani et al., 2016). The nutritional composition of chickpea changes according to the growth conditions and variety. According to the USDA Food Composition Database, the macronutrient content of raw chickpeas are: 62.95% carbohydrates, 20.47% proteins and 6.04% fat. Carbohydrates include dietary fibre, oligosaccharides, starch and simple sugars, and the total dietary fibre composition in chickpea was reported to be 12.2 % (Wallace et al., 2016). Although lipids are present in low amounts, the chickpea is rich in nutritionally

important unsaturated fatty acids such as linoleic and oleic acids (Jukanti et al., 2012).

The chickpea is consumed to a significant degree in the Middle Eastern diet. Foods based on chickpeas are prepared by a wide range of recipes and preparation methods include soaking, grinding, sprouting, fermentation, boiling, mashing, roasting, frying and steaming treatments (Deshpande and Damodaran, 1990; Köksel et al., 1998). Chickpea flour addition to bread formulations improves the protein nutritional quality of produced bread (Estevez et al., 1987; Mohammed et al., 2012; Pathania et al., 2017). Moreover, in some Mediterranean countries, fermented chickpea is used as a leavening agent for the production of traditional breads and rusks (Hatzikamari et al., 2007a).

2.3.2. Chickpea Bread

Chickpea bread is produced in some Mediterranean and Balkan countries. Fermented chickpea liquid starter and dough are produced by the fermentation of chickpeas and used as a leavening agent for the production of chickpea bread. In Turkey, this bread type has been known especially in the Aegean and Thrace Regions and also some parts of the Middle Anatolia and Mediterranean Regions for a long time (Figure 2.4). Chickpea bread is traditionally produced at homes in those regions and also in small-scale bakeries (Hatzikamari et al., 2007a).

Figure 2.4. Chickpea breads from different regions

For the production of the chickpea liquid starter, coarsely ground chickpeas are put into a jar or bottle and then hot water is added. Chickpea fermentation is conducted in a hot location for around 16-18 hours. Bakeries put chickpea liquid jars in a hot place in the bakery and normally use blankets to prevent them cooling down. After 16-18 hours, a thick foam layer and the smell of chickpea liquid indicate the end of fermentation (Figure 2.5). This liquid is then used completely, or in some bakeries used after seperating the chickpeas for dough production. For that purpose, chickpea liquid is mixed with wheat flour and hot water. In some bakeries, boiled water is used instead of hot water. The resulting chickpea dough is kept in a warm place for a few hours and then used as a leaving agent in bread production.

Figure 2.5. Chickpeas and fermented liquid starter

Sensorial properties and shelf life are improved by using chickpea dough as the leavening agent; however, traditional chickpea bread is only known in some regions. As a result, studies on chickpea bread and fermentation are very limited.

2.3.3. Studies on Chickpea Bread

Chickpea dough is used in the production of some bread types and also different traditional bakery products. For example, chickpea leavened *simit* bread is well known in the Aegen region. In a study, the effect of chickpea type on the chemical, physical and microbial properties of the commercially produced chickpea fermentation liquid and *simit* bread were investigated (Kasım, 2014). The study optimized the production conditions and reported that different types of chickpeas resulted in end products with differing properties.

Chickpea bread has a different aroma as a result of the fermentation of different microorganisms (Özkaya, 1992). Hancıoğlu-Sıkılı (2003) investigated the aroma profile of chickpea breads produced using starters under laboratory conditions and reported higher levels of some carbonil compounds in chickpea breads than any other bread types (Hancıoglu-Sıkılı, 2003). Özkaya (1992) produced breads with different types of leaveners and reported that chickpea bread has a distinct aroma when it was compared with the bread produced using commercial instant yeast (Özkaya, 1992).

In the study of Baykara (2006), similar results were obtained. In this study, bread was manufactured using three different bread leaveners including commercial press yeast (*S. cerevisiae*), traditional leavener prepared with chickpea, and commercial press yeast (0.5%) + chickpea leavener. Sensorial evaluation results showed that bread samples made with press yeast (0.5%) + chickpea leavener were preferred to breads made with the other two leaveners (Baykara, 2006). Similar sensorial results were obtained in the study of Narlıoğlu (2013). In that study, a commercial yeast, a chickpea dough and a combination of them were used in the production and from sensorial point of view, a combination of two leaveners were preferred. In addition, results showed that during storage, water loss was the lowest in the chickpea doughs (Narlıoglu, 2013).

Çebi (2014) investigated the effects of different strains isolated from chickpea fermentations on the volatile profile, texture and color properties of

chickpea bread. It was reported that using a starter culture had statistically significant effects on crumb hardness, cohesiveness and chewiness values of the breads. 58 volatile compounds belonging to different chemical groups were determined in the dough, crumb and crust of bread produced with chickpea dough with the selected strains

2.3.4. Chickpea Fermentation Microflora

Studies on chickpea fermentations identified the microflora as some LAB, yeasts, *Bacillus* and *Clostridium* spp. (Katsaboxakis and Mallidis, 1996; Hancıoglu-Sıkılı, 2003; Hatzikamari et al., 2007b). Katsaboxakis and Mallidis (1996) isolated species belonging to *Lactobacillus, Corynebacterium, Micrococcus, Pediococcus, Bacillus* and *Clostridium* genera during the fermentation of coarsely ground chickpeas in water at 32, 37 and 42°C. Hatzikamari et al. (2007b) indicated that *Bacillus* and *Clostridium* species have an effect on the enzymatic and chemical changes observed during chickpea fermentations. These species degrade the compound into chickpea water and produce gas. In that study, at the beginning of the fermentation, *Bacillus* species, *B. cereus, B. thuringiensis* and *B. licheniformis* and then *Clostridium* species, *Cl. perfringens* and *Cl. beijerinckii* were identified. It was reported that *B. cereus* and *Cl. perfringens* grew predominantly during fermentation and did not seem to form toxins, hence any health hazard after consumption of the bread, properly baked, seems improbable.

In another study conducted in Turkey, chickpea liquid starters and doughs collected from bakeries were investigated for their microflora. *E. mundtii, E. gallinarum, E. casseliflavus, Lb. plantarum, Lb. pentosus, Lb. sanfranciscensis, Lb. viridescens, Lb. bifermentans, Pd. urinaeequi, St. thermophilus* and *Lc. lactis* subsp. *cremoris* and yeast *S. cerevisiae* were identified. The chickpea dough was then produced by using selected LAB starters (Hancıoglu-Sıkılı, 2003).

Çebi (2009) produced chickpea dough using traditional procedure under laboratory conditions and isolated and identified the LAB devoloped during the fermentation of chickpea liquid starter and the dough. The isolated LAB strains belonged to *Lactobacillus*, *Lactococcus* and *Weissella* genera. Species isolated from the chickpea liquid starter were identified as *Lc.* spp. *lactis*, *Lb. brevis* and *Lb.plantarum,* and those isolated from chickpea dough were identified as *Lc. lactis, Lb. brevis*, *Lb. plantarum, Lb. pentosus* and *W. confusa.*

Erginkaya et al. (2016) reported the dominant microflora in chickpea fermentations to be LAB, yeasts, aerobic and anaerobic spore-forming bacteria. They produced chickpea bread under laboratory conditions and supported *Bacillus* and *Clostridium* species in chickpea fermentations.

3. MATERIALS AND METHOD

3.1. Materials and Sampling

In the present study, six commercial bakeries in different cities were selected based on their traditional production of natural sourdough and chickpea dough. A total of 20 samples were collected including sourdough (8), chickpea liquid starter (6) and chickpea dough (6) samples at two different times as shown in Table 3.1.

Table 3.1. Sampling dates and locations

City	Type of the sample	1. sampling	2. sampling
Mersin	Sourdough	26/04/2016	08/11/2016
Antalya	Sourdough	02/05/2016	02/01/2017
Ankara	Sourdough	09/06/2016	10/02/2017
Birgi Town/Ödemis/İzmir	Chickpea liquid starter and dough	25/05/2016	23/02/2017
Söke/Aydın	Chickpea liquid starter and dough	25/05/2016	23/02/2017
Nevşehir	Chickpea liquid starter and dough	01/04/2016	14/11/2016

3.1.1. Sourdough Samples

Whole-meal wheat sourdough samples were collected from Pikan Bakery in Antalya, Gattini Bistro in Mersin and Canberk Food Company in Ankara. In addition, a rye sourdough sample was taken from one of the locations together with whole-meal wheat sourdough. Collected sourdoughs were Type I sourdoughs produced without baker's yeast. The sourdough sample was taken aseptically before the daily refreshment step, and put into sterile jars (Figure 3.1). Samples were kept at 4°C until analyses. All samples were subjected to chemical and microbiological analyses in the Industrial Microbiology Laboratory at the Food Engineering Department in Çukurova University within 24 hours.

Figure 3.1. Sourdough samples were taken into sterile jars

3.1.2. Chickpea Liquid Starter and Dough Samples

The chickpea liquid starter and dough samples were collected from Cumhuriyet Bakery in Soke/Aydın, Tokoglu Bread in Birgi Town/Odemis/Izmir and Yayla Bakery in Nevsehir. All of the bakeries have been producing chickpea bread for years and are well-known in their regions. Chickpea liquid starter samples were obtained by seperating chickpeas from the fermentation liquid at the end of the fermentation (Figure 3.2). Chickpea dough samples were collected by taking a piece of a final leavened dough (Figure 3.3).

Figure 3.2. Fermented chickpea liquid starter and obtained liquid after separation of the chickpeas

Figure 3.3. Chickpea dough

Samples were taken aseptically, placed into sterile jars and kept at 4°C until analyses. All samples were subjected to chemical and microbiological analyses in the Industrial Microbiology Laboratory at the Food Engineering Department in Çukurova University (within 24 hours).

3.2. Production of Sourdough and Chickpea Dough under Laboratory Conditions

Sourdough and chickpea dough samples were produced as controls on a laboratory scale and chemical and microbiological analyses were also performed on the control samples. All analyses were performed in duplicate.

3.2.1. Laboratory Sourdough Production and Sampling

Sourdough production under laboratory conditions proceeded according to the traditional (sourdough Type I) protocol without using starter culture or baker's yeast (Figure 3.4.). Doughs were prepared with boiled and cooled tap water and whole-meal wheat flour from local company belonging to the same production batch. For dough preparation, whole-meal wheat flour (216.21 g) and boiled and cooled tap water (183.79 mL) were mixed manually to produce 400 g of dough

with a dough yield [(dough weight/flour weight)×100] of 185. Each sourdough was fermented at 28°C for 24 h in glass jars covered with a lid. The resulting sourdoughs were propagated over a period of 7 days according to the daily back-slopping (refreshment) procedure and the sourdough from the previous day's fermentation was used as the starter (20% [wt/wt] of inoculum) to ferment a new mixture of flour (172.98 g) and tap water (147.02 mL), resulting in a dough yield of 185 (Figure 3.5). Sourdough production was carried out in duplicate.

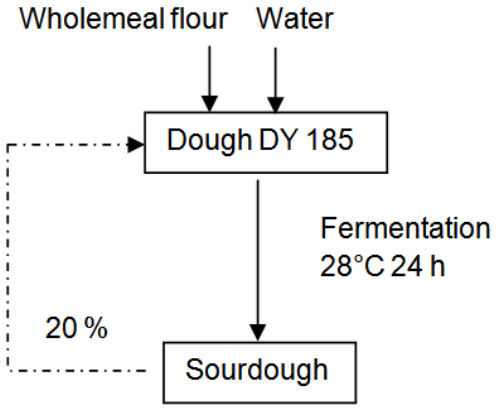

Figure 3.4. Sourdough production under laboratory conditions

The first sample (0 h) was taken from the flour and water mixture, unfermented dough, after mixing. During the back-slopping procedure, sampling was performed on the sourdoughs immediately before the daily refreshment step. Both total titratable acidity (TTA) and pH measurements were carried out on all samples collected after 4 (4 h), 8 (8 h) and 12 (12 h) hours of the experiment and once every 24 hours of until the last refreshment of the sourdough production. The samples collected at 0 h, 1 (1d), 2 (2d), 4 (4d) and 7 (7d) days of daily back-slopping were also subjected to plate counting and isolation of presumptive LAB and yeasts in addition to TTA and pH measurements. Furthermore, carbohydrate, organic acid and ethanol analyses were conducted on these samples. Samples were analysed in duplicate.

Figure 3.5. Laboratory produced sourdough sample

3.2.2. Laboratory Chickpea Liquid Starter and Dough Production and Sampling

Production of chickpea liquid starter and dough is shown in Figure 3.6. Chickpeas (*Koçbaşı* variety) were ground for the production of chickpea liquid. Then 50 g of chickpeas were put into sterile glass jars and mixed with 400 mL of boiled and cooled tap water (50°C). Fermentations were conducted in glass jars covered with a lid during 18 hours at two different temperatures, 32 and 37°C. After the typical smell of the chickpea liquid and foam formation occured, fermentation was terminated. At the end of the fermentation, chickpeas were seperated and the liquid was used for the production of the chickpea dough. 150 mL of liquid is mixed with 200 g of flour (DY 175) from local company and fermented at 32 and 37 °C for 4 hours. Chickpea liquid starter and dough production were carried out in duplicate. Chickpea liquid starter production is shown in Figure 3.7. Chemical and microbiological analyses were performed in duplicate on the chickpea liquid starter (CLS) and chickpea dough (CD) samples fermented at two different temperatures. Samples were collected at the beginning and end of the fermentations, 0 and 18 h, in chickpea liquid starter and 0 and 4h in chickpea dough. The collected samples were subjected to plate counting and isolation of presumptive LAB and yeasts besides the TTA and pH measurements

and also carbohydrate, organic acid and ethanol analyses. Samples were analyzed in duplicate.

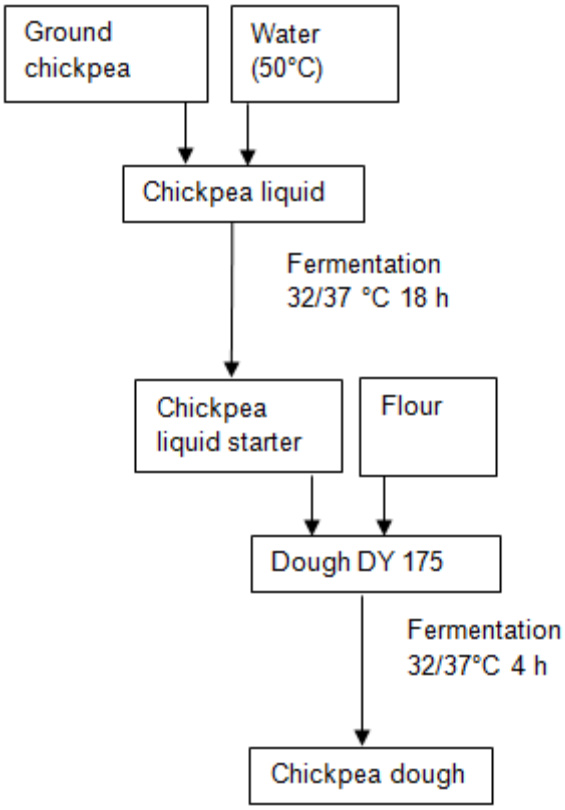

Figure 3.6. Chickpea liquid starter and dough production

Figure 3.7. Chickpea liquid starter production under laboratory conditions

3.3. Determination of pH, Total Titratable Acidity, Carbohydrates, Organic Acids and Ethanol

Chemical analyses were conducted on the sourdough, chickpea liquid starter and dough samples that were collected from different bakeries and also samples produced on laboratory scale. For each sample pH, TTA, carbohydrate, organic acid and ethanol analyses were conducted. All analyses were performed at least in duplicate.

3.3.1. Determination of pH

The pH measurement of the samples was performed using a digital glass pH meter (Mettler Toledo, SevenCompact™ pH Ion S220, Switzerland) previously calibrated with 3 standard solutions at pH 4, 7 and 11. For the determination of pH, 10 g of sample was homogenized with 90 mL of distilled water, using a magnetic plate stirrer for 3 min, and the pH was measured by inserting the probe into the mixture (Lopez et al., 2001).

3.3.2. Determination of Total Titratable Acidity

Total titratable acidity of the samples was determined after homogenization of 10 g of sample with 90 mL of distilled water on a magnetic plate stirrer. The mixture was then titrated with 0.1 N NaOH to a final pH of 8.5. The TTA was expressed as the amount (mL) of 0.1 M NaOH needed to achieve the pH of 8.5 (Lopez et al., 2001).

3.3.3. Assessment of Carbohydrates, Organic Acids and Ethanol Content Using High Performance Liquid Chromatography (HPLC)

3.3.3.1. Determination of Carbohydrates, Organic Acids and Ethanol

Maltose, sucrose, glucose, fructose, ethanol and lactic and acetic acids were determined using HPLC. The liquid chromatographic apparatus (LC-20 AD, Shimadzu, Kyoto, Japan) consisted of a pump system, an on-line degasser (DGU-$20A_5$), a column oven (CTO-10ASVP), a refractive index detector (RID-10A) for sugar and ethanol analysis and a UV/Vis detector (SPD-20A) monitored at 210 nm for the analysis of organic acids. The injection volume was 20 µL. Chromatographic separation was performed using an Aminex HPX-87H column (300 x 7.8 mm, Bio-Rad, Hercules, CA, USA) under the following conditions: flow rate 0.5 mL/min and column temperature 50°C. The mobile phase was 5 mM H_2SO_4. Specific Shimadzu software was used for data evaluation. All of the analyses were performed in duplicate and the results are expressed as means ± standard deviation.

3.3.3.2. Preparation of the Standards

Stock standard solutions were prepared individually from HPLC grade standards obtained from Sigma-Aldrich. Seven-point standard curves were constructed from standard solutions. The method's limit of detection (LOD), limit of quantification (LOQ) and recovery were determined for maltose, sucrose, glucose, fructose, lactic acid, acetic acid and ethanol. The LOD and LOQ values

were estimated as 3 and 10 times the standard deviation derived from analyses of 10 injections at the lowest calibration levels, respectively. For the recovery test, the dough sample was spiked with standards during the homogenization step at final concentrations in the linear range of the calibration curves. Spiked and unspiked samples of the dough were analysed under the same conditions. Six replicates were used for the determination of recovery and results were calculated for each standard based on the following formula:

$$\%\, recovery = \frac{C_s - C_u}{C_a} \times 100$$

C_s= Concentration of the analyte in spiked sample

C_u= Concentration of the analyte in unspiked sample

C_s= Concentration of the analyte added

3.3.3.3. Extraction Procedure

Ten g of sample was homogenized with 90 mL 25 mM phosphate buffer (pH 5.6) according to the extraction method of Paramithiotis et al. (2006). The mixture was centrifuged (12,000 rpm, 10 min, at 4°C; Kubota 7780, Japan). One mL of the supernatant was mixed with 50 µl of perchloric acid and kept at 4°C for 24 h. Protein agglomerates were removed by centrifugation (12,000 rpm, 60 min, 4°C; Hettich® Universal 320R, Germany) and the supernatant was filtered through a 0.45 µm PVDF Syringe Filter (Isolab) and injected into the HPLC system.

3.4. Microbiological Analyses

Sourdough (SD) and chickpea dough (CD) samples (10 g) were suspended with 90 mL of sterile 0.85% (wt/vol) NaCl solution in a sterile stomacher bag and homogenized for 3 min using a bag mixer (Interscience, model 400 P, France) and a 10-fold dilution series of the samples was made by using 9 mL of a sterile 0.85% (wt/vol) NaCl solution. Chickpea liquid starter (CLS) samples were serially diluted

by using 9 mL of a sterile 0.85% (wt/vol) NaCl solution. From each homogenate, 10-fold dilution series were prepared and aliquots (0.1 mL) of decimal dilutions were spread onto selected agar media, as shown in Table 3.2 by the spreading plate method for microbial counts of LAB, yeasts, total mesophilic aerobic bacteria, molds (filamentous fungi) (Madigan et al., 2012). For coliform group bacteria, liquid media (broth) was used to conduct the Most Probable Number (MPN) method instead of the spreading plate method (Clesceri et al., 1998). Selected media were supplemented with different sterile filtered (Millex-GS, 0.22 μm filter) antibiotics according to the target organism. For that purpose, cycloheximide (0.1 g/L, Sigma), sodium propionate (2 g/L, Sigma-Aldrich) and oxytetracycline (0.1 g/L, Sigma) antibiotics were used to prevent yeast, mold and bacteria growth, respectively.

Table 3.2. Media used for the enumeration of microorganisms in the sourdough, chickpea liquid starter and dough samples

Sample	Presumptive group of microorganism	Media	Incubation conditions
Sourdough Chickpea liquid starter Chickpea dough	LAB	mMRS agar	30°C, 48-72 hours anaerobic
Sourdough Chickpea liquid starter Chickpea dough	LAB	gM17 agar	30°C, 48-72 hours anaerobic
Sourdough	LAB	SDB agar	30°C, 48-72 hours anaerobic
Sourdough Chickpea liquid starter Chickpea dough	Total yeasts	YPD agar	28°C, 48-72 hours
Sourdough Chickpea liquid starter Chickpea dough	non- *Saccharomyces* yeasts	L-lysine agar	28°C, 48-72 hours
Sourdough Chickpea liquid starter Chickpea dough	Total mesophilic aerobic bacteria	PCA agar	30°C, 72 hours
Sourdough Chickpea liquid starter Chickpea dough	Mold	MEA agar	28°C, 3-5 days
Sourdough Chickpea liquid starter Chickpea dough	Presumptive coliform	LST broth	37°C, 24-48 hours
Chickpea liquid starter Chickpea dough	Total mesophilic aerobic bacteria (Presumptive *Bacillus* spp.)	Nutrient agar	37°C, 18 hours

3.4.1. Viable Counts of Presumptive LAB

Enumeration of presumptive LAB was estimated by plating serially diluted SD, CD and CLS samples onto different media including modified de Man Rogosa Sharpe (mMRS) (Merck) agar containing 1% maltose (w/v) and 5% fresh yeast

extract solution (v/v) and modified glucose M17 (gM17) (Merck) agar containing 0.5% glucose. In additon, the microbial suspensions of sourdough samples were plated on sourdough bacteria agar (SDB) (Obis et al., 2001; Valmorri et al., 2006; Settanni et al., 2011). SDB agar medium contained 2% maltose (w/v), 0.6% pancreatic digest of casein (w/v), 0.3% yeast extract (w/v), 10% fresh yeast extract solution (v/v), 0.03% Tween 80 (v/v) and 1.5% agar (w/v) (Kline and Sugihara, 1971). All media were supplemented with cycloheximide (0.1 g/L) and sodium propionate (2 g/L) to prevent growth of yeasts and molds, respectively. Incubation of plates was performed anaerobically by using Anaerocoult A (Merck 1.13829) in sealed jars at 30 °C for 48-72 hours. Each plate (mMRS, M17 and also SDB for sourdoughs) was counted and results are expressed as CFU/g or mL (colony forming units per gram or mL sample).

From the selected plates, 10-15 colonies/plate were randomly picked and streaked onto a single agar plate containing appropriate agar media for isolation by the plate-streaking technique. Streaked plates were incubated at 30°C for 48 hours anaerobically and then colonies were examined. When all of the colonies on the plate had the same general appearance, a colony was picked and subsequently transferred into the corresponding broth media and incubated at 30°C for 48 hours. Each colony that had a different appearence on a plate was streaked again onto a separate plate until a pure culture was obtained. Then, isolated LAB colonies were further subjected to Gram stain and catalase tests, and Gram (+) and catalase (-) isolates were transferred into the corresponding broth media containing 40% (v/v) sterile glycerol solution and stored at -25 °C.

3.4.2. Viable Counts of Presumptive Yeasts

Cell densities of total yeasts were estimated by plating serially diluted samples on Yeast Extract Peptone Dextrose (YPD) (Sigma) agar medium. YPD agar medium was supplemented with oxytetracycline (0.1 g/L) and sodium propionate (2 g/L) to prevent the growth of bacteria and molds, respectively.

Incubation of plates was performed at 28 °C for 48-72 h. Each plate was counted and results are expressed as CFU/g or mL (colony forming units per gram or mL sample).

From the selected plates, 10-15 colonies/plate were randomly picked and streaked onto YPD agar media for isolation by the plate-streaking technique. Streaked plates were incubated at 28°C for 48 hours and then colonies were examined. When all of the colonies on the plate had the same general appearance, a colony was picked and subsequently transferred into the corresponding broth media and incubated at 28°C for 48 hours. Each colony that had a different appearence on a plate was streaked again onto a separate plate until a pure culture was obtained. Pure cultures were transferred into the corresponding broth media containing 40% (v/v) sterile glycerol solution and stored at -25 °C.

3.4.3. Viable Counts of Presumptive Non-*Saccharomyces* Yeasts

Enumeration of non-*Saccharomyces* yeasts were estimated by plating serially diluted samples on L-lysine agar medium supplemented with oxytetracycline (0.1 g/L) and sodium propionate (2 g/L) to prevent the growth of bacteria and molds, respectively. Incubation of plates was performed at 28 °C for 48-72 h. Each plate was counted and results are expressed as CFU/g or mL (colony forming units per gram or mL sample).

From the selected plates, 10-15 colonies/plate were randomly picked and streaked onto YPD agar media for isolation by the plate-streaking technique. Streaked plates were incubated at 28°C for 48 hours and then colonies were examined. When all of the colonies on the plate had the same general appearance, a colony was picked and subsequently transferred into the corresponding broth media and incubated at 28°C for 48 hours. Each colony that had a different appearence on a plate was streaked again onto a separate plate until a pure culture was obtained. Pure cultures were transferred into the corresponding broth media containing 40% (v/v) sterile glycerol solution and stored at -25°C.

3.4.4. Viable Counts of Total Mesophilic Aerobic Bacteria

Total mesophilic aerobic bacteria were enumerated by plating serially diluted samples on Plate Count Agar (PCA) (Merck) supplemented with cycloheximide (0.1 g/L) and sodium propionate (2 g/L) to prevent the growth of yeasts and molds, respectively. Incubation of plates was performed at 30 °C for 72 hours. Each plate was counted and results are expressed as CFU/g or mL (colony forming units per gram or mL sample).

3.4.5. Viable Counts of Molds

Molds were enumerated by plating serially diluted samples on Malt Extract agar (MEA) (Merck) medium supplemented with oxytetracycline (0.1 g/l) and cycloheximide (0.1 g/l) to prevent growth of bacteria and yeasts, respectively.Incubation of plates was performed at 28 °C for 3-5 days. Then plates were counted and results were expressed as CFU/g or ml (colony forming units per gram or ml sample).

3.4.6. Total Presumptive Coliform Count

Total coliform counts were performed using the MPN technique. One mL aliquots of decimal dilutions of the samples were inoculated into 3 tubes containing 10 mL of Lauryl Sulfate Tryptose (LST) broth (Merck) with Durham tube. The tubes were incubated at 37°C for 24 h. After 24 hours, the tubes were removed from the incubator and the inner durham tubes were examined for gas production. Growth and gas production in the tubes showed presumptive coliforms and these tubes were recorded as positive and estimated using the MPN method. Gas-negative tubes were re-incubated for an additional 24 h and then examined again at 48 h (Feng et al., 2002). The indole test was conducted by adding 0.2-0.3 mL of Kovacs' indole reagent (Merck) to the gas-positive tubes and development of a distinct red color in the upper layer was recorded as positive showing the growth of

an indole positive culture. Indole-positive tubes were reported as presumptive *Escherichia coli* and evaluated using the MPN method (Halkman, 2005).

3.4.7. Viable Counts of Presumptive *Bacillus* spp.

Presumptive *Bacillus* spp. were enumerated by plating serially diluted samples onto Nutrient agar (NA) (Merck) and incubated at 37°C for 18 hours (Hatzikamari et al., 2007b). Then plates were counted and results were expressed as CFU/g or mL (colony forming units per gram or mL sample).

In laboratory produced samples, heat treatment was applied to samples for the enumeration of spore-forming bacteria. Before inoculation, 1:10 dilutions of the samples were heated to 80°C for 10 minutes. Dilutions were then cooled down to 37°C and inoculated onto petri dishes using the spread plating method. Heat application enabled survival of only spore-forming bacteria, resulting in the enumeration of aerobic spore-forming bacteria, most probably *Bacillus* spp., as reported by other researchers (Halkman, 2005; Erginkaya et al., 2016).

3.5. Molecular Identification of LAB Isolates

Potential LAB isolates were subjected to genotypic characterization by RAPD-PCR analysis and identification by sequence analysis of 16S rRNA genes. LAB isolates were grown overnight in corresponding broth media at 30°C, cells were havested and genomic DNA was extracted using an InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol.

3.5.1. DNA Extraction

Stored LAB isolates were activated and genomic DNA was prepared from LAB isolates after overnight growth at 30°C in a microcentrifuge tube containing broth media. For the genomic DNA extraction, overnight grown LAB culture was centrifuged (Thermo Scientific™ MicroCL 17 microcentrifuge, Germany) at 13,300 rpm for 3 min to pellet the cells (Figure 3.8). The supernatant was discarded

without disturbing the cell pellet. The pellets were then resuspended in sterile ultra-distilled water, vortexed at high speed for 10 s and centrifuged again. This step was repeated three times and the supernatant was removed. Then 150-200 µL of InstaGene matrix kit was added to the pellet using 1,000 µL pipette tip, vortexed for 10 s and incubated at 56 °C for 30 min. Following the incubation, the tubes were vortexed for 10 s and placed in a 100°C boiling waterbath for 8 min. The tubes were then vortexed at high speed for 10 s and centrifuged at 13,300 rpm for 3 min. The resulting supernatant including crude cell extract was stored at -20 °C. The stored DNA extract was used for further PCR assays.

Figure 3.8. Centrifugation in the microcentrifuge to pellet the cells

3.5.2. Randomly amplified polymorphic DNA (RAPD) PCR analysis

Differentiation of the LAB isolates was performed using RAPD-PCR analysis in a 25-µL reaction mix using the M13 primer with the sequence 5'-GAGGGTGGCGGT TCT-3' (Stenlid et al., 1994; Settanni et al., 2012). The PCR mix for the M13 primer was prepared as follows:1.25 µL (50 ng) DNA, 2.5 µL Dream Taq buffer (10x +20mM $MgCl_2$Thermo Scientific), 2 µL dNTP (2.5 mM, Thermo Scientific), 1 µL $MgCl_2$ (25 mM, Thermo Scientific), 0.2 µL M13 primer (100 µM, Thermo Scientific), 0.2 µL Dream Taq DNApolymerase (5 U/µL, Thermo Scientific) and sterile distilled water in 25-µL mixture.

The concentrations of DNA extracts were determined using a Qubit 3.0 Fluorometer using Qubit® dsDNA BR Assay Kit (Life Technologies, Invitrogen). DNA amplifications with the M13 primer were performed in the thermocycler (Techne TC-Plus 02, UK) programmed as follows: initial denaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 1 min; annealing at 42°C for 20 sec and extension at 72°C for 2 min; plus a final extension step at 72°C for 10 min.

RAPD fragments were separated using 1.2% (w/v) agarose (Sigma) gel electrophoresis prepared with 1 x TBE diluted from 5 x TBE that contained 54 g/L (w/v) Trisma base (Sigma), 27.5 g/L (w/v) boric acid (Merck) and 7.44 g/L (w/v) EDTA (Titriplex® III, ethylenedinitrilotetraacetic acid disodium salt dihydrate, Merck). SYBR Safe™ DNA gel stain (Invitrogen) was used for visualization of DNA bands under UV light. DNA samples were loaded onto the agarose gel with DNA loading dye (6X LD, Thermo Scientific). One kb Gene ruler (Thermo Scientific) and O'Gene Ruler mix (Thermo Scientific) DNA ladders were used as the molecular size markers to determine the size of the amplified DNA fragment. The electrophoresis was run in 1 x TBE at 120 V and then visualized (Vilber Lourmat Infinity V X 2, France) in the gel Image system as shown in Figure 3.9. RAPD-PCR profiles were analysed using band pattern analysis employing the software package (Infinity V X 2). Images of amplification fragments were scored as band absent (0) or present (1) and data were entered into a binary matrix. Similarity indices of band profiles were calculated on the basis of the Jaccard coefficient. Dendrograms were constructed by means of the unweighted pair group method with arithmetic average (UPGMA) and 1 or 2 LAB isolates in each cluster were identified by 16S rRNA gene sequencing.

Figure 3.9. Gel Image System (Vilber Lourmat Infinity)

3.5.3. 16S rRNA Gene Sequence Analysis

Molecular identification of LAB with different RAPD-PCR profiles was carried out using 16S rRNA gene sequencing. PCR amplification was performed using primers fD1(5'- AGAGTTTGATCCTGGCTC AG-3') and rD1 (5'-AAGGAGGTGATCCAG CC-3') (Weisburg et al., 1991). The PCR mix was prepared as follows: 5 µL DNA, 6 µL Dream Taq buffer (10x +20 mM $MgCl_2$, Thermo Scientific), 5 µL dNTP (2.5 mM, Thermo Scientific), 1.2 µL $MgCl_2$ (25 mM, Thermo Scientific), 0.08 µL fD1 primer (100 µM, Thermo Scientific), 0.08 µL rD1 primer(100 µM, Thermo Scientific) and 0.5 µL Dream Taq DNA polymerase (5 U/µL, Thermo Scientific) in a 50-µL mixture. Amplification was performed in the thermocycler (Techne TC-Plus 02, UK) which was programmed as follows: initial denaturation at 95°C for 3 min; 30 cycles of denaturation at 94°C for 1 min; annealing at 54°C for 45sec and extension at 72°C for 2 min; plus a final extension step at 72°C for 7 min.

PCR products were separated by electrophoresis on a 1.5% (w/v) agarose (Sigma) gel stained with SYBR Safe™ DNA gel stain (Invitrogen) and subsequently visualized by Vilber Lourmat Infinity (V X 2, France). PCR amplicons were sent to BM Laboratuvar Sistemleri (Ankara) for sequencing. The

ABI chromatograms of the sequences were examined, multiple alignments were performed using ClustalW Multiple alignment (Bioedit version 7.0.9) and then resultant sequences were compared by Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) with nucleotide sequences deposited at the database National Center for Biotechnology Information (NCBI) (Altschul et al., 1997). Species identity was determined by comparison to reference sequences of the 16S rRNA gene sequences with a threshold of 98% (Yarza et al., 2014).

Phylogenetic analyses were performed using the sequences from at least 1,400 bp DNA fragments that were generated using the fD1 and rD1 primers. Phylogenetic trees were constructed based on aligned sequences using the two possible tree reconstruction methods, i.e., UPMGA and Minimum evolution, with MEGA 7.0 software.

3.6. Molecular Identification of Yeast Isolates

Potential yeast isolates were subjected to genotypic characterization by ITS region amplification of the 5.8S rRNA gene, its Restriction Fragment Length Polymorphism (RFLP) analysis and identification by sequence analysis of the D1/D2 domain of the 26S rDNA gene. Yeast isolates were grown for 24-36 h in YPD broth media at 28°C, cells were harvested and genomic DNA was extracted using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol.

3.6.1. DNA Extraction

Stored yeast isolates were activated and genomic DNA was prepared from yeast isolates after 24-36 h growth in YPD broth media at 28°C. For the genomic DNA extraction, yeast culture grown in a microcentrifuge tube was centrifuged (Thermo Scientific™ MicroCL 17 microcentrifuge, Germany) at 13,300 rpm for 3 min to pellet the cells. The supernatant was discarded without disturbing the cell

pellet. The pellets were then resuspended in sterile ultra-distilled water, vortexed at high speed for 10 s and centrifuged again. This step was repeated three times and the supernatant was removed. Then, 50 µL of freshly prepared lyticase (L4025, ≥200 units/mg solid, Sigma-Aldrich, MO, USA) solution (4 U/µL) was added to the pellet and incubated at 37°C for 1 h to digest the yeast cells. The pellets were then centrifuged at 13,300 rpm for 3 min. Supernatant was discarded and the pellets were resuspended in sterile ultra-distilled water, vortexed at high speed for 10 s and centrifuged again. This step was repeated twice. The supernatant was removed and 120-200 µL of InstaGene matrix kit (Bio-Rad Laboratories, USA) was added to the pellet using 1,000 µL pipette, vortexed 10 s and incubated at 56°C for 30 min. Following incubation, the tubes were vortexed for 10 s and placed in a 100°C boiling waterbath for 8 min. The tubes were then vortexed at high speed for 10 s and centrifuged at 13,300 rpm for 3 min. The resulting supernatant including crude cell extract was stored at -20°C. The stored DNA extract was used for further PCR assays.

3.6.2. ITS Region Amplification of the 5.8S rRNA Gene

Differentiation of the yeasts was performed via internal transcribed spacer (ITS) region amplification of the 5.8S ITS rRNA region using primers ITS1 (5'-TCCGTAGGTGAACCTGCG G-3') and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as described previously (Esteve-Zarzoso et al., 1999). The PCR mix for ITS region amplification was prepared as follows: 2.5 µL (50-100 ng) DNA, 5 µL Dream Taq buffer (10x +20 mM $MgCl_2$, Thermo Scientific), 5 µL dNTP (2.5 mM, Thermo Scientific), 2.5 µL $MgCl_2$ (25 mM, Thermo Scientific), 0.126 µL ITS1 primer (100 µM, Thermo Scientific), 0.126 µL ITS4 primer (100 µM, Thermo Scientific) and 0.150 µL Dream Taq DNA polymerase (5 U/µl) and sterile distilled water in a 50-µL mixture. 5.8S ITS rRNA regionamplifications were performed in the thermocycler (Techne TC-Plus 02, UK) which was programmed as follows: initial denaturation at 95°C for 5 min; 35

cycles of denaturation at 94°C for 1 min; annealing at 55°C for 2 min and extension at 72°C for 2 min; plus a final extension step at 72°C for 10 min.

PCR products were separated on a 1.5% w/v agarose gel stained with the SYBR Safe™ DNA gel stain (Invitrogen). The electrophoresis was run in 1× TBE at 110 V and subsequently visualized (Vilber Lourmat Infinity V X 2, France). The sizes of the fragmentswere determined using a standard molecular weight marker (100 bp/plus ladder, Thermo Scientific) (Settanni et al., 2011).

3.6.3. RFLP (Restriction Fragment Length Polymorphism) Analysis

PCR products of the 5.8S ITS region were digested using the restriction endonucleases *Hae* III, *Hha* I and *Hinf* I (Thermo Scientific). Digestions with three different endonucleases were performed seperately by adding 10 µL of the amplified DNA to 15 µL of the restriction enzyme mixture including 2.5 µL restriction enzyme buffer (Buffer R for *Hae* III and *Hinf* I and Buffer Tango for *Hha* I), 11.5 µL sterile distilled water and 1 µL (10 U/µL) restriction enzyme. The mixtures were then put into a water bath at 37°C for 10-16 hours.

Restriction fragments were analysed through 2% (w/v) agarose gel in 1 x TBE buffer and stained with SYBR Safe™ DNA gel stain (Invitrogen). The electrophoresis was run in 1 x TBE at 120 V and subsequently visualized (Vilber Lourmat Infinity V X 2, France).The sizes of the fragments were determined using a standard molecular weight marker (50 bp ladder, Thermo Scientific) (Settanni et al., 2011).

Yeast sharing identical restriction patterns were classified into groups and 1 or 2 isolates were chosen as a representative of each group for sequence analysis of the D1/D2 domains of the 26S rRNA gene.

3.6.4. 26S rRNA Gene Sequence Analysis

Sequence analysis of the D1/D2 domain of the 26S rDNA gene was used to differentiate yeast isolates. Amplification of the D1/D2 domains of 26S rRNA was

carried out using NL1 (5'-GCA TAT CAATAAGCGGAGGAA AAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACG G-3') primers as described previously (Kurtzman and Robnett, 1998). The PCR mix for D1/D2 domain of the 26S rDNA gene sequence analysis was prepared as follows: 5 µL (50-100 ng) DNA, 5 µL Dream Taq buffer (10x +20 mM $MgCl_2$, Thermo Scientific), 0.5 µL dNTP (2.5 mM, Thermo Scientific), 2.5 µL $MgCl_2$ (25 mM), 0.1 µL NL1 primer (100 µm, Thermo Scientific), 0.1 µL NL4 primer (100 µm, Thermo Scientific) and 0.5 µL Dream Taq DNApolymerase (5 U/µl, Thermo Scientific) and sterile distilled water in 50-µL mixture. Amplifications of the D1/D2 domains of 26S rRNA were performed in the thermocycler (Techne TC-Plus 02, UK) which was programmed as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 94°C for 1 min; annealing at 52°C for 45 sec and extension at 72°C for 1 min; plus a final extension step at 72°C for 7 min.

PCR products were separated by electrophoresis on a 1.5% (w/v) agarose (Sigma) gel stained with the SYBR Safe™ DNA gel stain (Invitrogen) and subsequently visualized (Vilber Lourmat Infinity V X 2, France). PCR amplicons were sent to BM Laboratuvar Sistemleri (Ankara) for sequencing. Resultant sequences were compared using the BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with nucleotide sequences deposited at the database National Center for Biotechnology Information (NCBI) (Altschul et al., 1997). The sequence alignments were evaluated using ClustalW with type strains and their closest relatives (Bioedit version 7.0.9) (Thompson et al., 1997; Francesca et al., 2014). Phylogenetic analyses were performed using the sequences obtained from the 26S rRNA gene sequence analysis. Phylogenetic trees were constructed based on aligned sequences using the phlogenetic tree reconstruction method, UPMGA, with MEGA 7.0 software.

3.7. Functional Characterization of Selected LAB Isolates

Strains of the LAB species frequently isolated in sourdough and chickpea fermentations were selected for the evaluation of the functional properties to be used as a starter culture in sourdough and chickpea fermentations. Functional analysis were performed on selected strains at least in duplicate.

3.7.1. Acidification Activity

The acidification test of selected LAB strains was performed in sterile flour extract (SFE) liquid broth according to a previously described method (Alfonzo et al., 2013). For the preperation of SFE, 200 g of white wheat flour was suspended in 1 L of distilled H_2O and sterilized at 121°C for 20 min. The supernatant was then used as the liquid media in subsequent experiments.

Overnight grown LAB cultures in MRS broth were harvested by centrifugation at 13,300 rpm for 3 min (Thermo Scientific MicroCL 17, Germany), washed with Ringer's solution and resuspended in the same solution to an optical density at 600 nm of 1.00 (Shimadzu UV-1700, Japan) to standardize bacterial inocula. Twenty mL of SFE was inoculated with 1% (v/v) of the solution consisting of the cell suspension and incubated at 30°C. The acidifying capacity of LAB was monitored during their incubation by pH measurements taken at 2 h intervals for the first 8 h of incubation and then at 24 ,48 ,72 h and 7 d after inoculation. Uninoculated SFE was used as the control.

3.7.2. Determination of Lactic and Acetic Acids

According to the acidification capability, some strains were selected and also analysed for their ability to produce lactic and acetic acids following 8 h of fermentation in sterile flour extract. For that purpose, acidified SFE (aSFE) of the selected strains were used for further analysis. Perchloric acid was used for protein precipitation in the samples following storage at 4°C for 24 h. Protein agglomerates were removed by centrifugation (13,300 rpm, 60 min, 4°C; Hettich® Universal

320R, Germany) and the supernatant was filtered through a 0.45 µm PVDF Syringe Filter (Isolab) and injected to the HPLC system.

3.7.3. Quantitative EPS Analysis

Overnight grown LAB cultures in MRS broth were harvested by centrifugation at 13,300 rpm for 3 min (Hettich® Universal 320R, Germany), washed with Ringer's solution and resuspended in the same solution to an optical density at 600 nm of 1.00 (Shimadzu UV-1700, Japan) to standardize bacterial inocula. For EPS yield determination, MRS broth supplemented with 50 g/L sucrose was inoculated with the cell suspension solution 1% (v/v) and incubated at 30°C (Tayuan et al., 2011). Sucrose was separately autoclaved and then added to the sterilized MRS medium (Malik et al., 2015). EPS were extracted from the 72 hour-old bacteria culture and boiled at 100°C for 10 min to inactivate EPS degrading enzymes (Kusmiati et al., 2016). After cooling, 1 mL of the cell culture was treated with 17% (v/v) of 85% trichloracetic acid solution. The solution was incubated at 4°C overnight and then centrifuged (14,000 rpm, 4°C, 30 min) to remove cells and protein (Frengova et al., 2000; Onbasli and Aslim, 2008). Each supernatant was treated with three-volumes of ethanol (95%) and left overnight at 4°C (Joshi and Koijam, 2014). The precipitated EPS was centrifuged at 14,000 rpm at 4°C for 20 min and the supernatant was discarded (Abdelnasser et al., 2017). The precipitate of pure EPS was dried at 60°C for 24 h in the same centrifuge tubes to minimize EPS loss and total EPS yields were determined gravimetrically by measuring the dry mass (Osińska-Jaroszuk et al., 2014; Huang et al., 2017).

3.7.4. EPS Production on Agar Medium

Selected bacteria cultures were streaked onto MRS agar medium supplemented with 50 g/L sucrose and incubated at 30°C for 72 hours. The formation of mucoid or viscous colonies on the agar was considered to be EPS production (Lule et al., 2015).

3.7.5. Antimicrobial Activity Against Selected Species

Selected strains were evaluated for their antimicrobial activity using the dual culture overlay technique against *B. subtilis, B. lincheniformis, Escherichia coli, Penicillium expansum* and *Penicillium digitatum*. Firstly, the selected LAB strains to be tested for bacteriocin production were grown on the surface of mMRS containing 1.5% agar at 30°C for 24 h anaerobically. The indicator strains, *B. subtilis* and *B. lincheniformis* were grown in Nutrient broth at 37°C, *Escherichia coli* in Brain Heart Infusion broth at 37°C and the molds *Penicillium expansum* and *Penicillium digitatum* in Malt extract broth at 28°C until reaching OD_{600}=1.0. Indicator strains were inoculated (1%) onto soft agar medium (containing 0.75% agar) specific for each strain and the soft media were poured onto the plates where growth of the producers had occurred and the plates were incubated at the optimal growth temperature and time for the indicator strains. After incubation, the plates were controlled for zone formation. A detectable clear zone around the colonies of the producer strain was scored as positive inhibition (Schillinger and Lücke, 1989; Corsetti et al., 2004).

3.7.6. Protease Capacity

Protease activity of selected LAB strains was assessed on mMRS agar containing 2% skim milk powder. The medium was sterilized by autoclaving at 115°C for 10 min. Isolates were incubated at 28°C for 8 days. After incubation, the formation of halo zones around microbial colonies indicated protease capacity (Palla et al., 2017).

3.7.7. Growth at Different Conditions

Selected strains were evaluated for growth at different temperatures, pH levels, salt concentrations and carbohydrate sources. For growth at different temperatures, strains (OD=1) were inoculated onto mMRS broths (1%). For the groth at differnt temperatures, inoculated mMRS broths were incubated at 15, 28,

37 and 45°C for 2-7 days. For tolerance to different pH values, strains (OD=1) were inoculated onto mMRS broth (1%) prepared at pH 3.5, 4.5 and 6.5 with filter sterilized 5 N HCl and 2 N NaOH solutions and incubated at 30°C for 3 days. For tolerance to different salt concentrations, strains (OD=1) were inoculated onto mMRS broth (1%) containing 4, 6 and 8% NaCl (w/v) and incubated at 30°C for 3 days. Precipitation and turbidity in the broth media was accepted as growth of the strain at the condition.

The ability to ferment various carbohydrates was evaluated using MRS broth prepared without glucose and meat extract. Each sugar solution (1%, w/v) was added to MRS broth media via filter sterilization. Tested carbohydrates were D (+) glucose monohydrate (Sigma-Aldrich), D (-) fructose (Merck), D (+) galactose (Fluka), lactose monohydrate (Merck), sucrose (Merck), maltose monohydrate (Merck), L (+) rhamnose monohydrate, raffinose (Difco), D (-) mannitol (Merck), D (+) mannose (Fluka), D (-) arabinose (Fluka) and D (+) xylose (Sigma Aldrich). The control broth lacked sugar addition. Chlorophenol red (0.004 %, w/v) was added as the indicator and conversion of the color from red-purple to yellow indicated low pH values due to the growth and production of lactic acid (Schillinger and Lücke, 1987).

3.7.8. Enzyme Profile

For the enzyme profile assessment, the API ZYM enzyme (Biomerieux, France) testing system was used according to the manufacturer's instructions. First of all, 5 mL of distilled water was distributed into the incubation wells of the incubation tray to create a humid atmosphere. Then selected bacterial cell suspensions (5-6 McFarland turbidity) were inoculated into cupules of the incubation tray and incubated at 37°C for 4-4.5 h. After incubation, 1 drop of ZYM A reagent and 1 drop of ZYM B reagent was added to each cupule, and color changes were recorded and evaluated.

3.8. Production of Experimental Sourdoughs and Chickpea Liquid Starters with Selected Strains

Among mostly isolated LAB species, two and one strains were characterized for use in sourdough and chickpea fermentations, respectively.

Sourdough production under laboratory conditions was performed according to the traditional (sourdough Type I) protocol using selected strains individually and also in combination. A control sourdough was produced without using the selected starter culture. For dough preparation, whole-meal wheat flour (216.21 g) and boiled and cooled tap water (183.79 mL) were mixed manually to produce 400 g of dough with a dough yield [(dough weight/flour weight)×100] of 185. Strains (OD=1) were inoculated at a concentration of 1% (v/w) to the dough. Each sourdough was fermented at 28°C for 24 h in glass jars covered with a lid. The resulting sourdoughs were propagated until reaching constant acidity by a daily back-slopping (refreshment) procedure and the sourdough from the previous day's fermentation was used as the starter (20% [wt/wt] of inoculum) to ferment a new mixture of flour (172.98 g) and tap water (147.02 mL), resulting in a dough yield of 185. Sourdough productions were carried out in duplicate. Experimental sourdough productions are shown in Figure 3.10.

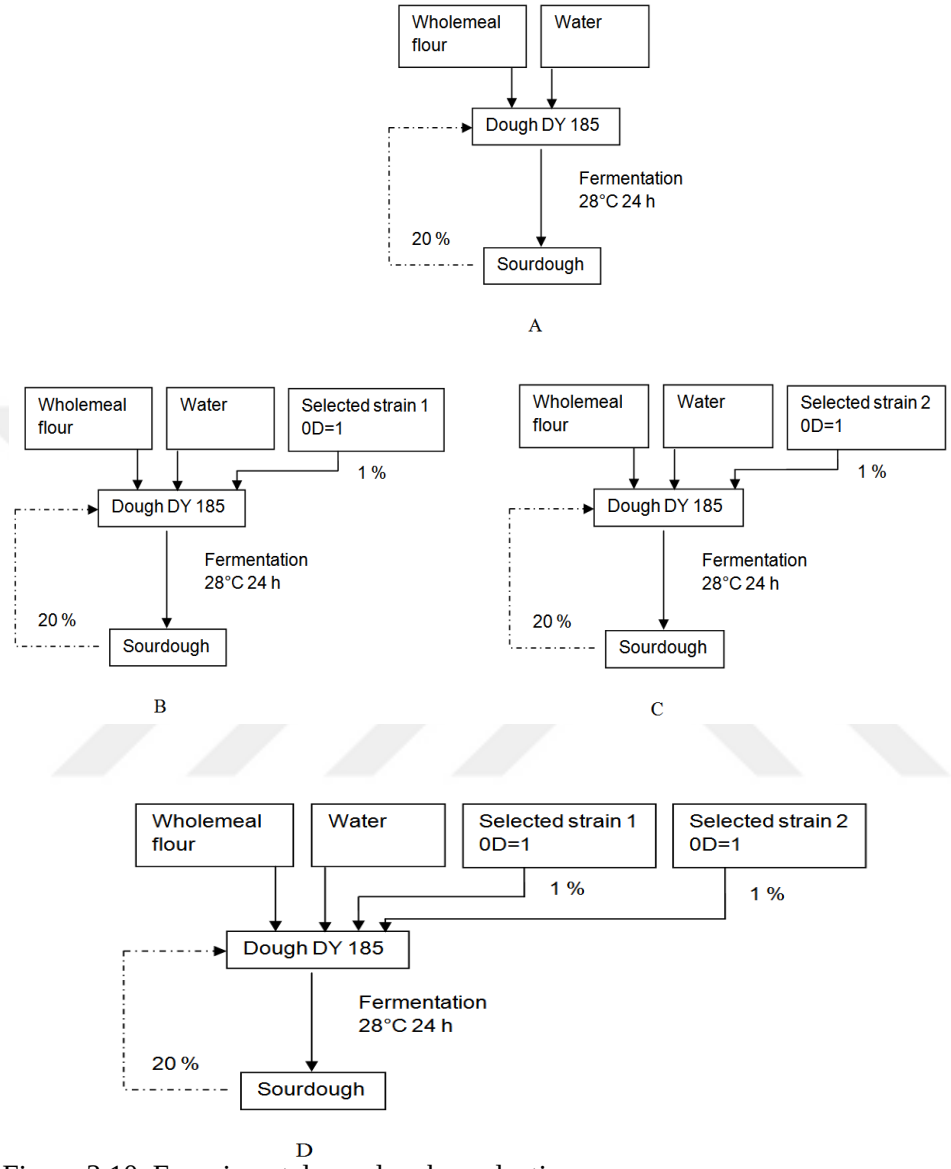

Figure 3.10. Experimental sourdough production
A) Control B) Selected strain 1 C) Selected strain 2 D) Dual-combination of selected strains

The first sample (0 h) was taken from the flour and water mixture, unfermented dough, after mixing. Both TTAand pH measurements were carried out

on all samples collected after 4, 8 h and 12 h of the experiment and once every 24 hours of until the last refreshment of the sourdough production. During the back-slopping procedure, sampling was performed on the sourdoughs immediately before the daily refreshment step and the samples were subjected to plate counting, TTA, pH measurements, carbohydrate, organic acid and ethanol analyses. Samples were analysed in duplicate. Also sourdoughs were examined for their generation of volatile organic compounds (VOCs) by SPME-GC-MS at the beginning and end of the fermentations. Samples were analyzed in duplicate.

Chickpeas (*Koçbaşı* variety) were ground for the production of chickpea liquid. Then 50 g of chickpeas were put into sterile glass jars and mixed with 400 mL of boiled and cooled tap water at 37°C as 50°C is very high for the strain inoculation. The selected strain (OD= 1) was inoculated at a concentration of 1% (v/w) into the chickpea liquid starter under aseptic conditions. The beginning of the fermentation for the selected strain was immeadiately after starter inoculation. The control chickpea liquid starter was produced without inoculating a starter culture. Fermentations were conducted in glass jars covered with a lid at 37 °C for 18 h. At the end of the fermentation, chickpeas were seperated and the liquid was used for the production of the chickpea dough. 150 mL of liquid is mixed with 200 g of flour (DY 175) and fermented at 37 °C for 4 hours. Productions were carried out in duplicate. Experimental chickpea liquid starter and dough productions are shown in Figure 3.11.

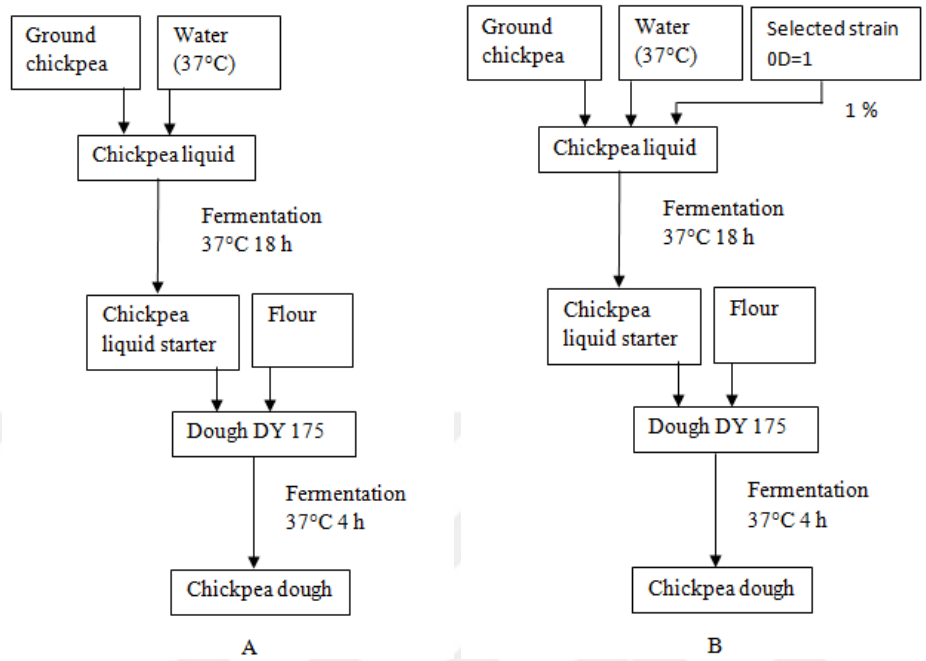

Figure 3.11. Experimental chickpea liquid starter and dough production
A) control production B) production with selected strain

The samples were taken at the beginning (0 h) and end of the fermentations (18 h for chickpea liquid starters, 4 h for chickpea doughs). Samples were subjected to plate counting, TTA, pH, carbohydrate, organic acid, ethanol and VOC analyses at the beginning and end of the fermentations. Samples were analyzed in duplicate. For chickpea liquid starter samples, pH, spore-forming bacteria and bacteria grown on NA were also monitored every 2 hours during 10. Samples were analyzed in duplicate.

3.9. VOC Analysis by SPME-GC-MS in Experimental Samples

Experimental sourdough, chickpea liquid starter and dough samples were examined for their generation of VOCs with the modified method of Settanni and others (2013). VOCs were determined applying the solid phase micro extraction (SPME) isolation technique. Each sample (3 g) was heated to 30°C in a vial for 30 min. Then headspace was collected by a fiber (85 µm Carboxen\PDMS) at 30°C for 30 min. The SPME fibre was directly inserted into the GC/MS (Agilent 7000

Series Triple Quad) equipped with a HP - 5MS capillary column (30 m, 0.250 mm i.d., film thickness 0.25 mm, %5 phenyl methyl-poly-cyloxane). Separation was achieved by using the following temperature program: initial 40°C with a 4 min hold and ramped to 90°C at 3°C/min, 130°C at 4°C/min, 240°C at 5°C/min and held for 8 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Ionizing energy was 70 eV and MS were at the full-scan mode with scan range of 50–600 m/z. The identification of VOCs was achieved by using the National Institute of Standards and Technology (NIST 14L) reference library and VOCs were expressed as relative peak areas (peak area of each compound/total area*100).

3.10. Statistical Analysis

Data of the analysis were subjected to one-way analysis of variance (ANOVA) and multiple comparison of means by Duncan's procedure at a $p<0.05$ using the IBM SPSS 20 software. Multivariate statistical analyses were carried out to investigate the correlations between the characteristics and the samples. XLSTAT 2018 software for excel was used for data processing and graphic construction. Dissimilarity index calculation and dendrogram construction were carried out using DARwin (6.0.15) software package. Phylogenetic trees were constructed from molecular sequences to investigate the phylogenetic relations between strains by Mega 7.0 software.

4. RESULTS AND DISCUSSION

4.1. Sourdough Samples

4.1.1. Chemical Characteristics of Sourdough Samples

Whole-meal wheat and rye sourdough samples were collected from three different bakeries located in different cities at two different times. Codes were assigned to each collected sample as letters and numbers without expressing the bakery names due to the special request by the bakeries. Sourdough samples were coded as SD-M1, SD-M2, SD-T1, SD-T2, SD-K1, SD-K2, SD-R1 and SD-R2, with SD denoting sourdough, and a randomly chosen letter, and 1 or 2 indicating the first or second sampling. R coded sourdough was rye sourdough and the other were wheat sourdough samples. The first sourdough samples (coded as 1) were collected from bakeries in the spring or at the beginning of summer and the second samples (coded as 2) were collected at the end of the autumn or in winter, resulting in sourdoughs with different characteristics.

4.1.1.1. pH

Results of the pH measurements of the 8 sourdoughs, including the two sampling are shown in Table 4.1. The pH levels of the collected sourdough samples ranged from 3.71 to 3.96 and the pH exhibited a mean value of 3.87. The lowest pH level was measured in the SD-T2 as 3.71, on the other hand, the pH of the first sampling of this sourdough, SD-T1, was 3.93. The highest pH value was measured as 3.96 in the rye sourdough sample. The pH of the rye sourdough of the second sampling, SD-R2, was 3.91. As it can be seen, pH values showed differences among sourdoughs and sampling times.

Table 4.1. The pH levels of the samples

Sample	pH	Std. deviation
SD-M1	3.86^{bc}	0.04
SD-M2	3.85^{bc}	0.01
SD-T1	3.93^{d}	0.03
SD-T2	3.71^{a}	0.04
SD-K1	3.91^{cd}	0.05
SD-K2	3.82^{b}	0.01
SD-R1	3.96^{d}	0.02
SD-R2	3.91^{cd}	0.03

$^{a-d}$Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

Testing of the homogenity of variances showed that variances can be treated as equal ($p> 0.05$) and a parametric ANOVA test was conducted. According to the statistical results, the differences between the samples collected from different bakeries were significant ($p<0.05$). The pH differences between samples could be due to the different microflora in the sourdough samples, and different production methods and incubation conditions since the samples were collected from different cities. These parameters directly affect the pH of the end product and therefore sourdoughs produced in different places exhibited different biochemical patterns. On the other hand, differences were observed between some samples collected from the same bakery at two different times. There weren't any significant differences in SD-M and SD-R sourdough samples collected at two different times. pH values of the SD-M1 and SD-M2 coded sourdough samples were found as 3.86 and 3.85, respectively. On the other hand, differences in the pH levels of SD-T and SD-K sourdough samples were statistically significant. pH values of the SD-K1 and SD-K2 coded sourdough samples were found to be 3.91 and 3.82, respectively. SD-T samples, including the first and second sampling as SD-T1 and SD-T2, showed differences among pH values. The fermentation conditions changed in that bakery, as mentioned by the owner, between the time interval of the first and second sampling. They tried to stabilize the conditions and for that purpose made some modifications during the fermentation; hence the differences observed between the first and second sampling could be a result of the modifications.

In the present study, pH results of the sourdough samples were in consistent with the pH values determined in other studies. Actually, sourdough shows great variation due to the artisan and region-dependent handling, including production method, flour that is used in the production, type of sourdough, amount of the sourdough inoculum, fermentation conditions, back-slopping times etc. Therefore, pH values, TTA and microbiological flora might be differ among different sourdough samples.

In the study of 19 Italian sourdoughs used for the manufacture of traditional breads, pH values were ranged from 3.70 to 4.28 (Minervini et al., 2012a). It was indicated that 13 of the 19 sourdoughs had pH values of less than 4.0, therefore, the pH values of many sourdoughs were in consistent with our results. In another study, conducted in 18 Italian sourdoughs, pH values were in the range of 3.90 and 5.01. Not all of them but many of the values were higher than the pH values in the present study (Lattanzi et al., 2013). In another study, 20 wheat sourdough samples were collected from central Italy, and exhibited a wide range of pH values, 3.46-5.23 (Valmorri et al., 2010). Ventimiglia et al. (2015) analysed 15 sourdoughs produced in southern Italy and reported pH levels in the range of 3.81-4.77. Lhomme et al. (2015) collected 16 sourdoughs from different regions of France and reported pH values in the range of 3.23- 4.01 and the highest pH was observed in the rye sourdough as it was detected in our study. Some studies have reported higher pH values. In the study of Zhang et al. (2015), 25 traditional sourdough samples were aseptically collected from China and mean pH values were in the range of 3.76 and 5.51. As it can be seen, pH values exhibit a wide variation among sourdoughs in different regions due to differences in the production methods and conditions.

4.1.1.2. Total Titratable Acidity

Total titratable acidity is given as mL of 0.1 N NaOH consumed and results of the 8 sourdoughs including both samplings are shown in Table 4.2. Acidity

levels of the collected sourdough samples ranged from 6.78 to 23.93 mL 0.1 N NaOH /10 g dough. The lowest and highest acidity values were calculated in the SD-M2 and SD-K1 samples, respectively. As expected, acidity content was significantly ($p<0.05$) different between sourdough samples.

Table 4.2. Mean TTA levels of the sourdough samples

Sample	TTA (mL 0.1 N NaOH /10 g dough)	Std. deviation
SD-M1	8.35^{a}	0.05
SD-M2	6.78^{b}	0.07
SD-T1	8.30^{a}	0.1
SD-T2	10.98^{e}	0.43
SD-K1	23.93^{f}	1.11
SD-K2	18.05^{g}	0.35
SD-R1	16.15^{c}	0.15
SD-R2	13.70^{d}	0.3

$^{a-g}$Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

As it can be seen, acidity values exhibited differences among sourdoughs and sampling times. Testing the homogenity of variances showed that variances were unequal and the differences between the samples were significant ($p<0.05$).

Acidity levels of sourdoughs collected from the same bakery at two different times were significantly different. However, among the samples the highest acidity was observed in SD-K sourdough at both sampling times. SD-K samples were characterized by higher acidity than other samples. Rye sourdough followed SD-K in terms of acidity. SD-M sourdough samples showed the lowest acidity level at both sampling times. The acidity content of the sourdoughs, except SD-T, decreased in the second sampling that was collected in winter, as can be seen in Figure 4.1. As expected, a decreasing temperature affected the fermentation of the sourdough resulting in lower acidity end products.

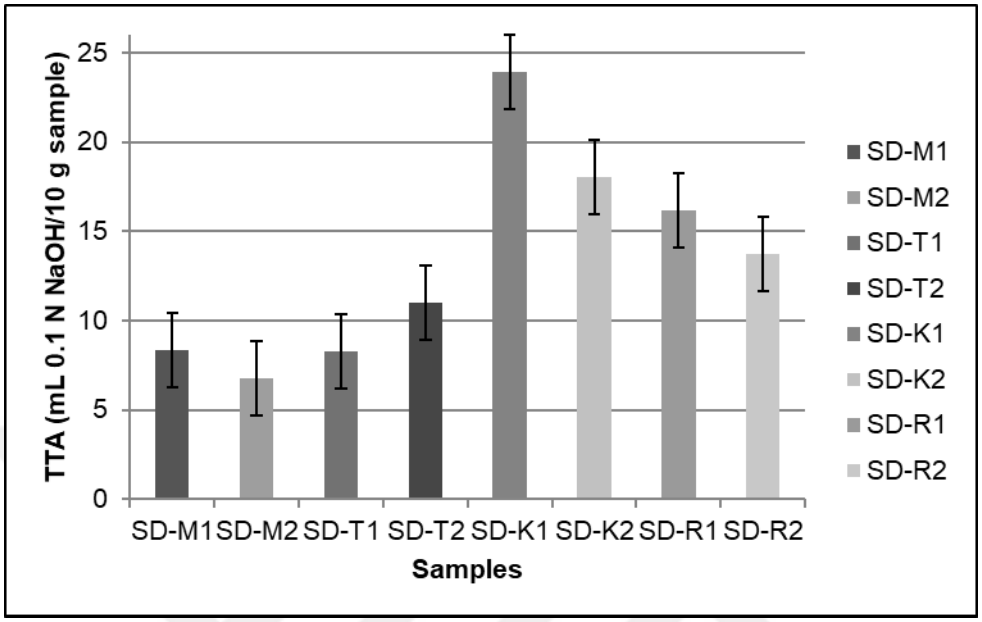

Figure 4.1. Acidity levels of the sourdough samples

Total acidity values of the sourdoughs showed a wide variation and were in the range of 6.78-23.93 mL 0.1 N NaOH. The median value for the acidity was determined to be 12.42 mL 0.1 N NaOH. In the study of Lhomme et al. (2015), TTA exhibited a median value of 16.2 mL 0.1 N NaOH. In another study, an acidity range of 12.3-13.0 mL NaOH was reported (Tamani et al., 2013). Viiard et al. (2012) reported the pH and acidity values to be between 3.5-3.7 and 19.0-22.0 mL 0.1 N NaOH in the rye sourdough samples. In another study, final acidity values of the rye sourdoughs fermented at 25°C, refreshed 12 times and fermented at 30°C, refreshed 24 times were reported to be in the range of 15.2-17.7 and 20.3-26.4 mL 0.1 N NaOH, respectively (Meroth et al., 2003). Ventimiglia et al. (2015) reported the acidity values of 15 sourdoughs produced in southern Italy to be in the range of 6.0-14.7 mL 0.1 N NaOH.

Under laboratory conditions, sourdough was produced at 28°C by propagating over a period of 7 days using the daily back-slopping (refreshment) procedure. The first sample (0 h) was taken from the flour and water mixture, unfermented sourdough,

immediately after mixing. During the back-slopping procedure, sampling was performed on the sourdoughs before each daily refreshment step. pH and TTA of the sourdough samples were determined at 4, 8 and 12 h of the fermentation and then once every 24 hours for 7 days.

The pH of the prepared sourdough did not change during the first 12 h of fermentation, as shown in Figure 4.2. The pH started to drop slowly, ending at pH 4.58 after the first day of fermentation. On the second day, it decresed to 3.99 and at the end of the fermentation, it was determined to be 3.60. TTA data had a reverse relationship with pH and were correlated linearly with pH values. TTA was stable during the first 12 hours, but then started to increase and reached 8.72 mL 0.1 N NaOH/10 g dough on the first day. The acidity level continued to increase the next day and was determined to be 14.74 mL. During the following days, the acidity continue to increase, but not greatly, reaching at final value of 17.56 mL 0.1 N NaOH.

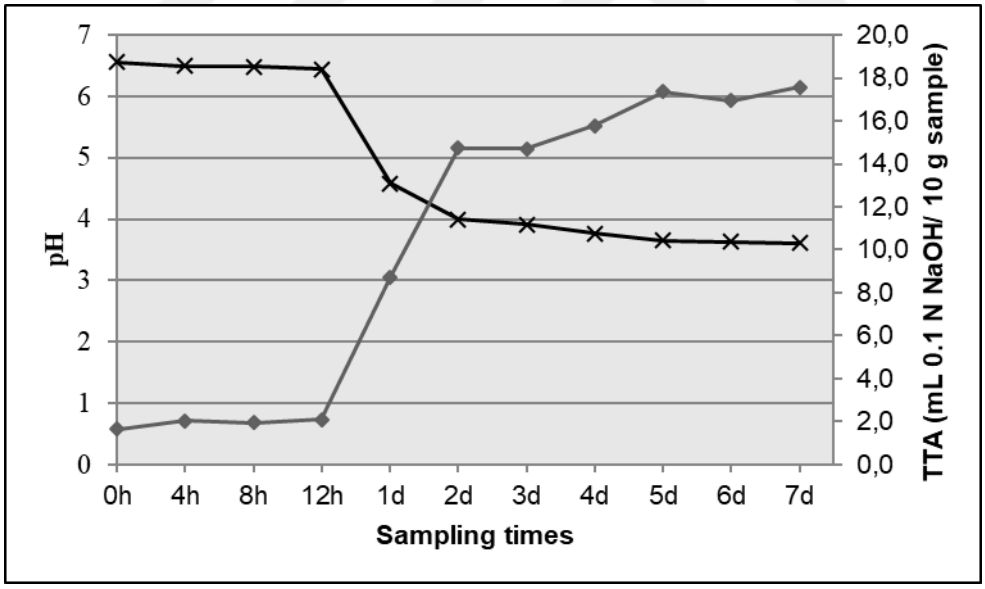

Figure 4.2. Changes in pH during a 7-day sourdough fermentation with daily back-slopping (Symbols: × shows changes in pH, ♦ shows changes in acidity levels)

During sourdough fermentation, TTA increases and pH decreases as a result of the produced organic acids. Depending on the applied fermentation conditions such as refreshment time, type of flour, temperature, dough yield and starter culture addition, the pH and TTA values can differ in sourdoughs. In the present study, sourdough produced under laboratory conditions showed pH and acidity patterns that are similar to some other studies (Van Der Meulen et al., 2007; Taccari et al., 2016; Fujimoto et al., 2018). Fujimoto et al. (2018) produced sourdough by back-slopping at 28°C for 5 days. In their study, the pH declined to less than 4.0 on day 2. On the 5th day, pH values of 3.62 and 3.61 for the two produced sourdoughs are in agreement with our results. Some studies have investigated sourdough production under laboratory conditions with starter additions. Paramithiotis et al. (2005) prepared sourdoughs with various starter culture combinations via propagation every 24 hours at 25 and then 30 °C. After 24 hours, pH and acidity levels of the sourdoughs were reported in the range of 3.57-3.85 and 10.1-12.5 mL 0.1 N NaOH, respectively.

The flour type is important and directly affects the properties of the sourdough. Taccari et al. (2016) studied the fermentation of Type I sourdough propagated for 20 days with daily back-slopping under laboratory and artisan conditions at room temperature with three different flour types. They reported the pH and TTA values in the ranges of 3.72 to 4.02 and 9.40 to 18.10 ml, respectively. They determined the highest acidity in the sourdough samples produced using whole-meal flour.

4.1.1.3. Carbohydrate, Organic Acid and Ethanol Contents of the Sourdoughs

The content of maltose, sucrose, glucose, fructose and ethanol was determined by HPLC using an RID detector. Lactic and acetic acid contents were determined by HPLC with a UV detector. Retention times detected by the RID detector were 9.393, 9.470, 11.168, 12.591 and 24.056 for maltose, sucrose, glucose, fructose and ethanol, respectively. Retention times detected by the UV

detector were 16.291 and 17.902 for lactic and acetic acid, respectively. Standards are shown with their chromatogram images in Appendix 1, 2 and 3. Discrimination of the maltose and sucrose peaks was difficult as their retention times were close to each other and they were eluted together. Therefore, the results of these compounds are given together as maltose+sucrose.

The concentration ranges of the standards were 0.081-2.512, 0.082-2.543, 0.083-2.554, 0.079-2.445, 0.091-2.605, 0.113-3.257 and 0.30-9.80 g/L for maltose, sucrose, glucose, fuctose, lactic acid, acetic acid and ethanol, respecively. Seven point linear calibration curves (R^2> 0.998) were constructed for all standards according to the mean of three consecutive injections versus mean area. Calibration curves are given in Appendix 4.

The LOD-LOQ values were calculated by multiplying 3 and 10 times the standard deviation of the 10 standard injections and determined to be 0.032-0.108, 0.028-0.110, 0.023- 0.075, 0.029-0.096, 0.031-0.103, 0.030-0.100 and 0.095-0.316 for maltose, sucrose, glucose, fructose, lactic acid, acetic acid and ethanol, respectively. A recovery test was conducted for each standard based on the six injections of the spiked and unspiked samples. Recovery levels of the samples were 104.79, 101.66, 116.94, 92.73, 99.55 and 84.60 % for maltose+sucrose, glucose, fructose, ethanol, lactic acid and acetic acid, respectively.

Mean values of the carbohydrate and ethanol content of the collected sourdough samples are given in Table 4.3. Mean-median values of the content of maltose+sucrose, glucose, fructose and ethanol were 2.43-1.62, 1.57-1.70, 2.67-2.13 and 9.59-10.16 g/kg, respectively. A sourdough sample chromatogram image is shown in Appendix 5.

Table 4.3. Mean carbohydrate and ethanol content (g/kg) of the sourdough samples

Sourdoughs	Maltose+sucrose	Glucose	Fructose	Ethanol
SD-M1	5.47^{d}±1.13	2.30^{e}±0.14	2.30^{c}±0.14	14.70^{d}±1.05
SD-M2	2.32^{c}±0.02	1.39^{b}±0.05	1.96^{b}±0.08	14.94^{d}±1.63
SD-T1	6.24^{e}±0.02	2.16^{de}±0.06	2.79^{d}±0.21	9.97^{c}±0.04
SD-T2	1.37^{ab}±0.09	0.84^{a}±0.17	0.98^{a}±0.13	10.80^{c}±0.49
SD-K1	1.28^{ab}±0.06	1.54^{b}±0.04	0.78^{a}±0.02	4.39^{a}±1.13
SD-K2	<LOQ	0.81^{a}±0.16	0.78^{a}±0.06	10.35^{c}±0.08
SD-R1	0.91^{a}±0.19	2.00^{cd}±0.23	6.96^{f}±0.14	6.66^{b}±1.89
SD-R2	1.87^{bc}±0.02	1.86^{c}±0.01	4.84^{e}±0.25	4.87^{ab}±0.075

Results indicate mean values± SD, Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

In the SD-K2 sample, the maltose+sucrose content was below the quantification limit. This sample also had the lowest glucose and fructose content. In the other samples, maltose+sucrose, glucose and fructose contents ranged from 0.91 to 4.47, 0.84 to 2.30 and 0.78 to 6.96 g/kg, respectively. Median values of maltose+sucrose, glucose, fructose, lactic acid and acetic acid were determined to be 0.83, 1.67, 2.09, 6.27 and 1.35 g/L, respectively. According to the statistical results, the differences in sugar content between the samples collected from the different bakeries were significant ($p<0.05$). The carbohydrate and organic acid differences among samples could be due to the different flours used in the production of sourdoughs together with different microflora and fermentation conditions as the samples were collected from different cities. In the samples collected from the same bakery at two different sampling times, the differences in carbohydrate content were significant, except glucose in SD-R and fructose in SD-K samples. SD-T samples, including the first and second sampling as SD-T1 and SD-T2, showed a big difference in sugar content and this could be related to the changing conditions during the production, as indicated previously. In the study of Lattanzi et al. (2013), maltose, glucose and fructose concentrations in sourdoughs used for the manufacture of traditional Italian breads were in the range of 0.3-28.7,

0.4-25.8 and 0.3-10.1 g/L, respectively. Median values of the sourdoughs were reported to be 5.6, 3.4 and 3.5 g/L, respectively.

The sugar content changes according to bacterial consumption and hydrolysis by flour enzymes; hence flour used for production and the microorganisms directly affect the content in the dough (Paramithiotis et al., 2006; Hansen, 2012). Sugars levels in the samples can decrease and increase as a result of bacterial consumption and hydrolisation by flour enzymes, respectively. Therefore, it is difficult to discuss the consumption ratio of sugars by microorganisms. It has been reported previously that carbohydrates are continuously liberated during fermentation, especially by endogenous flour enzymes, and it was not possible to estimate their consumption at the end of fermentation (Lattanzi et al., 2013). Korakli et al. (2001) reported maltose and glucose accumulation after 24 h fermentation in doughs because of the amylase and α-glucosidase activities of flour.

The mean-median content of lactic acid and acetic acid of the collected sourdoughs were 7.3-6.16 and 1.40-1.42 g/kg, respectively. In terms of mmol/L, lactic and acetic acids varied from 57 to 156 and 9 to 39 mM, respectively. The lowest and highest lactic and acetic acid concentrations were determined in the SD-M1 and SD-K1 sourdough samples, respectively. According to the statistical results, the differences between the content of lactic and acetic acid in the samples collected from different bakeries were significant ($p<0.05$). Lactic acid concentrations in SD-K and SD-T and acetic acid concentrations in SD-T samples at two different sampling times were not significant. Conversely, in other sourdough samples, differences in the two sampling times were significant. The mean organic acid content (g/kg) of the sourdough samples is shown in Table 4.4.

Table 4. 4. Mean organic acid content (g/kg) of the sourdough samples

Sourdoughs	Lactic acid	Acetic acid	FQ
SD-M1	5.15^{a}±0.85	0.58^{a}±0.09	5.90
SD-M2	6.25^{ab}±0.22	1.53^{bc}±0.51	2.73
SD-T1	5.89^{ab}±0.31	1.20^{b}±0.18	3.28
SD-T2	6.07^{ab}±0.40	1.63^{bc}±0.38	2.48
SD-K1	14.12^{d}±0.98	2.40^{d}±0.06	3.92
SD-K2	6.90^{b}±0.27	1.80^{c}±0.06	2.56
SD-R1	5.31^{a}±0.18	0.76^{a}±0.20	4.66
SD-R2	9.12^{c}±0.13	1.32^{b}±0.12	4.61

Results indicate mean values± SD, Different superscript letters within a column indicate asignificant difference (Duncan $p<0.05$)

In the laboratory poduced sourdough, the concentration of carbohydrate, organic acids and ethanol cotents were determined in the unfermented dough and also 1, 2, 4 and 7 refreshment steps of the backslopped sourdough during 7 days (Table 4.5).

Table 4.5. Mean carbohydrate, organic acid and ethanol content (g/kg) of the sourdough produced under laboratory conditions

Day	Maltose+ Sucrose	Glucose	Fructose	Lactic acid	Acetic acid	Ethanol
0d	18.46±0.69	6.86±0.11	8.76±0.21	<LOQ	<LOQ	<LOQ
1d	18.46±0.43	6.82±0.21	3.60±0.08	3.68±0.35	2.45±0.11	3.09±0.35
2d	18.76±0.75	15.26±0.26	2.06±0.10	8.62±0.42	3.30±0.01	3.14±0.30
4d	17.08±0.18	11.77±0.16	2.25±0.10	11.31±0.40	2.22±0.06	3.11±0.17
7d	<LOQ	1.30±0.08	1.34±0.07	14.96±0.49	0.91±0.14	15.01±1.08

Results indicate mean values± SD

In the laboratory produced sample, maltose+sucrose, glucose and fructose concentrations were determined to be 18.46, 6.86 and 8.6 g/kg in the unfermented dough. The maltose+sucrose concentration did not change over 4 days and then decreased below the LOQ at the 7th backslopping. The fructose concentration decreased during fermentation and was determined to be 1.34 g/kg at the last

refreshment. On the other hand, the gluose concentration increased after the first day and then decreased to 1.30 g/kg at the last refreshment. Lactic acid increased and reached up to 14.96 g/kg. Acetic acid increased until 2nd day of backsloping and then decreased. Ethanol was determined to be 15.01 g/kg at the last refreshment. Figure 4.3. shows the analyte concentrations at different backslopping during a 7-day sourdough fermentation with daily back-slopping times in terms of mol/g.

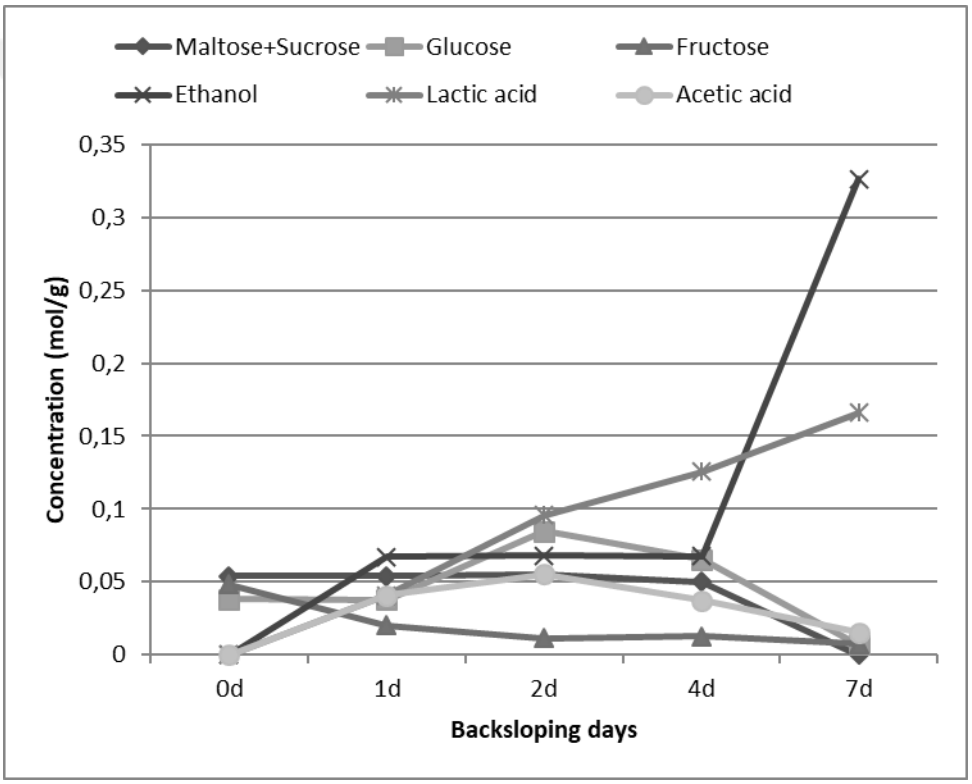

Figure 4.3. Changes in carbohydrate, organic acid and ethanol contents under laboratory conditions

According to the carbohydrate analysis, the maltose+sucrose content was around 53 mM for 4 days and was below the quantification limit at the final refreshment. Glucose and fructose were found at residual concentrations of less than 0.008 mmol/g (<8 mM) on the final refreshment day. In a study, the occurence

of maltose at concentrations of more than 60 mM was reported at the first refreshment step. In the same study, maltose concentrations were determined to be less than 20 and 40 mM on the 7th day refreshment at 23 and 30°C fermentations, respectively (Vrancken et al., 2011).

Final metabolite content of the sourdoughs change according to the metabolism of the flora in the fermentations. In the present study, metabolite contents were started to increase following fermentation. Ethanol is produced as the metabolite of yeast metabolism and its content was determined to be high at the final refreshment of the laboratory produced sourdough in this study.

The results of the present study are in agreement with other studies. In a study, the content of lactic and acetic acid in traditional organic sourdoughs collected from French bakeries were reported to be in the range of 3.29-11.44 and 0.66-2.03 g/kg, respectively (Lhomme et al., 2016). In another study conducted on sourdough samples prepared under laboratory conditions, lactic and acetic acid levels at the 10^{th} day of propagation were reported to be 88 mM for rye and 24 and 30 mM for wheat sourdoughs (Ercolini et al., 2013). Minervini et al. (2012a) reported lactic and acetic acid contents of sourdoughs used for traditional Italian breads to be in the range of 63.7-94 mM and 6-20.6 mM, respectively. Median values were reported as 80 mM for lactic acid and 18 mM for acetic acid (Minervini et al., 2012a). In this study, median values were calculated as 71 mM for lactic acid and 22.5 mM for acetic acid. Paramithiotis et al. (2006) reported the lactic and acetic acid contents in terms of mmol/g to be in the range of 0.04-0.11 and nd-0.02 in the sourdoughs produced with different yeast and LAB cultures. In this study, the content of lactic and acetic acids were in the range of 0.057-0.156 and 0.009-0.039 mmol/g, respectively.

The molar ratio between lactic and acetic acids, known as the fermentation quotient (FQ), represents an important parameter as it affects the aroma of the sourdoughs (Corsetti and Settanni, 2007). In the present study, FQ levels were determined in the range of 2.48-5.90 as shown in Table 4.4. The optimumin FQ

range is considered to be 2.0–2.7 (Hammes and Ganzle, 1998). For rye sourdough, the optimal FQ was reported to be in the range of 1.5–4.0 (Spicher, 1983). In the present study, rye sourdough samples at both sampling times exhibited the optimum FQ value. Among wheat sourdoughs, the SD-M1 sample displayed the highest FQ, which was above 4.00, indicating a low concentration of acetic acid with respect to lactic acid. The second sampling of the wheat sourdoughs, SD-M2, SD-T2 and SD-K2 showed optimum FQ levels. Corona et al. (2016) determined lactic acid, acetic acid and also FQ of the sourdough after 16 hours fermentation as 7.01 mg/g, 0.72 mg/g and 6.49, respectively. Another study of the same research group reported the lactic acid, acetic acid and FQ levels of the 15 collected sourdoughs produced in southern Italy in the range of 1.97-9.41 mg/g, 0.36-1.46 mg/g and 0.91-6.80, respectively (Ventimiglia et al., 2015). On the other hand, the FQ of the laboratory produced sourdough was high and determined as 10.84. Suitable conditions such as propagation ratio, fermentation time and temperature enables the growth of LAB resulting in increased metabolite production.

The mean ethanol content of the sourdough samples ranged from 4.87 to 14.94 g/kg. The highest ethanol content was determined in the SD-M samples at both sampling times; whereas the lowest ethanol content was determined in the SD-K1 sample. On the other hand, the first sampling of SD-K was 10.80 g/kg. Ethanol concentrations in the samples at both sampling times were not significant ($p>0.05$); except for the SD-K sample. Paramithiotis et al. (2006) reported the ethanol contents to be nd-0.41 mmol/g in the sourdoughs produced with different yeast and LAB cultures and the highest ethanol contents were observed in the sourdoughs produced with yeast mono-cultures, as ethanol is the main yeast metabolite in addition to being a product of heterofermentative metabolism. Minervini et al. (2012a) reported the ethanol content of the sourdoughs used for traditional Italian breads in the range of 0.05-0.50 M. In this study, the ethanol content ranged between 0.10-0.32 M, in terms of molarity, which was in agreement with previous studies.

The carbohydrate concentration in dough fermentations varies depending on the activity of cereal enzymes and the flora. Flour contains different sources of fermentable carbohydrates such as maltose, sucrose, glucose and fructose (Chavan and Chavan, 2011). During dough fermentation, sucrose is rapidly coverted into glucose and fructose, and maltose is hydrolysed to glucose by the endogenous enzymes in the flour and microbial enzyme activity (Swanson, 2008). Although the concentration of fermentable carbohydrates in wheat and rye flours is relatively low, wheat and rye contain about 60–70% starch and starch degradation at the dough stage is the predominant source of fermentable carbohydrates and reducing sugars (Ganzle, 2014a; Struyf et al., 2017). therefore, higher levels of glucose, fructose and maltose are detected compared with wheat flour (Codina et al., 2013). During fermentation, fermentable carbohydrates are consumed and their content is decreased. As it can be seen, the carbohydrate content in doughs is directly related to the metabolism of the sourdough microbiota and activity of cereal enzymes.

4.1.2. Microbiological Characteristics of Sourdough Samples

Cell densities of LAB, yeasts, total mesophilic aerobic bacteria and molds in the 8 sourdough samples studied are shown in Table 4.6.

Table 4.6. Mean values of cell counts (log CFU/g) of sourdough samples on different media

Samples	LAB		Yeasts			LAB/ yeasts*	TMAB	Molds
	mMRS	gM17	SDB	YPD	L- lysine		PCA	MXA
SD-M1	9.15	8.93	9.15	7.58	7.41	37:1	9.08	<1
SD-M2	10.99	10.48	10.87	6.75	6.97	8313:1	10.37	<1
SD-T1	7.39	6.83	6.73	8.87	8.22	0.02:1	6.90	<1
SD-T2	10.89	4.78	10.82	7.54	7.85	13:1	7.78	<1
SD-K1	6.47	7.23	11.84	6.97	3.59	1693:1	6.83	<1
SD-K2	5.60	5.32	11.96	7.69	2.70	271:1	4.54	2.2
SD-R1	11.67	10.29	11.59	10.02	8.02	145:1	11.48	<1
SD-R2	9.42	7.22	9.99	6.97	6.78	100:1	7.16	<1

*LAB and yeasts ratio was calculated according to the mean counts of three and two different media for LAB and yeasts, respectively.

4.1.2.1. Presumptive LAB Counts

The cell counts of presumptive LAB varied from 4.78 to 11.96 log CFU/g on different media. The highest cell density on mMRS agar was 11.67 log CFU/g in the rye sourdough sample, whereas the lowest cell density on mMRS agar was 5.60 in the SD-K2 sample. On the other hand, the highest cell count on SDB agar was in that sample at 11.96 log CFU/g. The lowest and highest cell counts on gM17 medium were 4.78 and 10.48 log CFU/g in the SD-T2 and SD-M2, respectively. Plate counts using three culture media showed different values of presumptive LAB, as shown in Figure 4.4. In the present study, the highest cell densities were counted on the SDB agar medium. Furthermore, other studies have reported higher cell counts on SDB medium (Minervini et al., 2012a; Lhomme et al., 2015; Ventimiglia et al., 2015). Apart from the culture medium, the 25th, median and 75th percentiles of the plate counts were determined to be 6.93, 9.29 and 10.88 log CFU/g, respectively. According to the culture medium, median and mean cell densities were 9.28-8.95, 7.23-7.63 and 10.85-10.37 log CFU/g as enumerated on mMRS, gM17 and SDB, respectively. These media contain different nutrients and the differences in cell densities on different media can be related to the qualitative and quantitative differences among media in terms of nutrients and also different metabolic capacities among strains harboured in each sourdough (Minervini et al., 2012a; Lattanzi et al., 2013). Minervini et al. (2012a) reported median plate counts of presumptive LAB in four culture media as 9.01 log CFU/g. Lhomme et al. (2015) determined median values of LAB cell densities in French sourdoughs to be 6.2, 9.2 and 8.2 log CFU/g as enumerated on mMRS, SDB, and MRS5, respectively. In another study on Italian sourdoughs, the cell density of LAB varied from 6.3 to 9.2 log CFU/g with the median value of 8.05 log CFU/g (Lattanzi et al., 2013). Ventimiglia et al. (2015) reported the cell counts of 15 sourdoughs produced in southern Italy in the range of 4.9-8.9, 4-8.5 and 4.5-9 log CFU/g as enumerated on MRS, M17 and SDB media, respectively. Palla et al.

(2017) determined the LAB counts on mMRS and SDB media to be 9.37 and 9.01 log CFU/g, respectively.

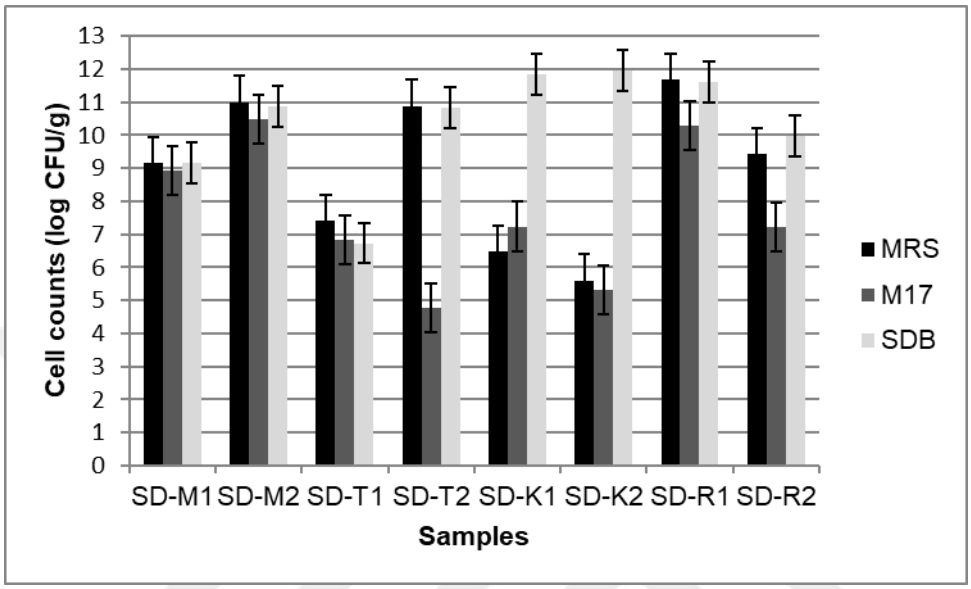

Figure 4.4. Presumptive LAB counts of sourdoughs on different media

4.1.2.2. Presumptive Yeast Counts

The cell counts of presumptive yeasts varied from 6.75 to 10.02 log CFU/g and 2.70 to 8.22 log CFU/g on YPD and L-lysine media, respectively. The highest cell density on YPD agar was counted in the rye sourdough sample, whereas the second sampling of rye sourdough showed lower yeast counts. Plate counts using two culture media showed different values of presumptive yeasts due to the qualitative and quantitative differences among media in terms of nutrients (Figure 4.5). Regardless of the culture medium, median and mean yeast counts were determined to be 7.48 and 7.12 log CFU/g.

In the study of Minervini et al. (2012a), malt extract and Sabouraud dextrose agar counts showed difference and regardless of the culture medium, the median value was reported as 7.30 log CFU/g. In another study, cell density of yeasts in traditional French sourdoughs was reported between 4.7-7.6 log CFU/g

with the median value of 6.5 log CFU/g (Lhomme et al., 2015). The median value of the cell density of yeasts was 7.03 log CFU/g in a study conducted on Italian sourdoughs (Lattanzi et al., 2013). Yeast cells were present at concentrations ranging from 5.03 to 8.61 CFU/g in traditional Italian wheat sourdoughs (Valmorri et al., 2010).

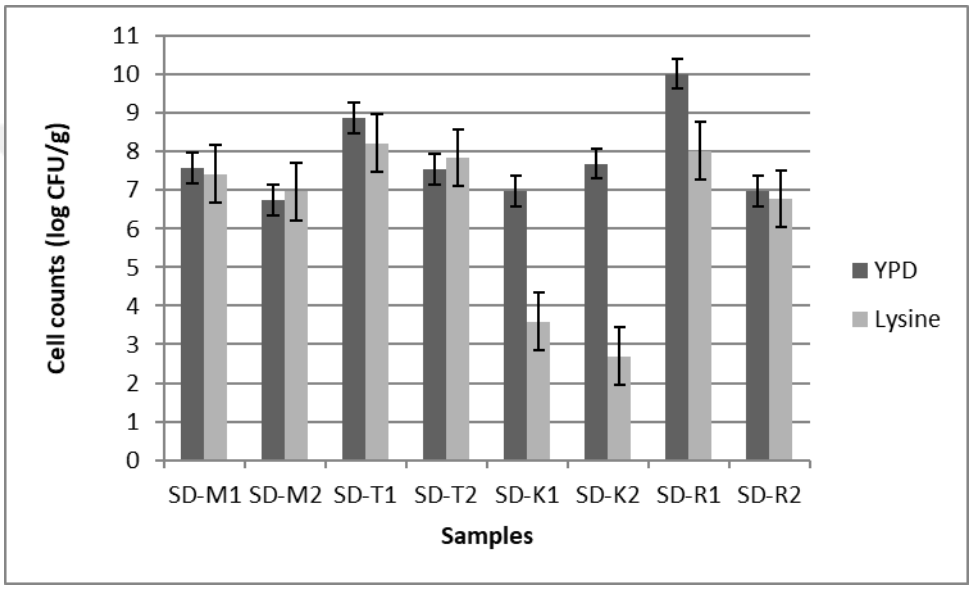

Figure 4.5. Presumptive yeast counts of sourdoughs on different media

The ratio between LAB and yeasts of the sourdough exhibited a wide variation. The LAB/yeast ratio was in the range of 0.02:1-8313:1. Similar results were obtained in other studies (Valmorri et al., 2010; Minervini et al., 2012a).

4.1.2.3. Enumeration of other Microorganisms

In the present study, mold, total mesophilic aerobic bacteria and presumptive coliform counts of the collected sourdough samples were investigated. Total mesophilic aerobic bacteria counted on PCA were in the range of 4.54-11.48 log CFU/g with the median value 7.47 log CFU/g as shown in Table 4.5. The rye

sourdough sample showed the highest bacterial count on PCA, which is in agreement with other counts of this sample. Total bacteria counts were in agreement with other studies (Saeed et al., 2009; Yagmur et al., 2016). Except SD-K2 sample, mold was not detected on the plates. For coliform, all of the tubes gave negative results (<3MPN/g).

Cell densities of LAB, yeasts, total mesophilic aerobic bacteria, molds and coliform bacteria were counted in the laboratory produced sourdough sample with daily back-slopping at 28°C. Microbial counts were determined at 0,1, 2, 4 and 7 d back-slopping days. Counts on mMRS, gM17, SDB, YPD, L-lysine and PCA media are shown in Figure 4.6.

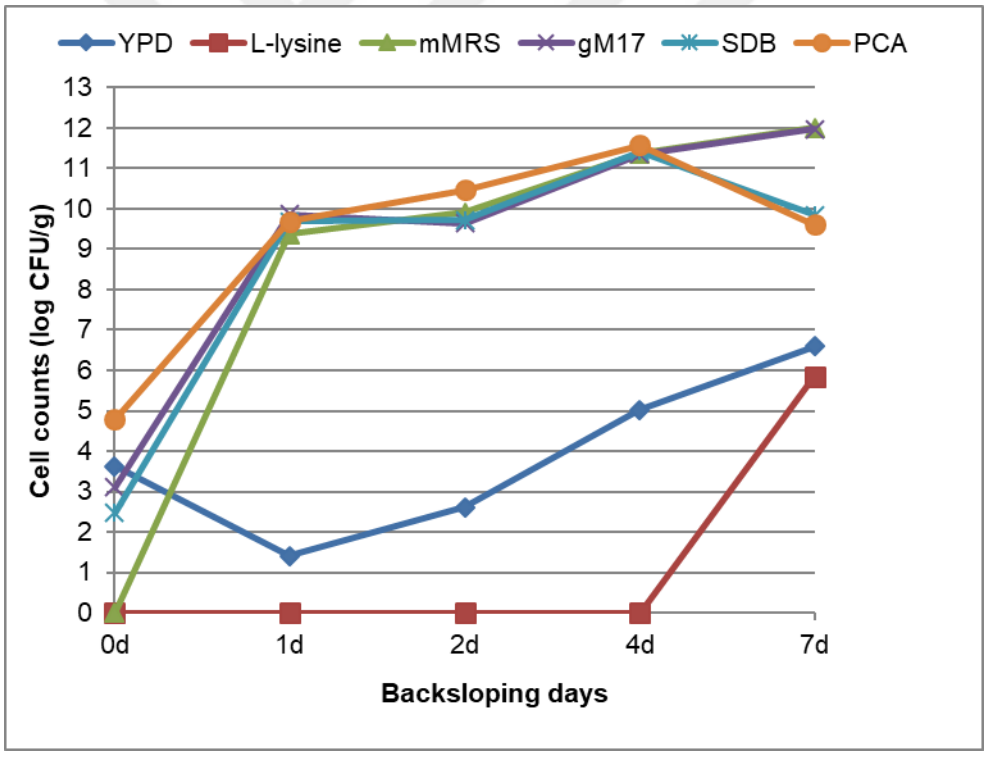

Figure 4.6. Cell counts on mMRS, gM17, SDB, YPD, L-lysine and PCA media of the laboratory scale sourdough sample

Presumptive LAB counts on three different media, namely mMRS, gM17 and SDB, at the first sampling in unfermented dough were determined to be <1, 3.11 and 2.47 log CFU/g, respectively. LAB counts increased rapidly following the next refreshment step in all three media. LAB counts on three different media on the following days were in consistent with each other and reached 12, 11.97 and 9.85 log CFU/g at the end of the fermentation on mMRS, gM17 and SDB, respectively. LAB counts of the present study were higher than previously reported studies. High LAB counts in the present study could be related to the high inoculum level, incubation temperature and refreshment time compared with other studies. In a study, LAB counts of a sourdough fermentation produced with backslopping during 10 days at different temperatures were reported (Vrancken et al., 2011). The backslopping procedure was repeated during 10 days by inoculating 800 grams of ripe sourdough into a 7 kg water-flour mixture. LAB counts at 23 and 30°C with backslopping every 24 h were determined to be around 9 log CFU/g at the last refreshment. Van der Meulen et al. (2007) investigated the microbial counts of laboratory sourdough fermentations (30°C) propagated over a period of 10 days with daily backslopping, without the addition of a starter culture, and reported low LAB and yeast counts at the beginning of the fermentation that were in agreement with our study. In their study, LAB counts rapidly increased, as shown in the present study, and reached 8–9 log CFU/g at the last backslopping; however, final LAB counts were less than the counts reported in this study (Van Der Meulen et al., 2007). The backslopping procedure was repeated by inoculating 800 grams of ripe sourdough into a 7.2 kg water-flour mixture. As it can be seen, the studies used a sourdough inoculum of around 10% for the refreshment of the next batch. However, in our study, a 20% inoculum was used for the refreshment, which resulted in higher LAB counts. Minervini et al. (2012b) produced sourdoughs at artisan and bakery levels by daily backslopping. Inoculum ratios were around 6–30%. LAB counts were between 6.5–8.5 log CFU/g at the 20^{th} and 80^{th} day of backslopping. The backslopping times were between 3-13 hours at 24–28°C and

less backslopping resulted in less LAB counts when compared to this study. In another study, rye and wheat sourdoughs were propagated with a 25% inoculum and cell densities of presumptive LAB reached values of more than 9 log CFU/g for wheat sourdoughs (Ercolini et al., 2013). The inoculum of the study was close to the inoculum level in the present study; however, the propagation time and temperature was 5 h at 25°C, which was less than used in this study.

In the present study, total presumptive yeast counts on YPD medium were below 4 log CFU/g at the beginning and showed fluctuations on the following days. The yeast population decreased at the first refreshment day but then increased again on the following days. At the last refreshment day, the yeast count was determined to be 6.6 log CFU/g. For non-*Saccharomyces* yeasts, the microbial count patterns were different. Until the last refreshment step, L-lysine counts were <1 log CFU/g and 7th backslopping day the count was determined as 5.85 log CFU/g. Van Der Meulen et al. (2007) reported the slow growth of yeasts compared with LAB. In some fermentations, the yeast population started to develop after 8 days of back-slopping. Final yeast counts in terms of logaritmic colony forming units were reported to be in the range of 5.95 and 7.53 log CFU/g (Van Der Meulen et al., 2007). Vrancken et al. (2011) reported the yeast counts in a sourdough fermentation with back-slopping during 10 days at different temperatures. Yeast counts at 23 and 30°C with back-slopping every 24 h were determined to be around 7 and 8 log CFU/g at the last refreshment, respectively. Yeast counts were around 4 log CFU/g at the first refreshment and then increased (Vrancken et al., 2011). Ercolini et al. (2013) reported the yeast count of wheat sourdough to be more than 4 log CFU/g at the beginning of sourdough production. It decreased on the 2nd day of refreshment, but then continued to increase and reached more than 6 log CFU/g on the 11th day of refreshment.

Total mesophilic aerobic bacteria counts on PCA medium were determined to be 4.78 log CFU/g in the unfermented dough and then increased to 11.58 log CFU/g at the 4th refreshment step of the sourdough On the other hand, counts

decreased to 9.6 log cfu/g on the last refreshment day. Mold and coliform bacteria counts are shown in Table 4.7. As it can be seen, no molds and presumptive coliform group bacteria were detected after the 2nd and 3rd refreshment steps, respectively. Indole testing of the coliform bacteria were negative.

Table 4.7. Mold and coliform bacteria

Refreshment day	Mold counts (log CFU/g)	Presumptive total coliform (MPN/g)/Indole test
0 day	2	23/-
1 day	1.5	20/-
2 day	<1	9.2/-
4 day	<1	<3
7 day	<1	<3

4.1.3. Multivariate statistical analysis of the sourdough samples

The microbiological and chemical parameters of sourdoughs were subjected to the multivariate analysis to evaluate the differences/variabilities among the samples. Agglomerative hierarchical clustering **(**AHC) classified the trials in accordance to their mutual dissimilarity and relationship and two main mega-clusters were generated basically with the cut-off line 0.789111 as shown in Figure 4.7. According to the AHC analysis, M1, M2 and T1 samples were included in a group. Laboratory produced sourdough, SD-L7, and SD-T2 samples were in another group. On the other hand, R and K sourdoughs were included as a one group and clustered separately from the other samples. Both sampling of the each sourdough belong to R, K and M bakeries were sorted into the same group as expected. On the other hand, SD-T1 and SD-T2 sourdoughs were clustered in different roots. As reported in the previous sections, properties of the SD-T samples were different in the second sampling since processing conditions changed. Laboratory produced sourdough was clustered with SD-T2 sample.

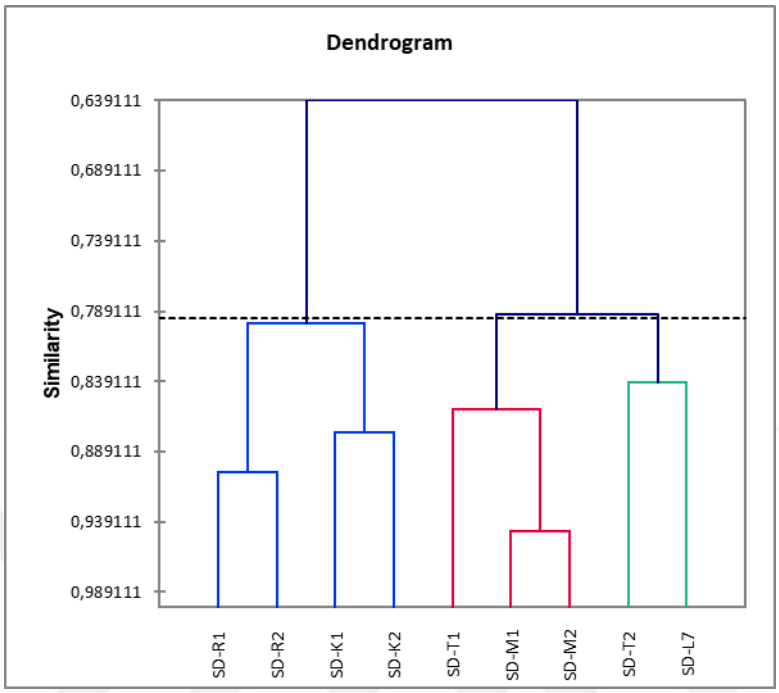

Figure 4.7. Dendrogram resulting from hierarchical cluster analysis on 13 variables determined on sourdoughs

Data of the microbiological and chemical inputs of sourdough samples were also subjected to principle component analysis (PCa) to expresss the important information as principal components. Four eigen-values were higher than 1 and correspond to 88.59% of the variance. The eigenvalues and the corresponding factors by descending order with the variability they represent is shown in Figure 4.8.

Factor 1 and Factor 2 explained 35.50 and 24.48% of total variability, respectively. A large part of the variability was taken into account by the two first axes since the percentage of variability represented by these two factors was 59.99% of total variability as shown in Figure 4.8. Correlation matrix (Pearson) is shown in Table 4.8.

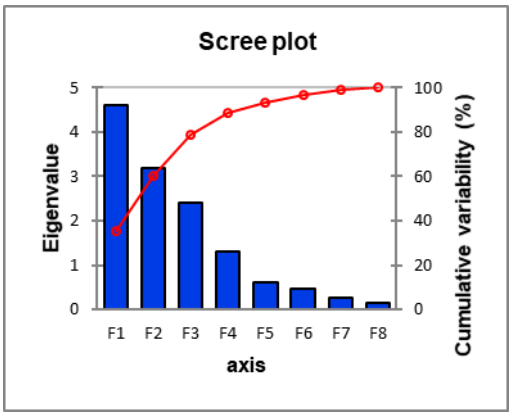

Figure 4.8. The eigenvalues and the corresponding factors by descending order with the variability they represent

Correlation circle (Figure 4.9A) composed of two distinct groups with regards to F1. The first group include acetic acid, TTA, lactic acid and SDB which were positively correlated. The second group contained other variables. Factor 2 has a lower incidence (24.48%) than Factor 1 but it was possible to explain rest of the variables as two district groups with regards to F2. The score plot (Fig. 4.9B) clearly shows the far distance among the sourdough samples collected from different bakeries. As it can be seen, close relation was found between SD-K1 and SD-K2 sourdoughs which were mainly characterized by acetic acid. Along Factor 1, SD-K, SD-T2 and SD-L7 sourdoughs differed from first sampling of the other sourdoughs. SD-L7, laboratory produced sourdough, was differed from the sourdoughs mainly along Factor 2 which has a lower incidence (24.48%) than Factor 1.

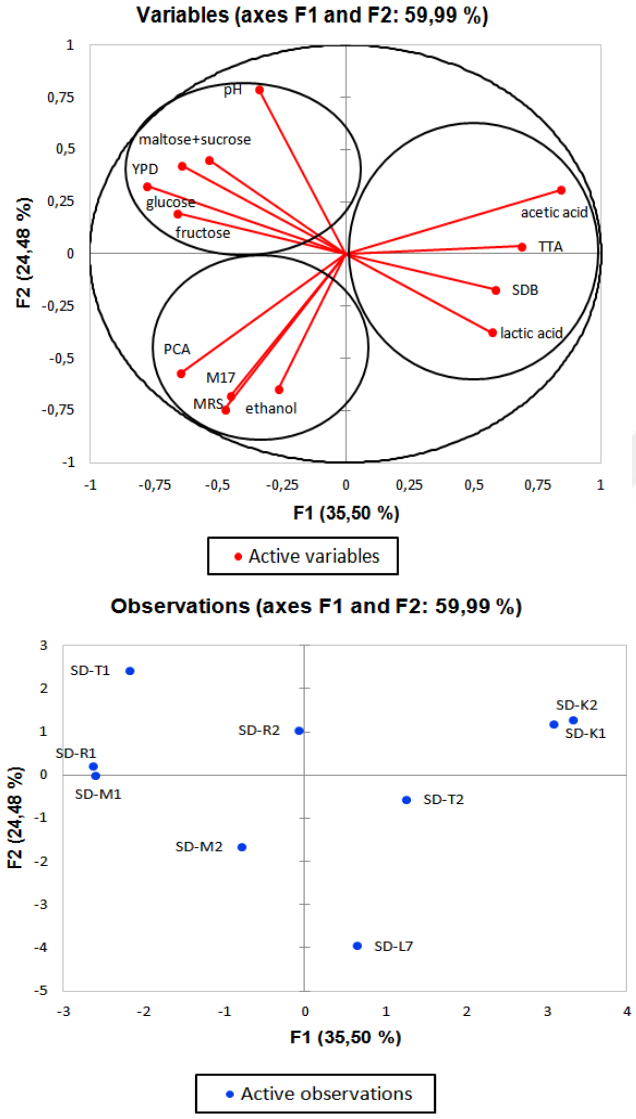

Figure 4.9. Loading plot (A) and score plot (B) resulting from principal component analysis of variables determined on sourdoughs

Table 4.8. Correlation matrix (Pearson (n)) of the variables

Variables	MRS	YPD	M17	SDB	PCA	pH	TTA	maltose+ sucrose	glucose	fructose	lactic acid	acetic acid	ethanol
MRS	**1**	0.022	0.640	0.003	0.857	-0.389	-0.318	-0.182	0.046	0.384	-0.032	-0.573	0.364
YPD	0.022	**1**	-0.054	-0.142	0.223	0.535	-0.093	0.236	0.416	0.656	-0.570	-0.382	-0.288
M17	0.640	-0.054	**1**	-0.043	0.809	-0.165	-0.051	-0.080	0.337	0.309	0.274	-0.547	0.407
SDB	0.003	-0.142	-0.043	**1**	0.014	-0.059	0.594	-0.820	-0.582	-0.067	0.189	0.465	-0.273
PCA	0.857	0.223	0.809	0.014	**1**	-0.031	-0.290	0.015	0.355	0.505	-0.114	-0.601	0.312
pH	-0.389	0.535	-0.165	-0.059	-0.031	**1**	-0.059	0.418	0.617	0.567	-0.420	0.064	-0.596
TTA	-0.318	-0.093	-0.051	0.594	-0.290	-0.059	**1**	-0.675	-0.303	-0.118	0.710	0.485	-0.557
Maltose+sucrose	-0.182	0.236	-0.080	-0.820	0.015	0.418	-0.675	**1**	0.722	0.083	-0.470	-0.350	0.194
Glucose	0.046	0.416	0.337	-0.582	0.355	0.617	-0.303	0.722	**1**	0.600	-0.226	-0.568	-0.134
Fructose	0.384	0.656	0.309	-0.067	0.505	0.567	-0.118	0.083	0.600	**1**	-0.350	-0.543	-0.405
Lactic acid	-0.032	-0.570	0.274	0.189	-0.114	-0.420	0.710	-0.470	-0.226	-0.350	**1**	0.354	-0.161
Acetic acid	-0.573	-0.382	-0.547	0.465	-0.601	0.064	0.485	-0.350	-0.568	-0.543	0.354	**1**	-0.436
Ethanol	0.364	-0.288	0.407	-0.273	0.312	-0.596	-0.557	0.194	-0.134	-0.405	-0.161	-0.436	**1**

Values in bold are different from 0 with a significance level alpha=0

4.1.4. Biodiversity of the LAB and Yeasts in Sourdough Samples

A total of 439 LAB and 235 yeast isolates were collected from sourdough samples including the laboratory-scale produced sourdough. 305 LAB and 181 yeast colonies were collected from bakeries and their distribution according to the sourdough is shown in Figure 4.10. Number of LAB isolates from SD-M and also SD-T samples were almost same at both sampling times.

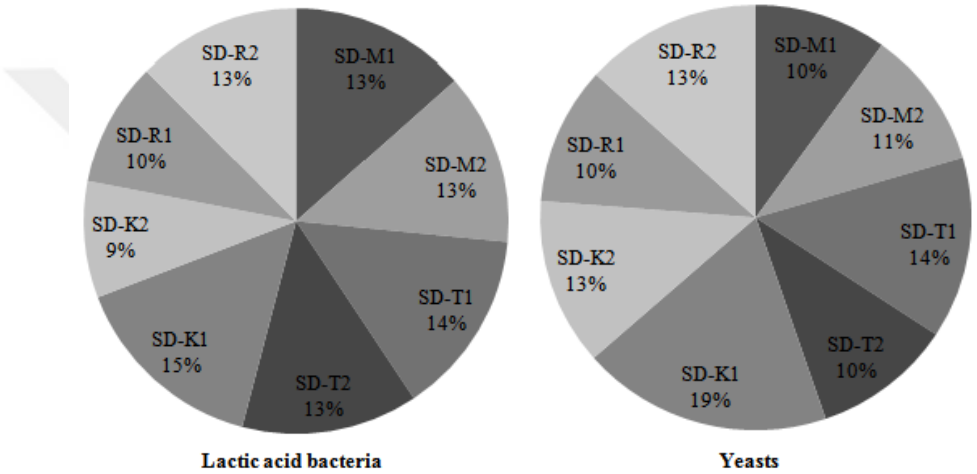

Figure 4.10. Distribution of presumptive LAB and yeast isolates from collected sourdoughs

In addition, 134 LAB and 54 yeast colonies were randomly picked from different days of the laboratory scale production as shown in Figure 4.11. The number of the collected LAB isolates was higher on the 4th and last day of the refreshment of the sourdough than on the initial days.

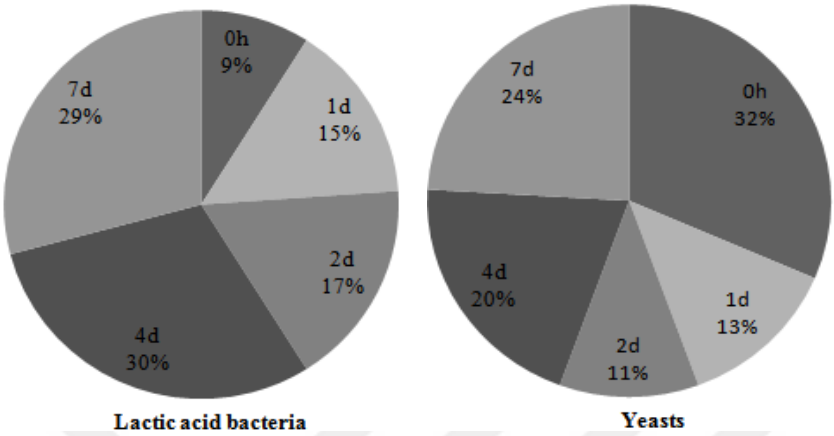

Figure 4.11. Distribution of presumptive LAB and yeasts isolates of sourdough produced under laboratory conditions

4.1.4.1. LAB Identification

A total of 305 colonies were picked from mMRS, gM17 and SDB media of 8 sourdough samples collected from bakeries. Furthermore, 134 colonies were collected from different days of the fermentation of the sourdough sample produced under laboratory conditions. All of the 439 presumptive LAB cultures were subjected to microscopic inspection and Gram-stain and catalase tests. After Gram-stain characterization and catalase testing, 389 strains were still considered putative LAB cultures (Gram-positive and catalase-negative). All of the LAB cultures were grown in the MRS or M17 broth 12-24 hours and subjected to DNA extraction by using Instagene matrix kit. Then genomic DNA of the isolates were subjected to RAPD analysis using M13 primer. Some strains showed weak band profile and were eliminated for further analysis. Bands were evaluated according to the DNA marker by using the Infinity gel documentation imaging system software. Band patterns of RAPD-PCR profiles of 299 strains were scored as band absent (0) or present (1) and data were entered into a binary matrix. The dissimilarity index was calculated on the basis of the Jaccard coefficient generated with the DARwin (6.0.15) software package. A dendrogram was also constructed based on the

genetic distances with the UPGMA method as shown in Figure 4.12. According to the calculated genetic distance matrix, a total of 102 strains were chosen for sequence analysis that had a genetic distance at the level 0.4≤. A gel image of the RAPD-PCR analysis with M13 primer of the strains is attached to Appendix 8.

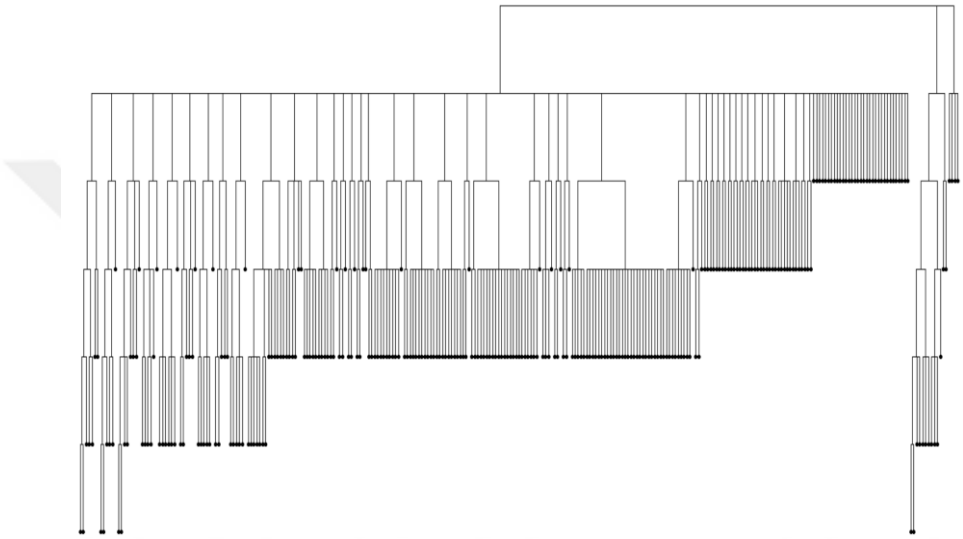

Figure 4.12. Dendrogram obtained from RAPD-PCR (M13 primer) band profiles of LAB isolates in sourdough fermentations

Selected strains were subjected to 16s rRNA gene sequencing analysis. Obtained sequences and their ABI chromatograms were examined using Bioedit Sequence Alignment Editor 7.2.6. (Hall, 1999). The sequences more than 1400 bp were compared by Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) with nucleotide sequences deposited at the database National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). Sequences with at least 98% identity to the sequences of the closest relative available within the NCBI database showed strains belonging to the same species. Strains with less than 98% identity were identified at the genus (94%<) and family (86%<) level (Yarza et al., 2014).

A total of 84 strains representing 178 isolates were confirmed to be members of the LAB group with a sequence length of more than 1250 bp. Based on the 16s rRNA sequence analysis, a total of 52 strains (1400 bp≤) representing 113 isolates were identified at the species level (98%≤). The identified strains along with their accession numbers are given in Table 4.9.

Table 4.9. Identified LAB isolates at the species level (sequence length 1400 bp ≤) (98% ≤) in sourdough samples

Strain	Number of isolates	Species	Similarity % (accession number of closest relative by GenBank)	Sequence length (bp)	Accession number
RL17	3	*Lb. paralimentarius*	99 (NR_114844.1)	1497	MH704092
XL23	1	*Lb. plantarum*	99 (NR_104573.1)	1485	MH704093
XL24	2	*Lb. plantarum*	98 (NR_115605.1)	1464	MH704094
BL45	2	*Lb. paraplantarum*	98 (NR_025447.1)	1490	MH704095
RL164	1	*Lb. plantarum*	98 (NR_104573.1)	1558	MH704096
XL170	2	*Lb. plantarum*	98 (NR_117813.1)	1541	MH704097
RL177	2	*Lb. brevis*	99 (NR_116238.1)	1489	MH704098
RL214	1	*Lb. acidophilus*	98 (NR_117062.1)	1566	MH704099
RL227	2	*Lb. paracasei*	98 (NR_025880.1)	1479	MH704100
RL233	3	*Lb. paralimentarius*	99 (NR_114844.1)	1463	MH704101
BL631	5	*Lb. sanfranciscensis*	99 (NR_116285.1)	1521	MH704102
BL635	1	*Lb. plantarum*	98 (NR_104573.1)	1523	MH704103
XL640	2	*Lb. pentosus*	98 (NR_029133.1)	1508	MH704104
RL658	10	*Lb. sanfranciscensis*	98 (NR_029261.2)	1527	MH704105
RL670	1	*Pd. inopinatus*	98 (NR_025388.1)	1499	MH704106
BL734	1	*Lb. paralimentarius*	98 (NR_114844.1)	1548	MH704107
BL735	1	*Lb. plantarum*	98 (NR_115605.1)	1529	MH704108
BL740	2	*Lb. paralimentarius*	98 (NR_114844.1)	1506	MH704109
BL741	4	*W. confusa*	98 (NR_113258.1)	1485	MH704110
RL749	1	*Lb. plantarum*	98 (NR_115605.1)	1552	MH704111
RL750	1	*Lb. sanfranciscensis*	98 (NR_116285.1)	1473	MH704112
RL826	1	*Lb. brevis*	98 (NR_044704.2)	1544	MH704113
RL833	4	*Lb. paralimentarius*	99 (NR_114844.1)	1502	MH704114
BL843	1	*Lb. sanfranciscensis*	98 (NR_116285.1)	1487	MH704115
BL848	1	*Lb. sanfranciscensis*	98 (NR_116285.1)	1433	MH704116
XL958	6	*Lb. paracasei*	98 (NR_025880.1)	1483	MH704117
XL959	1	*E. faecium*	98 (NR_114742.1)	1505	MH704118
XL963	2	*Lb. plantarum*	98 (NR_104573.1)	1527	MH704119
BL969	1	*Leu. citreum*	99 (NR_041727.1)	1477	MH704120
BL970	2	*Lb. sanfranciscensis*	98 (NR_117814.1)	1479	MH704121
RL975	1	*Leu. citreum*	98 (NR_041727.1)	1554	MH704122

Table 4.9. Continued

RL976	9	*Lb. sanfranciscensis*	98 (NR_029261.2)	1450	MH704123
RL982	2	*Lb. paralimentarius*	99 (NR_114844.1)	1425	MH704124
RL986	4	*Lb. sanfranciscensis*	98 (NR_116285.1)	1519	MH704125
RL989	1	*Lb. sanfranciscensis*	98 (NR_029261.2)	1514	MH704126
BL1023	3	*Lb. sanfranciscensis*	98 (NR_117814.1)	1555	MH704127
RL1042	1	*Lb. parabrevis*	98 (NR_042456.1)	1499	MH704128
RL1046	9	*Lb. plantarum*	98 (NR_104573.1)	1579	MH704129
XL1542	1	*Lc. lactis* subsp. *cremoris*	98 (NR_040954.1)	1411	MH704130
RL1545	1	*Leu. mesenteroides*	98 (NR_074957.1)	1414	MH704131
RL1546	1	*W.cibaria*	99 (NR_036924.1)	1403	MH704132
RL1551	2	*Lb. curvatus*	98 (NR_042437.1)	1400	MH704133
XL1558	1	*Leu. mesenteroides*	98 (NR_074957.1)	1404	MH704134
BL1577	1	*Leu. mesenteroides*	98 (NR_074957.1)	1512	MH704135
BL1578	1	*Lb. curvatus*	98 (NR_113334.1)	1587	MH704136
BL1579	1	*Leu. mesenteroides*	98 (NR_074957.1)	1496	MH704137
RL1617	1	*Lb. curvatus*	98 (NR_113334.1)	1450	MH704138
RL1624	1	*Lb. plantarum*	98 (NR_115605.1)	1551	MH704139
RL1628	1	*Lb. paralimentarius*	98 (NR_114844.1)	1583	MH704140
RL1633	1	*Leu. mesenteroides*	98 (NR_074957.1)	1528	MH704141
RL1639	2	*Lb. paralimentarius*	98 (NR_114844.1)	1417	MH704142
BL1649	1	*Pd. pentosaceus*	98 (NR_042058.1)	1526	MH704143

32 strains representing 65 isolates were identified only at the genus (94%≤) or family (86%≤) level as shown in Table 4.10.

Table 4.10. Identified LAB isolates at the genus (94%≤) or family level (86%≤) in sourdough samples

Strain	Number of isolates	Family/Genus	Similarity % (accession number of closest relative by GenBank)	Sequence length (bp)	Accession Number
XL21	2	*Lactobacillus* spp.	97 (NR_104573.1)	1512	MH704197
XL29	2	*Lactobacillus* spp.	97 (NR_025447.1)	1510	MH704198
BL47	3	*Lactobacillus* spp.	95 (NR_114844.1)	1489	MH704199
XL74	1	*Enterococcus* spp.	95 (NR_113933.1)	1489	MH704200
BL84	2	*Lactobacillaceae*	92 (NR_114844.1)	1562	MH704201
BL86	1	*Lactobacillus* spp.	96 (NR_115605.1)	1566	MH704202
XL168	12	*Lactobacillus* spp.	96 (NR_104573.1)	1431	MH704203
XL172	2	*Lactobacillus* spp.	94 (NR_104573.1)	1465	MH704204
XL238	1	*Lactobacillus* spp.	97 (NR_025880.1)	1645	MH704205
BL628	1	Lactobacillaceae	86 (NR_029261.2)	1472	MH704206
RL829	1	*Lactobacillus* spp.	94 (NR_044704.2)	1555	MH704207
RL835	1	*Lactobacillus* spp.	95 (NR_029261.2)	1437	-
RL837	1	*Lactobacillus* spp.	94 (NR_029261.2)	1426	MH704208
RL839	1	*Weissella* spp.	96 (NR_113258.1)	1472	MH704209
BL841	5	*Lactobacillus* spp.	96 (NR_029261.2)	1564	MH704210
XL962	1	*Enterococcus* spp.	94 (NR_114742.1)	1595	MH704211
XL965	2	*Lactobacillus* spp.	96 (NR_104573.1)	1601	MH704212
RL980	1	*Lactobacillus* spp.	97 (NR_114844.1)	1512	MH704213
RL988	1	*Lactobacillus* spp.	96 (NR_117814.1)	1580	MH704214
BL1028	2	*Lactobacillus* spp.	96 (NR_117814.1)	1449	MH704215
RL1043	1	*Lactobacillaceae*	92 (NR_042456.1)	1501	MH704216
XL1530	1	*Enterococcus* spp.	94 (NR_113904.1)	1438	MH704217
XL1531	4	*Enterococcaceae*	87 (NR_114742.1)	1255	MH704218
XL1532	2	*Enterococcaceae*	91 (NR_114742.1)	1406	-
XL1561	1	*Enterococcus* spp.	98 (NR_113904.1)	1340	MH704219
RL1570	2	*Weisella spp.*	98 (NR_113258.1)	1362	MH704220
RL1616	1	*Lactobacillaceae*	96 (NR_042058.1)	1650	MH704221
RL1622	2	*Pediococcus* spp.	95 (NR_042058.1)	1558	MH704222
RL1640	1	*Lactobacillaceae*	86 (NR_104573.1)	1407	MH704223
RL1641	1	*Lactobacillus* spp.	97 (NR_114844.1)	1528	MH704224
XL1677	1	*Enterococcus* spp.	96 (NR_114742.1)	1444	MH704225
XL1678	5	*Enterococcus* spp.	96 (NR_114742.1)	1505	MH704226

In the present study, 113 strains belonging to 18 LAB species were identified, as shown in Table 4.11. *Lb. sanfranciscensis* (32.7%) was the dominant species, followed by *Lb. plantarum* (18.6%) and *Lb. paralimentarious* (15.9%). In addition, *Lb. paracasei* (7.1%), *Leu. mesenteroides* (4.4%), *W. confusa* (3.5%), *Lb. curvatus* (3.5%) and *Lb. brevis* (2.7%) were found to be minor species.

Furthermore, *Lb. pentosus, Leu. citreum, Lb. paraplantarum, Lb. acidophilus, E. faecium, Pd. inopinatus, Lb. parabrevis, Lc. lactis* subsp. *cremoris, W. cibaria* and *Pd. pentosaceus* were only isolated from 1 or 2 samples.

Table 4. 11. Percentage of the isolated LAB species in sourdoughs

Species	Number of species	%
Lb. sanfranciscensis	37	32.7
Lb. plantarum	21	18.6
Lb. paralimentarious	18	15.9
Lb. paracasei	8	7.1
Leu. mesenteroides	5	4.4
W. confusa	4	3.5
Lb. curvatus	4	3.5
Lb. brevis	3	2.7
Lb. pentosus	2	1.8
Leu. citreum	2	1.8
Lb. paraplantarum	2	1.8
Lb. acidophilus	1	0.9
E. faecium	1	0.9
Pd. inopinatus	1	0.9
Lb. parabrevis	1	0.9
Lc. lactis subsp. *cremoris*	1	0.9
W. cibaria	1	0.9
Pd. pentosaceus	1	0.9
Total	113	100%

The number of *Lb. sanfranciscensis* was 37 out of 113 strains, i.e. 1/3 of the isolates were allotted to this species. *Lb. sanfranciscensis* was identified in all of the sourdoughs, except SD-M1 and SD-R1. *Lb. sanfranciscensis* was detected in T and K sourdough samples at both sampling times. On the other hand, it was only isolated at the 2nd sampling from M and R sourdoughs as shown in Table 4.12. In the laboratory produced sourdough, *Lb. sanfranciscensis* was not isolated during 7 days of fermentation. *Lb. plantarum* was detected in all collected sourdough samples except SD-T1 and SD-R2 samples. In addition, it was determined on the 4th day of the laboratory-produced sourdough. *Lb. paralimentarious* was isolated from all sourdoughs except the SD-K sample. It was also isolated at the 4th and 7th

day of the laboratory-produced sourdough. *Lb. paracasei* was only found in the SD-T samples at both sampling times. *W. confusa* was only identified in the SD-M2 sample. *Lb. brevis* was isolated from both sampling of the SD-R sourdough. Other isolated strains, *Lb. pentosus, Leu citreum, Lb. paraplantarum, Lb. acidophilus, E. faecium, Pd. inopinatus* and *Lb. parabrevis* were minor species isolated from different bakeries. *Leu. mesenteroides, Lb. curvatus, Lc. lactis* subsp. *cremoris, W. cibaria* and *Pd. pentosaceus* strains were not isolated from the collected samples and were only identified in the laboratory scale sourdough production.

Table 4.12. Number of LAB identified at the species level in sourdough samples

Species	M1	M2	T1	T2	K1	K2	R1	R2	L1	L2	L4	L7
Lb. sanfranciscensis		2/12	1/7	10/23	18/22	4/14		2/6				
Lb. plantarum	5/8	2/12		2/23	1/22	9/14	1/5				1/3	
Lb. paralimentarious	1/8	4/12	3/7	2/23			2/5	3/6			1/3	2/3
Lb. paracasei			2/7	6/23								
Leu. mesenteroides									1/4	3/6		1/3
W. confusa		4/12										
Lb. brevis							2/5	1/6				
Lb. curvatus									1/4	2/6	1/3	
Lb. pentosus					2/22							
Leu. citreum				2/23								
Lb. paraplantarum	2/8											
Lb. acidophilus			1/7									
E. faecium				1/23								
Pd. inopinatus					1/22							
Lb. parabrevis						1/14						
Lc. lactis subsp. *cremoris*									1/4			
W. cibaria									1/4			
Pd. pentosaceus										1/6		
Total LAB	8	12	7	23	22	14	5	6	4	6	3	3

The SD-M sample contained different species at both sampling times as shown in Figure 4.13. In the SD-M1 sample, *Lb. plantarum* was detected as the predominant species and *Lb. paralimentarious* and *Lb. paraplantarum* were detected as minor species. On the other hand, *Lb. paralimentarious* and *W. confusa* were co-dominant species in the SD-M2 sample and *Lb. sanfranciscensis* and *Lb. plantarum* were found as minor species.

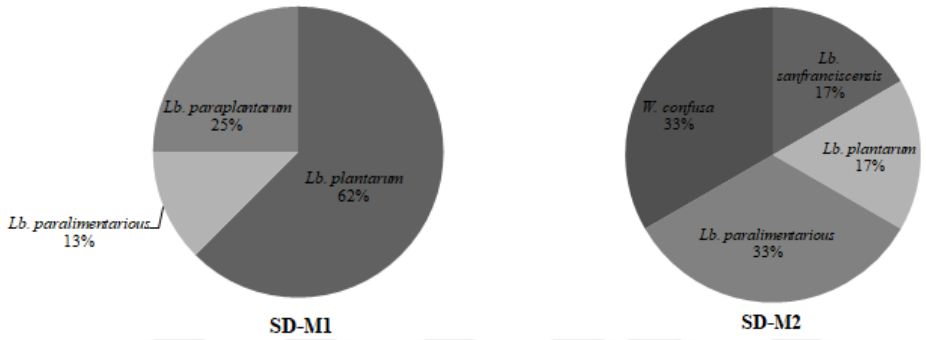

Figure 4.13. Species in SD-M sourdough samples at both sampling times

The SD-T sample contained *Lb. sanfranciscensis*, *Lb. paralimentarious* and *Lb. paracasei* at both sampling times as shown in Figure 4.14. In the SD-T1 sample, *Lb. paralimentarious* and *Lb. paracasei* were detected as the predominant species and *Lb. sanfranciscensis* and *Lb. acidophilus* were detected as minor species. On the other hand, *Lb. sanfranciscensis* was the dominant species at the second sampling. *Lb. paracasei* was also detected as the dominant species. The minor species of the SD-T2 sourdough was *Lb. plantarum, Lb. paralimentarious, Leu. citreum* and *E. faecium*.

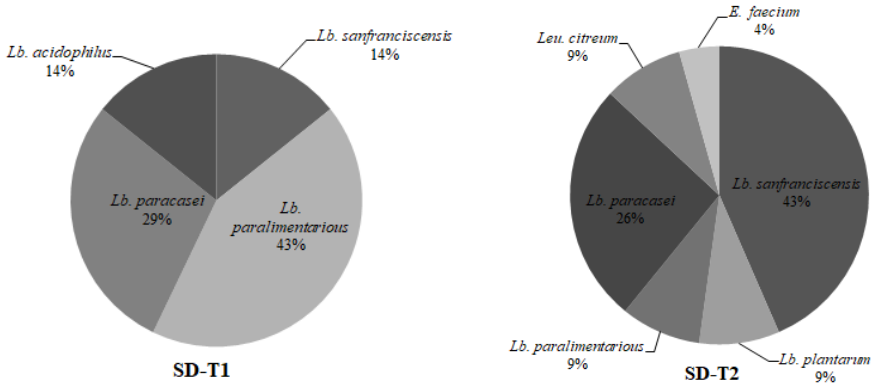

Figure 4.14. Species in SD-T sourdough samples at both sampling times

The SD-K sample contained *Lb. sanfranciscensis* as the dominant species at the first sampling as shown in Figure 4.15. In the SD-K1 sample, *Lb. sanfranciscensis* was the predominant species and *Lb. plantarum, Lb. pentosus* and *Pd. inopinatus* were detected as minor species. On the other hand, *Lb. plantarum* was the dominant species at the second sampling. *Lb. sanfranciscensis* and *Lb. parabrevis* were also detected in the SD-K2 sample.

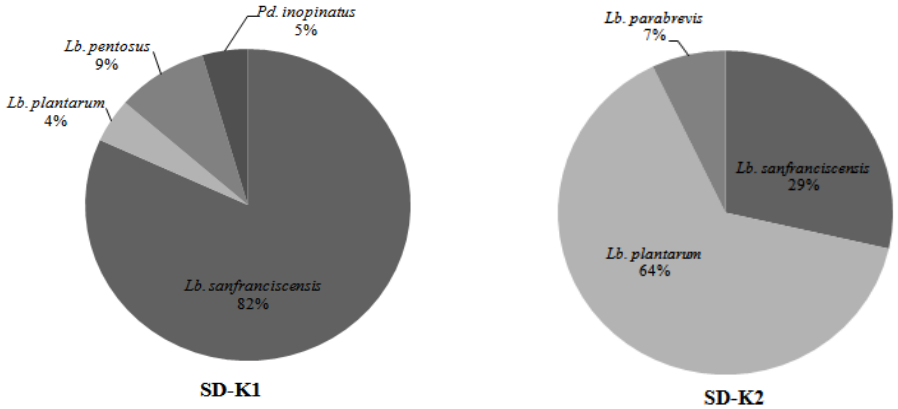

Figure 4.15. Species in SD-K sourdough samples at both sampling times

Figure 4.16 shows the species of the rye sample at two different sampling times. Rye sourdough contained *Lb. paralimentarious* as the dominant species at

both sampling. *Lb. paralimentarious* and *Lb. brevis* were co-dominant species and *Lb. plantarum* was the minor species in the SD-R1 sample. In the SD-R2 sample, the dominant species were *Lb. paralimentarious* followed by *Lb. sanfranciscensis* and *Lb. brevis.*

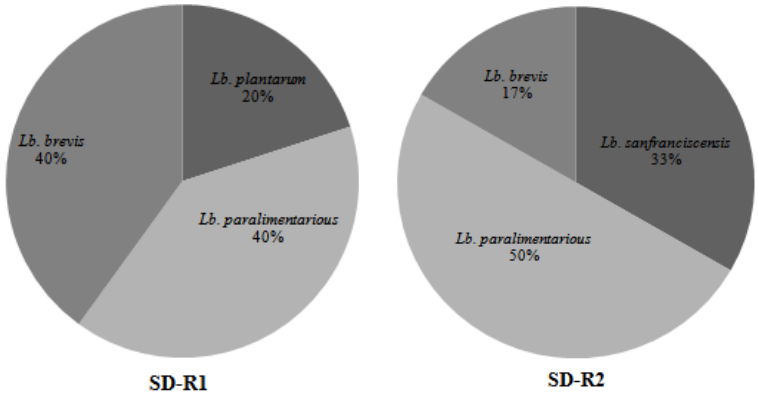

Figure 4.16. Species in SD-R sourdough samples at both sampling times

In laboratory produced sourdough, LAB identified at the species level were *Leu. mesenteroides, Lb. curvatus, Lc. lactis* subsp. *cremoris* and *W. cibaria* in the first refreshment of the sourdough. Following refreshment, *Leu. mesenteroides* and *Lb. curvatus* were detected but *Lc. lactis* subsp. *cremoris* and *W. cibaria* were not isolated. *Pd. pentosaceus* was also detected at the second refreshment. At the 4th refreshment, the LAB detected at the species level were *Lb. plantarum, Lb. paralimentarious* and *Lb. curvatus*. On the last day of refreshment, *Lb. paralimentarious* and *Leu. mesenteroides* were isolated. The number of identified species is shown in Figure 4.17. In the sourdough sample produced on laboratory scale, the well-known wheat sourdough LAB, *Lb. sanfranciscensi,* was not detected.

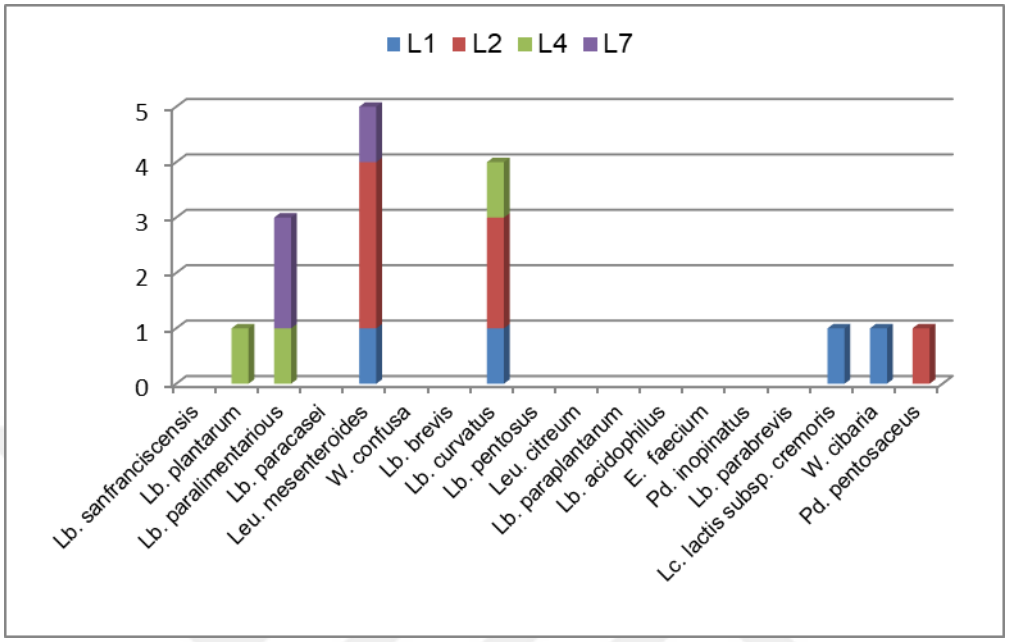

Figure 4.17. The number of identified LAB species in laboratory scale production

In the present study, the sourdough propagation was continued once in a 24 h during 7 days. However, the well-known wheat sourdough *Lb. sanfranciscensis* was not detected in any of the refreshments. The results of the present study confirm the results obtained from some other studies related to laboratory sourdough fermentations (Van Der Meulen et al., 2007; Vrancken et al., 2011). Vrancken et al. (2011) reported the absence of *Lb. sanfranciscensis* in laboratory sourdough fermentations performed under semi-sterile conditions and hypothesized the non-flour origin of this species. However, wheat LAB was monitored from ear harvest until the first step of fermentation and *Lb. sanfranciscensis* wasidentified as the only from durum wheat semolina (Alfonzo et al., 2013) but not from the ear, kernel, dough and also semolina in another monitoring study (Alfonzo et al., 2017). Minervini et al. (2012a) investigated the laboratory and artisan propagated sourdoughs and reported the LAB flora difference between artisan bakery and laboratory levels. In their study, strains of *Lb. sanfranciscensis* were found in some

sourdoughs. Results of the study showed the undoubted influence of the refreshment environment on the composition of sourdough yeast and LAB.

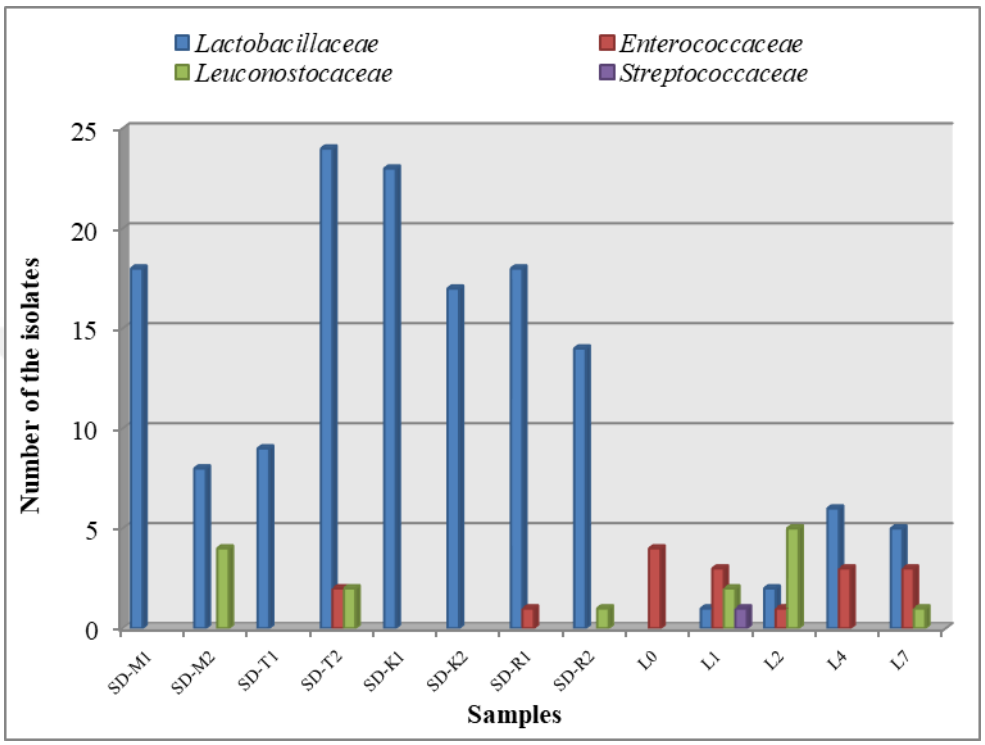

Figure 4.18. Distribution of the LAB strains at the family level in the sourdough fermentations

LAB detected in the sourdough samples at the family level are shown in Figure 4.18. The isolated strains from sourdough samples belonged to four families, i.e., *Lactobacillaceae*, *Enterococcaceae*, *Leuconostocaceae* and *Streptococcaceae*. As it can be seen, many of the isolated strains belonged to the *Lactobacillaceae* family. Among 178 isolates, the number of strains in the *Lactobacillaceae*, *Enterococcaceae*, *Leuconostocaceae* and *Streptococcaceae* families were 145, 17, 15 and 1, respectively. Distribution as a percentage is given in Figure 4.19.

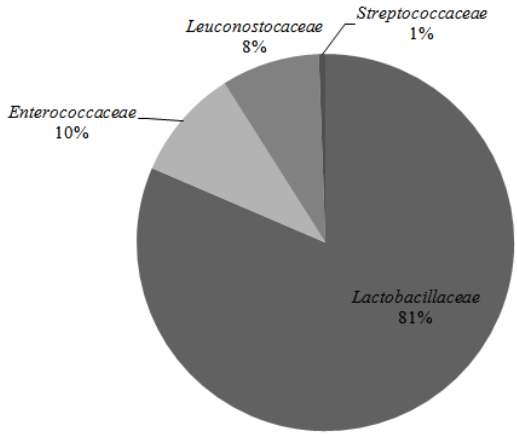

Figure 4 19. Frequency of the strains at the family level

LAB detected in the sourdough samples at the genus level are shown in Figure 4.20. The isolated strains from sourdough samples belonged to 6 genera, i.e., *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* and *Lactococcus.* As it can be seen, many of the isolated strains were *Lactobacillus* spp. Totally 166 strains were identified at the genus level and number of the *Lactobacillus, Enterococcus, Weissella, Leuconostoc, Pediococcus* and *Lactococcus* spp. were 135, 11, 8, 7, 4 and 1, respectively. Distribution as a percentage is given in Figure 4.21.

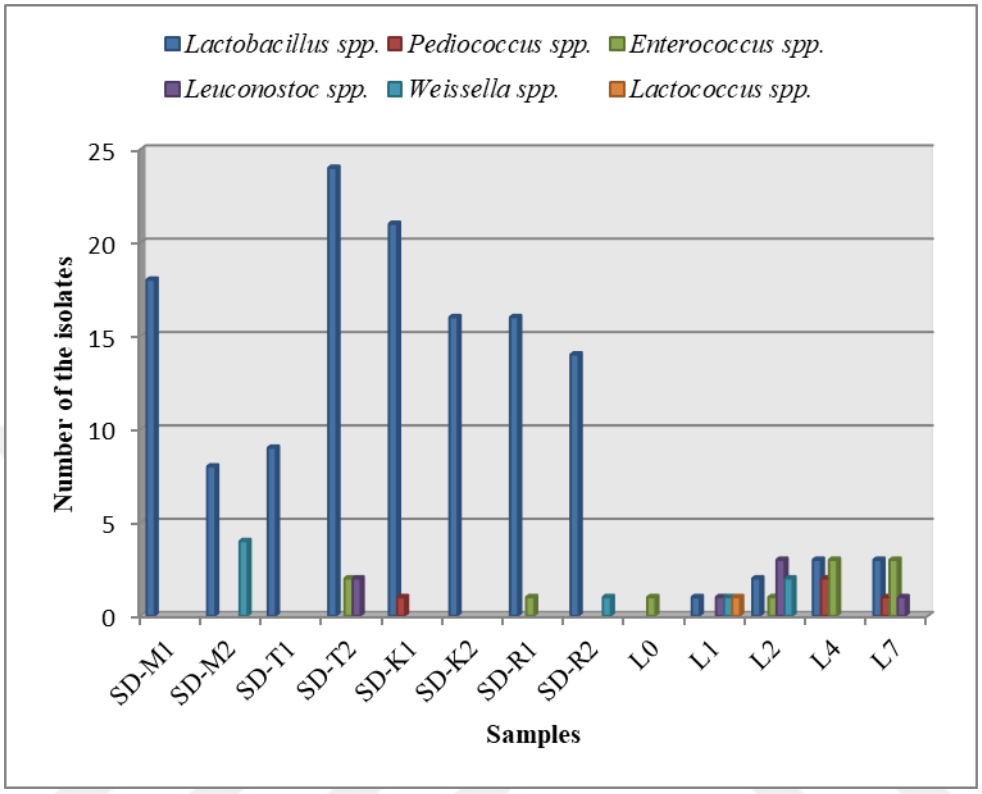

Figure 4. 20. Distribution of the LAB strains at the genus level in the sourdough fermentations

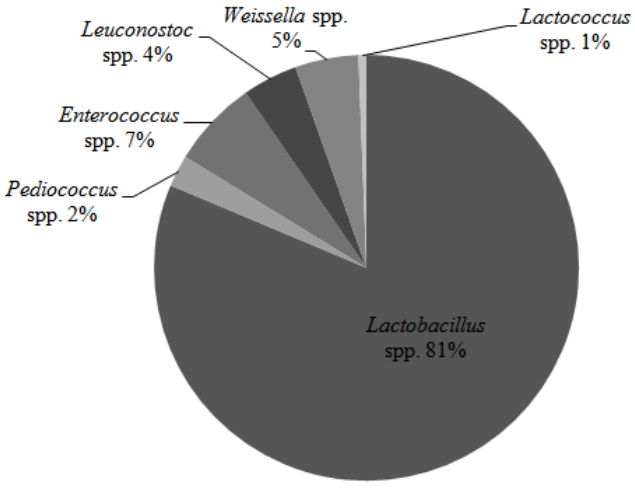

Figure 4.21. Frequency of the strains at the genus level

In the present study, 81% of the identified strains belonged to the genus *Lactobacillus* spp. Microbial patterns of the sourdoughs collected from different bakeries differed between each other. The predominant LAB species *Lb. sanfranciscensis* mainly dominated the sourdough ecosystem. As reported previously, this species is well known in natural sourdough habitats of the artisan and industrial bakeries (Kline and Sugihara, 1971; Corsetti et al., 2001; Meroth et al., 2003; Siragusa et al., 2009; Vrancken et al., 2011; Venturi et al., 2012; Lhomme et al., 2016). In both rye and wheat sourdoughs produced with continuous propagation by back-slopping procedures, *Lb. sanfranciscensis* was reported as probably the most adapted species in the sourdough microbiota (Gobbetti and Corsetti, 1997; Vogel et al., 2002; Vogel et al., 2011). As reported previously, its good adaptation to the sourdough environment can be related to the utilization of sourdough carbohydrates, activated proteolytic enzymes and synthesis of antimicrobial compounds (Gobbetti and Corsetti, 1997; Corsetti et al., 2001; Lattanzi et al., 2013). *Lb. sanfranciscensis* was followed by *Lb. plantarum* and *Lb. paralimentarius*. Minervini et al. (2012a) investigated the microbiota of 19 Italian sourdoughs and the most frequent LAB isolates belonged to *Lb. sanfranciscensis*, *Lb. plantarum* and *Lb. paralimentarius* accounting for 28, 16 and 14% of the total LAB isolates (Minervini et al., 2012a). As reported previously, *Lb. plantarum* is associated with *Lb. sanfranciscensis* in sourdoughs (Gobbetti, 1998). *Lb. paralimentarius* has been frequently reported in many sourdoughs (Cai et al., 1999; Minervini et al., 2012a; Taccari et al., 2016). Many sourdoughs contain associations of different hetero- and homofermentative LAB strains. It was reported that homofermentative LAB dominate in spontaneous fermentation processes and heterofermentative species drive sourdough fermentation processes produced via back-slopping (De Vuyst and Neysens, 2005). Most heterofermentative LAB, especially *Lactobacillus* spp., occur in stable sourdough ecosystems and the facultatively heterofermentative *Lb. paralimentarius* also seems to be optimally adapted to the sourdough ecosystem (Huys et al., 2013).

Other less predominant LAB species, including members of the genera *Weissella*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus* can be found in sourdoughs but at lower levels than *Lactobacillus* spp. (Corsetti and Settanni, 2007). According to Corsetti et al. (2007b), different non-*Lactobacillus* species are mainly found at the initial stages of sourdough production to prepare the environment for the growth of typical species of mature sourdoughs. In this study, besides the dominant *Lb. sanfranciscensis, Lb. plantarum, Lb. paralimentarius* species, also *Lb. paracasei, Leu. mesenteroides, W. confusa*, *Lb. curvatus* and *Lb. brevis* were found as minor species. Other less frequently isolated species were *Lb. pentosus, Leu. citreum*, *Lb. paraplantarum, Lb. acidophilus, E. faecium, Pd. inopinatus, Lb. parabrevis, Lc. lactis* subsp. *cremoris, W. cibaria* and *Pd. pentosaceus* in our study. The presence of isolated species in sourdoughs has been reported in other studies (Vogel et al., 1994; Corsetti et al., 2004; Gül et al., 2005; Iacumin et al., 2009; Minervini et al., 2012a; Rossi et al., 2012; Amari et al., 2013; Settanni et al., 2013; Rizzello et al., 2014; Lhomme et al., 2015; Yagmur et al., 2016; Alfonzo et al., 2017; Bartkiene et al., 2017). However, the species distribution and the dominant flora vary in the collected sourdough samples and showed the importance of the environment on the sourdough ecosystem. According to De Vuyst et al. (2017), sourdough ecosystem can contain a simple microflora characterized by *Lb. plantarum* or *Lb. sanfranciscensis* or a restricted LAB species diversity or with a complex microbial consortium including different LAB species generally less than three species. Besides geographical origin, sampling, isolation, and identification techniques are also important in the estimation of the sourdough ecosystem. However, the flour type, quality and the process parameters such as fermentation temperature, pH and pH evolution, dough yield, water activity, oxygen tension, back-slopping procedure and fermentation duration directly determine the dynamics and outcome of backslopped sourdough fermentation processes

Other species belonging to the genera *Enterococcus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus* and *Weissella* are generally determined at lower levels compared with *Lactobacillus* spp. as shown in the present study (Corsetti and Settanni, 2007). Corsetti et al. (2007b) reported the occurence of different non-*Lactobacillus* species during the first stages of sourdough fermentations, which prepare the environment for the establishment of typical species.

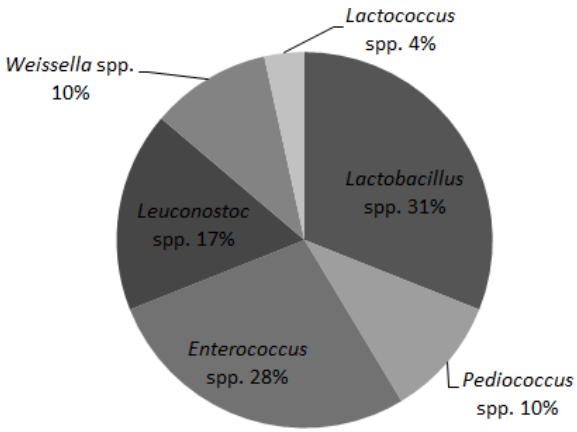

Figure 4. 22. Distribution of species in the laboratory sourdough production

In the present study, identified LAB in the sourdough produced at laboratory scale belonged to 6 genera including *Lactobacillus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus* and *Weissella*. The distribution of the genera in the laboratory sourdough production is shown in Figure 4.22. Van Der Meulen et al. (2007) reported the occurence of LAB species that were not specific for sourdough from the beginning until the 2nd day of the sourdough fermentation.

4.1.4.2. Phylogenetic Relation of the LAB Strains

Phylogenetic trees were constructed based on the 16S rRNA gene sequences (1400 bp≤) of the identified strains at the species level using two

possible tree reconstruction methods, minimum evolution and UPMGA, in MEGA 7 (Kumar et al., 2016).

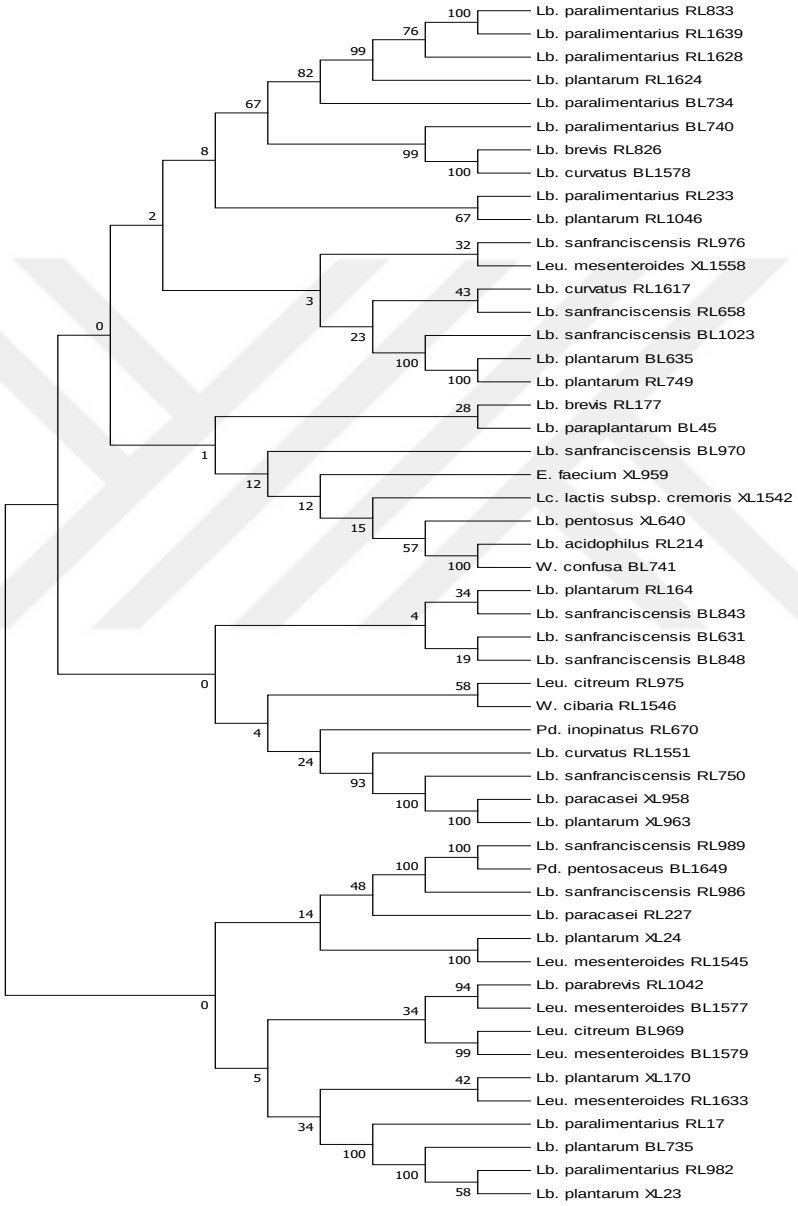

Figure 4.23. Evolutionary relationships of taxa by using the Minimum Evolution method

The evolutionary relationships of taxa that was constructed using the Minimum Evolution method is shown in Figure 4.23 (Rzhetsky and Nei, 1992). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the number of differences method (Nei and Kumar, 2000) and are in the units of the number of base differences per sequence. The analysis involved 52 nucleotide sequences. All ambiguous positions were removed for each sequence pair.

The second evolutionary history was inferred using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method as shown in Figure 4.24 (Sneath and Sokal, 1973). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the number of differences method (Nei and Kumar, 2000) and are in the units of the number of base differences per sequence. The analysis involved 52 nucleotide sequences. All ambiguous positions were removed for each sequence pair.

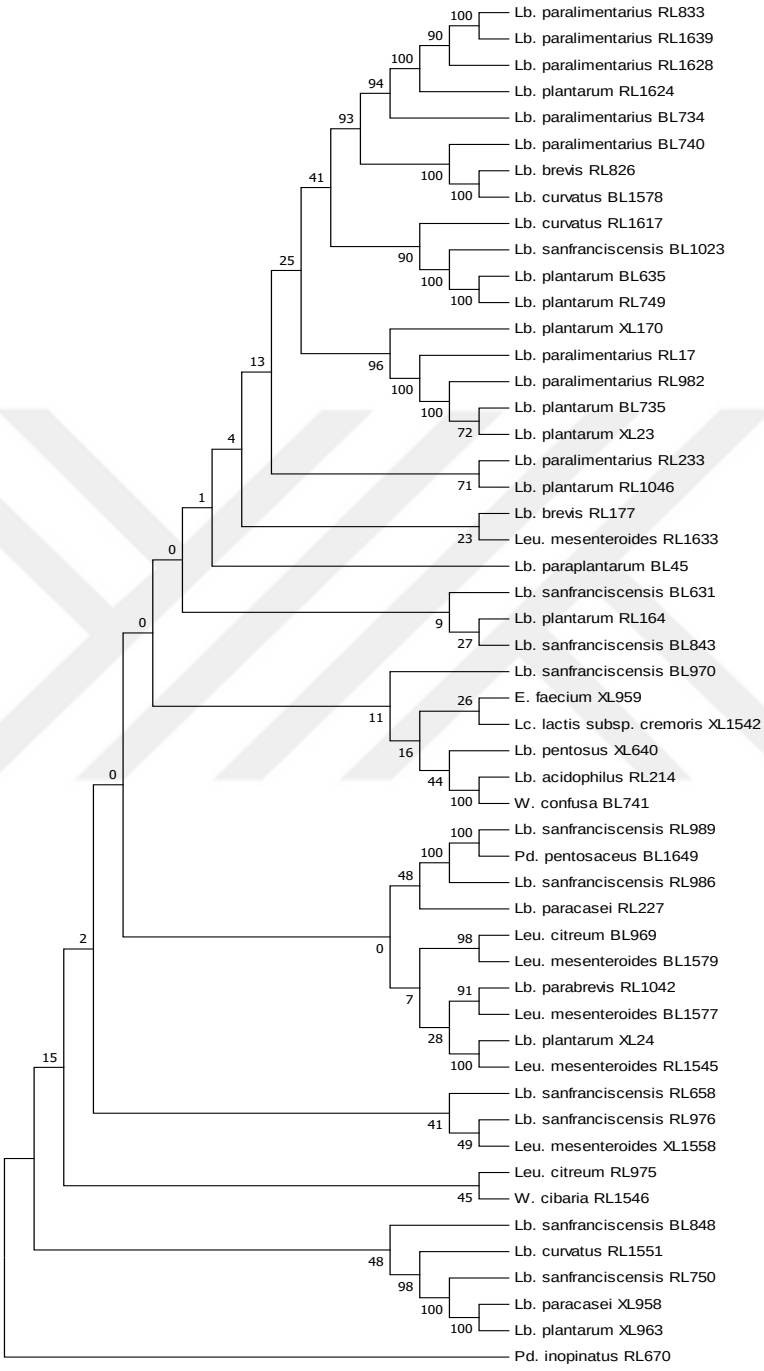

Figure 4.24. Evolutionary relationships of taxa by using the UPMGA method

4.1.4.3. Yeast Identification

A total of 181 presumptive yeast colonies were picked from YPD and L-lysine media of 8 sourdough samples collected from bakeries. In addition, 54 colonies were collected from laboratory scale sourdough production. All of the 235 presumptive yeast cultures were grown in YPD medium for 24-36 hours and subjected to DNA extraction using Instagene matrix kit. Before extraction, all of the isolated yeasts were treated with lyticase enzyme to degrade the cell walls. In total, 205 genomic DNA was extracted and subjected to 5.8S ITS rRNA region amplification using primers ITS1 and ITS4. A gel image of the 5.8S ITS rRNA region amplification is attached to Appendix 9. PCR products showing visible bands on the agarose gel were subsequently digested using the restriction endonucleases *Hae* III, *Hha* I and *Hinf* I. A gel image of the RFLP with restriction endonucleases Hae III, Hha I and Hinf I is attached to Appendix 10. A total of 7 profiles were determined according to the restriction fragments as shown in Table 4.13. Strains exhibited a unique restriction pattern for each species with the three endonucleases used.

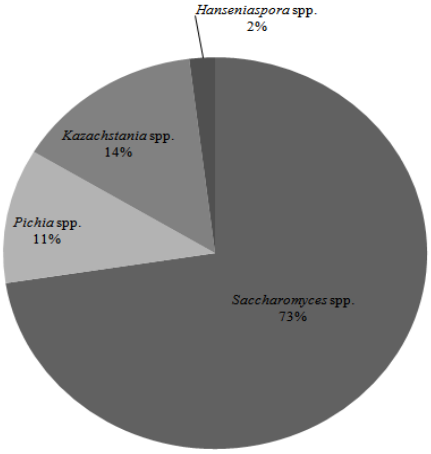

Figure 4. 25. Distribution of the genera of the identified yeast isolates

A total of 153 isolates belonging to 7 species were identified by 26S rRNA gene sequencing as shown in Table 4.14. For a species-level identification, identity more than 99% with the sequence length at least 400 bp was selected (Romanelli et al., 2010). Seven species were identified that belonged to 4 genera, i.e., *Saccharomyces, Hanseniaspora (H'spora), Pichia* and *Kazachstania,* as shown in Figure 4.22. One species was identified in the genera *Saccharomyces* and *Hanseniaspora.* On the other hand, two and three species belonged to the genera *Pichia* and *Kazachstania,* respectively.

Table 4.13. Restriction fragments of the identified yeast species from sourdoughs

RFLP Profile	Species	PCR products (bp)	Restriction fragments (bp)		
			Hae III	*Hha* I	*Hinf* I
I	*S. cerevisiae*	880	315+240+180+145	385+365+130	390+130
II	*P. membranifaciens*	500	320+90+50	175+110+90	275+200
III	*K. servazzii*	750	320+240+190	320+200+150	360
IV	*P. fermentans*	450	340+80+30	170+100+80	250+200
V	*H'spora valbyensis*	750	750	630+120	260+215+175+100
VI	*K. bulderi*	700	500+200	350+280	300+250+130
VII	*K. unispora*	750	300+220+180	310+200+160	385+360

Table 4.14. Accession numbers of the identified yeast species with their closest relatives and type strains

RFLP Profile	Species	N[1]	Strain[2]	Accession number	bp[3]	Closest relative Accession number / Identity(%)[4]	Type strain Accession number/ Identity(%)[5]	Divergent bases[6]
I	*S. cerevisiae*	111	PM 1	MH704179	598	*S. cerevisiae* SFM35 MG017576.1/99	*S. cerevisiae* NRRL Y-12632 NG_042623.1/99	7
II	*P. membranifaciens*	8	NM 1004	MH704180	600	*P. membranifaciens* CBS:598 KY108894.1/99	*P. membranifaciens* NRRL Y-2026 NG_042444.1/99	4
III	*K. servazzii*	7	PM 603 PM 604	MH704181 MH704182	614 624	*K. servazzii* NRRL Y-12661 NG_055029.1/99	*K. servazzii* NRRL Y-12661 NG_055029.1/99	5 3
IV	*P. fermentans*	9	PM 801 NM 816	MH704183 MH704184	594 603	*P. fermentans* lhWW149 MF462777.1/100 *P. fermentans* A16 KM589463.1/99	*P. fermentans* NRRL Y-1619 NG_055109.1/99	5 4

Table 4.14 (Continued)

V	*H'spora valbyensis*	3	NM 625 NM 626	MH704185 MH704186	586 621	*H'spora valbyensis* CBS:479 KY107857.1/99	*H'spora valbyensis* NRRL Y-1626 NG_042630.1/99	3 8
VI	*K. bulderi*	11	PM 190	MH704187	588	*K. bulderi* PD-1 MG983971.1/99	K. bulderi NRRL Y-27203 NG_055022.1/99	2
VII	*K. unispora*	4	PM 617	MH704188	562	*K. unispora* MG525064.1/99	*K. unispora* CBS 398 NG_055027.1/99	3

[1]Number of species [2]26S rRNA gene sequenced strain representing each RFLP profile, [3]sequence lengh, [4]Sequence identity in the D1/D2 region of isolates with species in the GenBank, [5]Sequence identity in the D1/D2 region of isolates with type strain of the same species in the GenBank, [6]Number of the divergent bases from type strain

In present study, 153 yeast strains belonging to 7 species were identified as shown in Table 4.15. *S. cerevisiae* (72.5%) was the dominant yeast species. Other isolated yeast species were *K. bulderi* (7.2%), *P. fermentans* (5.9%), *P. membranifaciens* (5.2%), *K. servazzii* (4.6%), *K. unispora* (2.6%) and *H'spora valbyensis* (2%).

Table 4.15. Percentage of the isolated yeast species in sourdoughs

Species	Number of the species	%
S. cerevisiae	111	72.5
K. bulderi	11	7.2
P. fermentans	9	5.9
P. membranifaciens	8	5.2
K. servazzii	7	4.6
K. unispora	4	2.6
H'spora valbyensis	3	2.0
Total	153	100%

The distribution of yeast species among sourdough samples is shown in Table 4.16. *S. cerevisiae* was the only species identified in SD-M sourdoughs at both sampling times. *S. cerevisiae* was isolated from thr SD-T2 sample as the dominant species. In addition, only one strain was determined as *K. bulderi* in the SD-T2 sample. *S. cerevisiae* and *K. bulderi* co-dominated the first sampling of the same bakery, SD-T1. In the SD-K1 sample, *S. cerevisiae*, *H'spora valbyensis*, *K. servazzii* and *K. unispora* were co-dominant. On the other hand, *S. cerevisiae* and *P. membranifaciens* were co-dominant in the SD-K2 sample. In rye sourdough, *S. cerevisiae* was dominant at the first sampling. *S. cerevisiae* and *P. fermentans* co-dominate the second sampling. In laboratory scale productions, none of the isolated strains were identified as yeast at the first 2 days. On the other, *S. cerevisiae* was isolated from 4 and 7 day refreshment.

Table 4.16. Distribution of the yeast species among sourdough samples

Yeast species	M1	M2	T1	T2	K1	K2	R1	R2	L4	L7
S. cerevisiae	13/13	18/18	13/23	17/18	7/21	9/17	16/16	13/22	1/1	4/4
P. fermentans								9/22		
K. bulderi			10/23	1/18						
K. servazzii					7/21					
P. membranifaciens						8/17				
K. unispora					4/21					
H'spora valbyensis					3/21					
Total LAB	13	18	23	18	21	17	16	22	1	4

In the present study, yeast diversity was less than that of the LAB microbiota, since only seven yeast species were identified in the collected sourdoughs. This finding is in consistent with the literature (Minervini et al., 2015). In the present study, *S. cerevisiae* was the most frequently isolated yeast species. Collected sourdoughs were produced without using baker's yeast. However *S. cerevisiae* was isolated from all of the samples including laboratory produced sourdough. As reported previously the presence of *S. cerevisiae* in the bakery sourdoughs can be related to contamination of the bakery environment with commercial baker's yeast (Vrancken et al., 2010; Minervini et al., 2015). On the other hand, baker's yeast was also not used for laboratory production. However, *S. cerevisiae* was detected at the 4th and 7th day of the refreshment steps. Flour could have been a source of *S. cerevisiae* in the laboratory scale production (Vrancken et al., 2010). Previous studies showed that *S. cerevisiae* is the most reported yeast species in both wheat and rye sourdoughs (Vernocchi et al., 2004; Vrancken et al., 2010; De Vuyst et al., 2016).

The SD-K sourdough sample showed a rich yeast biodiversity compared with other sourdough samples, as illustrated in Figure 4.26. Isolates of this bakery belonged to 4 different genera and 5 different species. Yeast species, determined at two different sampling times, exhibited differences. *S. cerevisiae*, *H'spora valbyensis*, *K. servazzii* and *K. unispora* were co-dominant in the SD-K1 sample and *S. cerevisiae* and *P. membranifaciens* were co-dominant in the SD-K2 sample. *K. bulderi* was identified in the SD-T sourdough samples. *P. fermentans* was only determined in the second sampling of the rye sourdough. The dominant yeast species in the collected sourdough samples was *S. cerevisiae*. However, the presence of other yeast species differed between bakeries. There were 7 yeast species over all 8 bakeries and 3 species representing 14.4% of all the identified strains belonged to *Kazachstania* clade including *K. servazzii*, *K. bulderi* and *K. unispora. Kazachstania* spp. have been previously isolated from sourdough and the bakery environment (Vrancken et al., 2010; Minervini et al., 2012a; De Vuyst et

al., 2014; Lhomme et al., 2015; Minervini et al., 2015; De Vuyst et al., 2016; Lhomme et al., 2016; Sarilar et al., 2017). As previously it was reported, *S. cerevisiae* may not compete with other sourdough and bread dough yeast species and *Kazachstania* spp. could be better adapted to these environments (Lhomme et al., 2015). *K. bulderi* (formerly known as *S. bulderi*), detected from maize silage as a novel species, was closely related to *S. barnettii* and *S. exiguus* (Middelhoven et al., 2000). In 2016, it was isolated from French sourdough for the first time (Lhomme et al., 2016). *K.unispora* (formerly known as *S. unisporus*) was previously determined in sourdoughs (Vrancken et al., 2010; De Vuyst et al., 2016). In the study of Vrancken et al. (2010), only one artisan sourdough isolate was identified as *K. unispora*. *K. unispora* and *K. servazzii* (formerly *S. servazzii*) have been reported in Italian sourdoughs (Di Cagno et al., 2014).

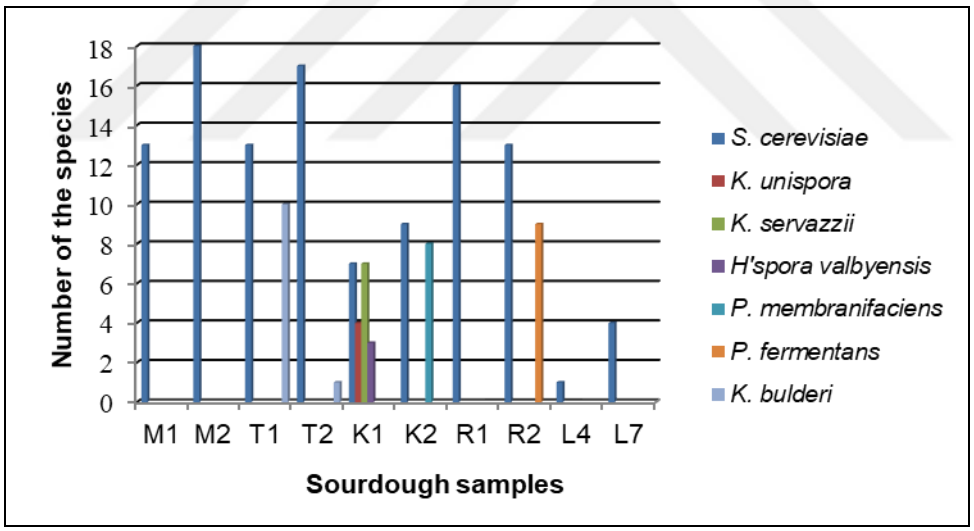

Figure 4.26. Distribution of yeast species in sourdough samples

Other yeast species *P. membranifaciens*, *P. fermentans* and *H'spora valbyensis* were isolated to a lesser extent from sourdough samples. In the present study, *P. membranifaciens* and *P. fermentans* were isolated from wheat and rye sourdough samples, respectively. *Pichia* spp. is rarely isolated from sourdough

fermentations (Paramithiotis et al., 2000; Vernocchi et al., 2004; Vogelmann et al., 2009; Yagmur et al., 2016). The occurence of *P. guiliermondii* in Turkish and Italian sourdoughs has been previously reported (Yagmur et al., 2016; Gaglio et al., 2017). Paramithiotis et al. (2000) isolated *P. membranfaciens* from Greek sourdoughs. Also presence of *P. membranifaciens* and *P. fermentans* species was reported in southern Italian sourdoughs (Succi et al., 2003). Recently, *P. membranifaciens* was also isolated from Chinese sourdoughs (Liu et al., 2018).

In the present study, *H'spora valbyensis* was isolated from sourdoughs for the first time. To my knowledge, the presence of this yeast species in sourdough fermentations has not been documented previously. *H'spora valbyensis* was isolated from industrial-scale Kombucha and cider fermentations (Coton et al., 2015; Coton et al., 2017).

The association of yeasts with LAB is necessary in order to protect the variety of regional specialities as previously reported (Corsetti and Settanni, 2007). De Vuyst et al. (2016) investigated the yeast diversity of sourdoughs and reported the adaptation of sourdough yeasts to the harsh conditions including nutrient starvation, acidic, oxidative, thermal, and osmotic stresses. Yeasts in sourdough fermentations primarily contribute to the leavening and flavor of sourdough products. Besides ethanol and carbon dioxide, some metabolites that affect flavour can be produced by yeast species. In addition, some functional properties such as vitamin production, improvement of the bioavailability of phenolic compounds, the dephosphorylation of phytic acid, the presence of probiotic potential and the inhibition of fungi and their mycotoxin production lead to nutritional and safety advantages.

4.1.4.4. Phylogenetic Relation of the Yeast Strains

Phylogenetic trees were constructed based on the 26S rRNA gene sequences (400 bp≤) of the identified yeast strains using a possible tree reconstruction method, UPMGA, in MEGA 7 (Kumar et al., 2016).

The evolutionary relation of the isolated yeast strans from sourdough samples was inferred using the UPGMA method as shown in Figure 4.27 (Sneath and Sokal, 1973). The bootstrap consensus tree inferred from 200 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (200 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Tamura et al., 2004). The analysis involved 10 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.

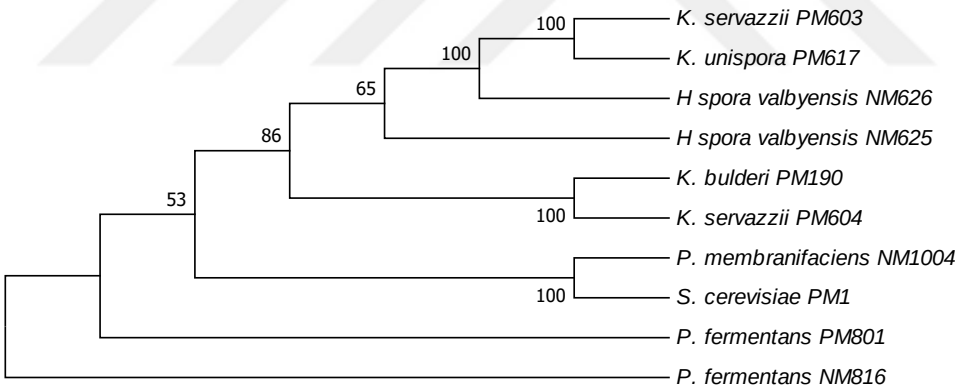

Figure 4. 27. Evolutionary relationships of yeast with UPMGA method

4.2. Chickpea Liquid Starter and Dough Samples

4.2.1. Chemical Characteristics of Chickpea Liquid Starter and Dough Samples

Chickpea liquid starter and dough samples were collected from three different bakeries located in different cities at two different times. The bakeries are

well-known in their regions and have been producing chickpea bread traditionally for many years. Chickpea liquid starter samples were obtained by seperating chickpeas from the fermentation liquid at the end of the fermentation. Chickpea dough samples were collected by taking a piece of leavened dough. Codes were assigned to each collected sample as letters and numbers without expressing the bakery names due to the special request by the bakeries. Chickpea liquid starter samples were coded as CLS-A1, CLS-A2, CLS-B1, CLS-B2, CLS-N1 and CLS-N2 and the chickpea doughs as CD-A1, CD-A2, CD-B1, CD-B2, CD-N1 and CD-N2. Codes were given with a randomly chosen letter for the bakery and number 1 or 2 indicating the first or second sampling. First samples (coded as 1) were collected from bakeries in the spring or at the beginning of summer and the second samples (coded as 2) were collected at the end of the autumn or in winter, resulting in chickpea fermentations with different characteristics.

4.2.1.1. pH

Results of the pH measurements of the 6 chickpea liquid starter samples, including both sampling are shown in Table 4.17.

Table 4.17. pH levels of the chickpea liquid starter samples

Sample	pH	Std. deviation
CLS-A1	5.13[b]	0.00
CLS-A2	4.82[a]	0.00
CLS-B1	5.28[c]	0.01
CLS-B2	5.13[b]	0.02
CLS-N1	5.50[d]	0.01
CLS-N2	5.67[e]	0.02

[a-d]Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

The pH levels of the chickpea liquid starter samples ranged from 4.82 to 5.67. and the mean and median pH values were 5.25 and 5.21, respectively. The lowest pH level was measured in the CL-A2 sample; whereas the highest pH value was measured as 5.67 in the CL-N2 chickpea liquid sample. The pH value of the first sampling of this bakery was the second highest pH as 5.50. There was a wide variation among pH levels of the chickpea liquid samples and sampling times. Testing of homogenity of variances showed that variances ccould be treated as equal ($p>0.05$) and a parametric ANOVA test was conducted. According to the statistical results, the differences between the samples collected from different bakeries were significant ($p<0.05$). The pH differences between samples could be due to the different microflora in the dough samples, different production methods and incubation conditions as the samples were collected from different cities. In addition, every bakery has its own traditional production parameters and these parameters directly affect the pH of the end product, therefore, chickpea liquids produced in different places exhibit different biochemical patterns. On the other hand, differences were observed between some samples collected from the same bakery at two different times.

Results of the pH measurements of the 6 chickpea dough samples, including both samplings are shown in Table 4.18.

Table 4.18. pH levels of the chickpea dough samples

Sample	pH	Std. deviation
CD-A1	5.53^{d}	0.14
CD-A2	5.20^{ab}	0.01
CD-B1	5.12^{a}	0.02
CD-B2	5.32^{bc}	0.12
CD-N1	5.52^{d}	0.07
CD-N2	5.43^{cd}	0.01

$^{a-d}$Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

The pH levels of the chickpea dough samples ranged from 5.12 to 5.53 and the mean and median pH values were 5.35 and 5.41, respectively. The lowest and highest pH levels were measured in the CD-B1 and CD-A1 samples, respectively. Testing of homogenity of variances showed that variances could be treated as equal ($p>0.05$) and a parametric ANOVA test was conducted. According to the statistical results, the differences between the samples collected from different bakeries were significant ($p<0.05$). The pH differences between samples could be due to the same reasons stated above, i.e., different microflora in the dough samples, different production methods and incubation conditions as the samples were collected from different cities. In addition, every bakery has its own traditional production parameters and these parameters directly affect the pH of the end product, therefore, chickpea liquids produced in different places exhibited different biochemical patterns. On the other hand, differences were observed between some samples collected from the same bakery at two different times. Among the chickpea dough samples, only CD-N samples collected at two different times did not show any significant difference between two samplings ($p>0.05$).

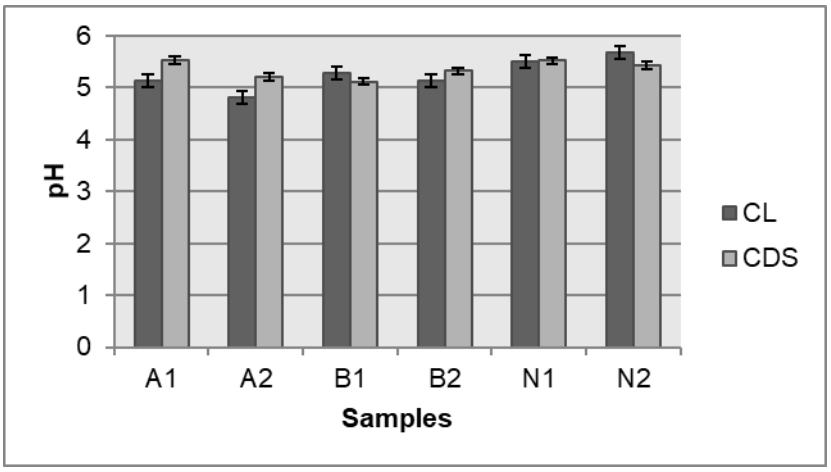

Figure 4.28. pH variation among chickpea liquid starter and dough samples

As it can be seen from Figure 4.25, the pH of chickpea dough starter samples were higher than the chickpea liquid in the A1, A2, B2 and N1 samples. Different production methods and incubation conditions directly affect the pH, therefore, chickpea dough starters produced in differing places exhibited different biochemical patterns. Similar results have been obtained in other studies. Erginkaya et al. (2016) reported higher pH levels in dough than the chickpea liquid starter. In the study of Hatzikamari et al. (2007a), the pH of the fermenting liquid was reported to be 5.35 after 18 h. Çebi (2009) also determined higher pH values in chickpea dough than the chickpea liquid starter. Katsaboxakis and Mallidis (1996), determined pH values at 32, 37 and 42°C to be 4.89, 4.66 and 4.60 at the end of a 30 hour fermentation of the coarsely ground chickpeas in the soak water, respectively. However, 12 and 24 hour fermentations of coarsely ground chickpeas in water resulted in pH values of 6.22 and 5.17, 5.61 and 4.61 and 5. 26 and 4.69 at 32, 37, and 42°C, respectively. As expected, the pH decreased with increasing time and temperature. The samples in this study were fermented for 16-18 hours at higher temperatures inside the bakery and reached lower pH values than reported at 12 hours; however, close pH values determined for 24 hour fermentation.

4.2.1.2. Total Titratable Acidity

Total titratable acidity was given as mL of 0.1 N NaOH consumed and results of the 6 chickpea liquid samples including both sampling are shown in Table 4.19.

Table 4.19. TTA levels of the chickpea liquid samples

Sample	TTA (mL 0.1 N NaOH /10 g sample)	Std. deviation
CLS-A1	2.24[c]	0.03
CLS-A2	3.20[e]	0.00
CLS-B1	1.70[a]	0.00
CLS-B2	1.65[a]	0.10
CLS-N1	2.43[d]	0.06
CLS-N2	2.02[b]	0.03

[a-d]Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

Acidity levels of the collected chickpea liquid samples ranged from 1.65 to 3.20 mL 0.1 N NaOH/10 g sample. In terms of per cent lactic acid, TTA values ranged from 0.15 to 0.29 %. The acidity content was significantly ($p<0.05$) different between chickpea liquid samples. The values also showed differences among samples at different sampling times. Testing of homogenity of variances showed that variances were unequal and the differences between the samples were significant ($p<0.05$). On the other hand, among the chickpea liquid samples, only CLS-B samples collected at two different times did not show any significant difference between two sampling ($p>0.05$).

TTA was given as mL of 0.1 N NaOH consumed and results of the 6 chickpea dough samples including both sampling are shown in Table 4.20.

Table 4.20. TTA levels of the chickpea dough samples

Sample	TTA (mL 0.1 N NaOH /10 g sample)	Std. deviation
CD-A1	3.03[a]	0.15
CD-A2	3.90[b]	0.00
CD-B1	5.40[d]	0.40
CD-B2	4.80[c]	0.00
CD-N1	3.17[a]	0.47
CD-N2	3.58[ab]	0.42

[a-d]Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

The acidity levels of the collected chickpea dough samples ranged from 3.03 to 5.40 mL 0.1 N NaOH/10 g sample. The median acidity level was determined to be 3.73 mL 0.1 N NaOH/10 g sample. In terms of per cent lactic acid, TTA values ranged from 0.29 to 0.49%. The acidity content was significantly ($p<0.05$) different among chickpea dough samples. Testing of homogenity of variances showed that variances were unequal and the differences between the samples were significant ($p<0.05$). The values also showed a significant difference among samples collected on two different sampling times, except CD-N samples, i.e., the CD-N sample did not show any significant difference between the two sampling ($p>0.05$).

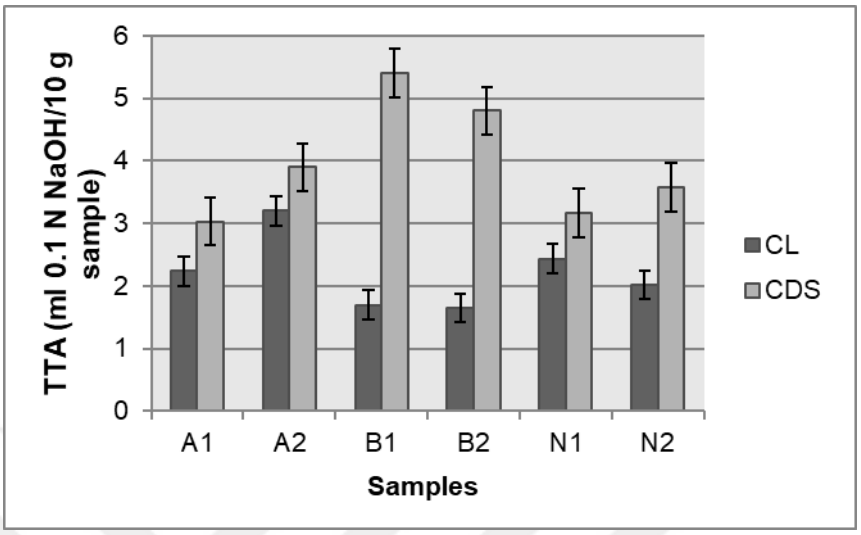

Figure 4.29. TTA variation among chickpea liquid starter and dough samples

In the A and N samples, the acidity values increased at the second sampling. The highest acidity levels of the dough samples were determined in the B sample (Fig 4.29) at both sampling times. Conversely, the lowest acidity among liquid samples was observed in the chickpea liquid samples taken from Bakery B at both sampling times. The production and incubation conditions at that bakery resulted in a chickpea dough with a higher acidity level than the fermented chickpea liquid starter. Hence, it is very difficult to find a correlation between the characteristics of liquid and dough samples collected from different bakeries. However, the samples collected from the same bakeries exhibited the same pattern at both sampling times in terms of pH and acidity. As it is known, acid production is related to the compounds and microbial flora in the sample. The flour used in the production contains varying levels of carbohydrate sources. Therefore, the raw material used in the production and microflora results in dough samples with different acidity level. The bakeries are located in different places and therefore different flours are used depending on the regions. In addition, production methods and incubation conditions can affect the pH and acidity levels in the samples.

Total acidity values of the chickpea liquid starter and dough samples displayed a wide variation. Hatzikamari et al. (2007a) reported the changes in total titratable acidity during a submerged chickpea fermentation liquid, at 37 °C for 18 h, with the final value as 0.34% lactic acid, which concurs with the present study. Another study reported the acidity value of the soak water, including coarsely ground chickpea seeds, as 0.04% at the beginning of the fermentation. The study reported the acidity values at the end of the 30 h fermentation as 0.22, 0.33 and 0.29% at 32, 37 and 42°C, respectively. On the other hand, 12 and 24 h fermentation of coarsely ground chickpeas in water resulted in acidity values of 0.07 and 0.20% at 32 °C, 0.10 and 0.35% at 37°C, and 0.20 and 0.31% at 42°C (Katsaboxakis and Mallidis, 1996). Total acidity values of this study was determined to be in the range of the values found at 37 °C and almost at 42°C, as expected.

The control chickpea liquid starter and dough samples were produced in duplicate under laboratory conditions. Fermentations were conducted at 32 and 37°C. Samples were taken at the beginning (CLS-0h) and end of the fermentation of the chickpea liquid starter (CLS-32 and CLS-37) and dough samples at both temperatures (CD-32-0h, CD-32, CD-37-0h and CD-37). The first samples were taken from the unfermented chickpea liquid immediately after water addition. As it can be seen in Figure 4.30, in the fermented chickpea liquid starters, the pH decreased and TTA increased during 18 hours of fermentation. At the end of the fermentation, the pH level at 32 and 37°C decreased to 4.91 and 4.75, respectively. The total titratable acidity value was higher in the chickpea liquid starter fermented at 37°C compared with 32°C. TTA values were 1.95 and 2.95 mL 0.1 N NaOH/10 g sample fermented at 32 and 37°C, respectively. Following chickpea liquid fermentations, the fermented liquid starter was used in chickpea dough production. Due to the addition of flour, the pH increased and acidity decreased. At the end of 4 hours of fermentation, the final pH values of both fermentations were close to each other as 4.84 at 32°C and 4.81 at 37°C. TTA values were 4.80 and 5.00 mL

0.1 N NaOH/10 g dough sample fermented at 32 and 37°C, respectively. Differences in the pH and TTA values of the chickpea liquid starter and dough samples fermented at two different temperatures were not statistically significant ($p>0.05$).

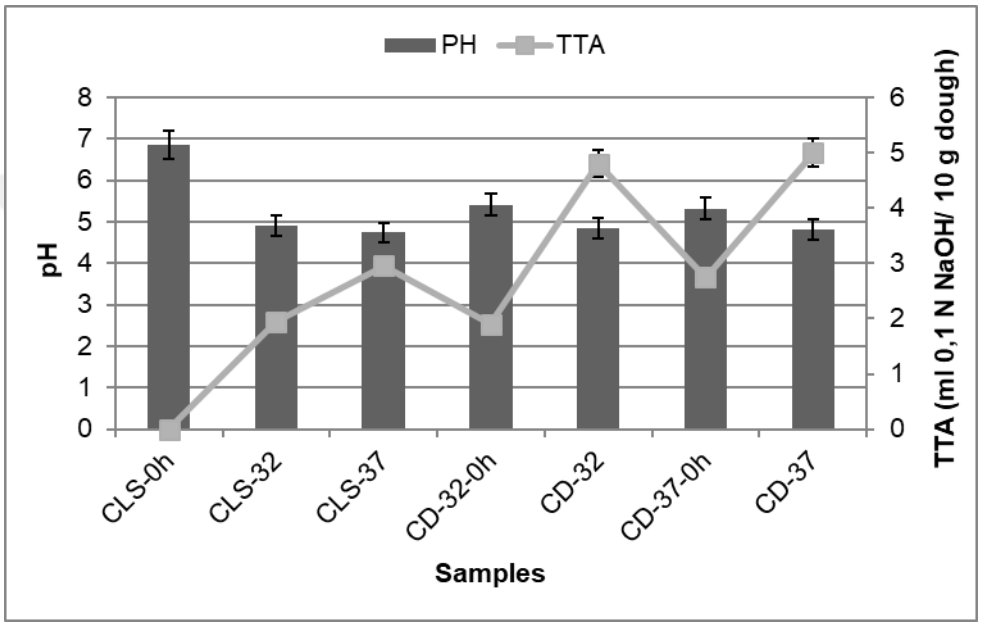

Figure 4. 30. pH and TTA levels of the chickpea liquid starter and dough samples produced under laboratory conditions
(CLS0h: unfermented chickpea liquid, CLS32: chickpea liquid starter fermented at 32°C, CLS37:chickpea liquid starter fermented at 37°C, CD-32-0h:unfermented dough produced with chickpea liquid starter fermented at 32°C, CD-37-0h:unfermented dough produced with chickpea liquid starter fermented at 37°C, CD-32: chickpea dough fermented at 32°C, CD-37: chickpea dough fermented at 37°C).

The pH and TTA values of the collected chickpea liquid starter samples were in the range of 4.82-5.67 and 1.65-3.20 mL 0.1 N NaOH/10 g liquid starter. Acidity values of the laboratory produced samples were in agreement with the collected samples; however, chickpea liquid starters produced under laboratory conditions were characterized by lower pH values. The pH and TTA values of the

collected chickpea dough samples were in the range of 5.12-5.53 and 3.03-4.80 mL 0.1 N NaOH/10 g dough. Under laboratory conditions, chickpea doughs exhibited higher acidity values compared with fermented chickpea liquid starters, which were in agreement with some of the collected samples. Acidity values of chickpea liquid starter and dough samples at 32-37°C were 0.18-0.27 % and 0.43-0.45 %, respectively. Hatzikamari et al. (2007a) reported the TTA for a submerged chickpea fermentation liquid fermented at 37°C for 18 h as 0.34 % lactic acid, which was higher than found in the present study. Katsaboxakis and Mallidis (1996) reported acidity values of the coarsely ground chickpeas after fermentation for 12-24 hours as 0.07-0.20% at 32°C and 0.10-0.35% at 37°C. The chickpea liquid acidity values of this our study were in agreement with that study at both temperatures. The pH values after 12-24 hours fermentation were 6.22-5.17 at 32°C and 5.61-4.61 at 37°C (Katsaboxakis and Mallidis, 1996). In this study, fermentations were conducted for 16–18 hours and the resulting pH values were in consistent with the pH values of the fermentations conducted at 37°C. In another study, the pH of the liquid was reported to be 5.35 after 18 h, which is higher than obtained in this study (Hatzikamari et al., 2007a). On the other hand, Erginkaya et al. (2016) reported the pH value of the unfermented and fermented chickpea liquids as almost 7 and below 5, respectively. The results of the chickpea liquid samples are in agreement with this study, both at the beginning and end of fermentation. However, the final pH value of the chickpea dough after 2 hours fermentation at 37°C was reported to be above 5 in their study In this study, the pH value of the dough was below 5 following 4 hours of fermentation at both temperatures. Increasing the time and temperature leads to a pH decrease and acidity increase, as differences in production methods, fermentation conditions, contents of the raw materials and also temperature of the water used in the production result in different final products with different biochemical patterns.

4.2.1.3. Carbohydrate, Organic Acid and Ethanol Contents of the Chickpea Liquid Starter and Dough Samples

The content of maltose, sucrose, glucose, fructose and ethanol were determined by HPLC with a RID detector and lactic and acetic acid levels were assessed with an UV detector. As reported in the previous section, discrimination of the maltose and sucrose peaks was difficult as their retention times were close to each other. Therefore, the results of these compounds are given in combination as maltose+sucrose. Chromatogram images of chickpea liquid starter and dough samples are shown in Appendix 6 and 7, respectively.

Mean values of the carbohydrate and ethanol content of the collected samples are given in Table 4.21. The mean-median values of maltose+sucrose, glucose, fructose and ethanol contents in chickpea liquid starter samples were 2.71-2.89, 4.60-4.58, 4.09-4.19 and 2.54-2.54 g/kg, respectively. The mean-median values of maltose+sucrose, glucose, fructose and ethanol content in chickpea dough samples were 25.22-24.69, 6.96-6.54, 6.95-7.04 and 2.66-2.67 g/kg, respectively.

Table 4.21. Mean carbohydrate and ethanol content (g/kg) of the chickpea liquid starter and dough samples

Samples	Maltose+sucrose	Glucose	Fructose	Ethanol
CLS-A1	$1.66^{a}\pm0.08$	$3.90^{b}\pm0.49$	$3.26^{b}\pm0.31$	$2.49^{ab}\pm0.06$
CD-A1	$20.38^{c}\pm1.76$	$5.70^{c}\pm1.10$	$4.35^{c}\pm0.65$	$2.81^{e}\pm0.07$
CLS-A2	$1.25^{a}\pm0.17$	$2.59^{a}\pm0.02$	$2.18^{a}\pm0.07$	$2.52^{ab}\pm0.08$
CD-A2	$29.26^{f}\pm0.76$	$7.38^{d}\pm0.60$	$6.56^{e}\pm0.33$	$2.80^{e}\pm0.08$
CLS-B1	$4.50^{b}\pm0.98$	$5.78^{c}\pm0.32$	$5.11^{d}\pm0.01$	$2.58^{bcd}\pm0.07$
CD-B1	$23.89^{de}\pm0.55$	$7.66^{d}\pm0.13$	$7.50^{f}\pm0.16$	$2.45^{a}\pm0.03$
CLS-B2	$3.06^{ab}\pm0.50$	$3.12^{ab}\pm0.53$	$2.42^{a}\pm0.24$	$2.55^{abc}\pm0.04$
CD-B2	$25.49^{e}\pm1.54$	$9.80^{e}\pm1.10$	$6.58^{e}\pm0.29$	$2.59^{bcd}\pm0.09$
CLS-N1	$3.10^{ab}\pm0.40$	$6.94^{d}\pm0.50$	$6.44^{e}\pm0.72$	$2.53^{ab}\pm0.02$
CD-N1	$29.38^{f}\pm1.18$	$5.54^{c}\pm0.27$	$8.44^{g}\pm0.20$	$2.65^{cd}\pm0.05$
CLS-N2	$2.71^{ab}\pm0.63$	$5.26^{c}\pm0.75$	$5.15^{d}\pm0.25$	$2.59^{bcd}\pm0.06$
CD-N2	$22.93^{d}\pm1.58$	$5.68^{c}\pm0.22$	$8.24^{g}\pm0.32$	$2.70^{de}\pm0.05$

Results indicate mean values± SD, Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

The carbohydrate content in the chickpea dough samples was generally determined higher than the chickpea liquid starter samples. In the chickpea liquid starter samples, the content of maltose+sucrose, glucose and fructose ranged from 1.25-4.50, 2.59-6.94 and 2.18-6.44 g/kg, respectively. In chickpea dough samples, the maltose+sucrose, glucose and fructose contents ranged from 20.38-29.38, 5.54-9.80 and 4.35-8.44 g/kg, respectively. Among the chickpea liquid starter samples, the highest maltose+sucrose content was measured in CLS-B1, and glucose and fructose contents were determined in the CLS-N1 sample. The CLS-A2 sample exhibited the lowest carbohydrate content. Among the chickpea dough samples, the highest maltose+sucrose and fructose contents were determined in the CD-N1 sample; however, the lowest glucose content was detected in that sample. The ethanol content of the samples was in the range of 2.45–2.81 g/kg.

The organic acid content of the chickpea liquid starter and dough samples are given in Table 4.22. The mean-median contents in the chickpea liquid starter samples were 0.39-0.25 for lactic acid and 0.99-0.94 g/kg for acetic acid, respectively. The mean-median values of lactic acid content in chickpea dough samples were 0.52-0.68 g/kg. The mean-median acetic acid levels were determined as 3.38-3.55 g/kg. In the samples collected from Bakery N, the lactic acid content was below the LOQ, except for the CD-N2 sample. Furthermore, CLS-A1 and CD-A2 samples contained lactic acid below the LOQ. Acetic acid was determined in all of the liquid samples in the range of 0.86-1.23 g/kg. With the exception of the CLS-B2, acetic acid content was higher than lactic acid in chickpea liquid samples. The levels of the acetic acid in the dough samples were below the LOQ.

Table 4.22. Mean organic acid content (g/kg) of the chickpea liquid starter and dough samples

Samples	Lactic acid	Acetic acid
CLS-A1	<LOQ	1.23^{c}±0.16
CD-A1	0.68^{b}±0.02	<LOQ
CLS-A2	0.90^{cd}±0.00	0.97^{ab}±0.12
CD-A2	<LOQ	<LOQ
CLS-B1	0.50^{a}±0.04	0.86^{a}±0.00
CD-B1	0.68^{b}±0.03	<LOQ
CLS-B2	0.93^{d}±0.01	0.90^{a}±0.01
CD-B2	0.85^{c}±0.15	<LOQ
CLS-N1	<LOQ	1.10^{bc}±0.10
CD-N1	<LOQ	<LOQ
CLS-N2	<LOQ	0.90^{a}±0.02
CD-N2	0.94^{d}±0.94	<LOQ

Results indicate mean values± SD, Different superscript letters within a column indicate a significant difference (Duncan $p<0.05$)

Control chickpea liquid starter and dough samples were produced at 32 and 37°C under laboratory conditions. Samples were taken at the beginning and end of fermentation of the chickpea liquid starter and dough samples at both temperatures. The maltose+sucrose was not detected in the chickpea liquid starter samples (<LOQ). The concentration of maltose+sucrose were 21.80 and 19.18 g/kg in the dough samples fermented at 32 and 37°C, respectively. The glucose and fructose content was higher in the chickpea liquid starter fermented at 32°C than at 37°C. On the other hand, the organic acid content was lower in the chickpea liquid starter fermented at 32°C than at 37°C. Similarly, the lactic acid content was higher in the doughs fermented at 37°C than at 32°C. Acetic acid was not determined in the dough samples. Between chickpea liquid starter samples fermented at two different temperatures, fructose and lactic acid content differences were statistically significant ($p>0.05$). Between chickpea dough samples, only the difference in the lactic acid content was statistically significant ($p>0.05$). Figure 4.31 shows the carbohydrate, ethanol and organic acid contents of the chickpea liquid starter and dough samples produced under laboratory conditions.

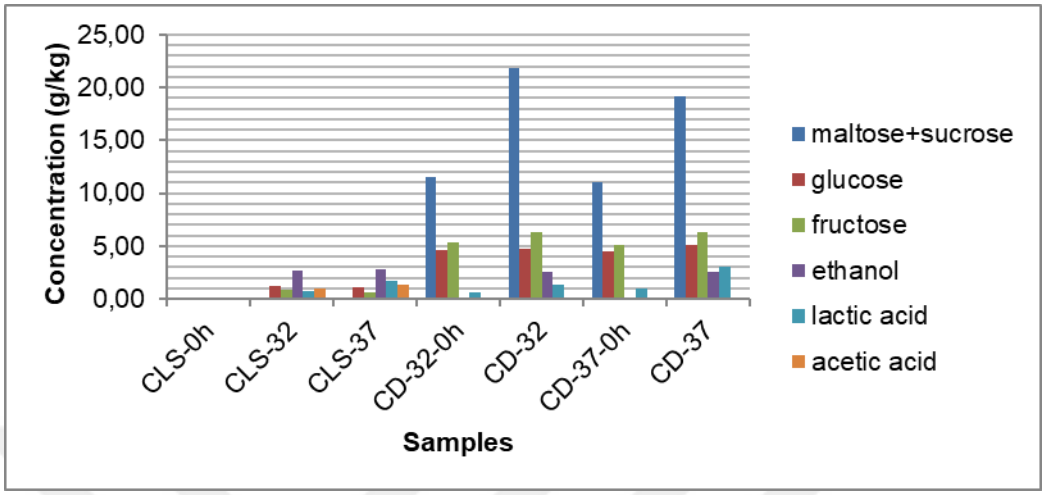

Figure 4.31. Carbohydrate, ethanol and organic acid contents of the chickpea liquid starter and dough samples produced under laboratory conditions
(CLS0h: unfermented chickpea liquid, CLS32: chickpea liquid starter fermented at 32°C, CLS37:chickpea liquid starter fermented at 37°C, CD-32-0h:unfermented dough produced with chickpea liquid starter fermented at 32°C, CD-37-0h:unfermented dough produced with chickpea liquid starter fermented at 37°C, CD-32: chickpea dough fermented at 32°C, CD-37: chickpea dough fermented at 37°C).

In the present study, the total fructose and glucose content was 1.79 g/kg at the end of the 18 h fermentation at 37°C. Hatzikamari et al. (2007a) investigated changes in chemical characteristics during a submerged chickpea fermentation at 37°C for 18 h and reported the reducing sugar content of 1.46 mg glucose/mL at the end of the chickpea liquid fermentation. The results of that study are almost in agreement with the results of the present study. In their study, the amount of reducing sugars gradually increased and then decreased at the end of the fermentation. They also reported the occurence of soluble starch. According to the Hatzikamari et al. (2007a), the increase in reducing sugars could be related to the enzyme activities and then decrease of them can be due to utilization as carbon source (Hatzikamari et al., 2007a). Another study investigated traditional chickpea fermentation in the Aegean region of Turkey and produced chickpea liquids fermenting at 42 °C for 16 hours. Final reducing sugar contents were in the range

of 1.78-2.32 mg/mL and changed according to the chickpea variety and (Kasım, 2014).

In the present study, variations among the carbohydrate concentrations in the collected samples were observed. The carbohydrate content of the chickpea liquids can vary according to the chickpea variety and fermentation time (Kasım, 2014). Samples were collected from different bakeries. The production method is usually the same in a particular bakery, however, differences in the chickpea fermentations conducted in the same bakery at two different times were observed. This could be related to the temperature change of the environment, as chickpea fermentations are conducted inside the bakery at the environmental temperature. Differences among different bakeries could be related to the using a different chickpea variety, the ratio of chickpeas to water, temperature of water, incubation time and temperature. In almost all of the chickpea dough samples, the carbohydrate contents were determined higher than determined in the liquid starters, which is related to the addition of flour during dough production as flour contains carbohydrates. However, the chickpea fermentation liquid also contained starch and carbohydrates, which originated from chickpeas (Kasım, 2014). The level of solubilized starch decreased and reducing sugars increased with the progression of the chickpea fermentation. However, compared with the dough fermentation, the liquid fermentation is conducted over longer periods of time; therefore, carbohydrates can be consumed via enzymatic and microbial activity. It was reported that an 18 h period results in considerable substrate modification in the chickpea liquid (Hatzikamari et al., 2007a).

4.2.2. Microbiological Characteristics of Chickpea Fermentations

Cell densities of LAB, yeasts, total aerobic bacteria and molds in the 12 collected chickpea liquid starter and dough samples are shown in Table 4.23.

Table 4.23. Mean values of cell counts (log CFU/g) of chickpea liquid starter and dough samples on different media

	LAB		Yeasts		Total aerobic		Molds
Sample	mMRS	gM1	YPD	L-lysine	NA	PCA	MXA
CLS-A1	1.60	4.40	5.18	5.00	5.30	5.30	0
CLS-A2	3.15	3.42	0	0	4.78	5.48	0
CLS-B1	5.69	5.58	2.55	2.30	2.20	3.45	1.60
CLS-B2	7.18	7.23	2.76	2.74	7.70	7.11	0
CLS-N1	5.45	5.65	5.85	4.34	5.99	5.45	3.58
CLS-N2	3.00	0	0	0	5.68	5.41	0
CD-A1	5.90	5.76	6.83	3.97	4.04	6.09	<1
CD-A2	4.30	4.60	3.86	3.45	4.78	4.20	2.30
CD-B1	6.86	6.68	4.00	2.62	3.53	5.49	1.78
CD-B2	6.41	7.85	3.73	3.53	7.39	8.08	2.15
CD-N1	5.32	5.51	5.58	3.88	5.30	5.49	4.40
CD-N2	6.89	7.16	2.20	<1	6.64	6.96	2.60

4.2.2.1. Presumptive LAB Cell Counts

Cell counts of presumptive LAB in collected chickpea liquid starters were found to be in the range of 1.60-7.18 log CFU/g on mMRS medium. On the other hand, cell counts of presumptive LAB cell counts on gM17 medium ranged from 0 to 7.23 log CFU/g for chickpea liquid starter samples. The mean, 25th, median, and 75th percentiles of the cell counts were determined to be 4.5, 4.3, 2.65 and 6.81 log CFU/g on mMRS and 4.38, 4.99, 2.56 and 6.04 log CFU/g on gM17. The highest cell densities on mMRS and gM17 agar media were counted in the CLS-B2 sample. In addition, the cell counts of the CLS-B1 sample was high compared with the other samples. The lowest cell density on mMRS agar was 1.60 for the CLS-A1 sample. Bacterial growth was not detected in CLS-N2 sample on gM17 agar.

The mean cell counts of presumptive LAB in collected chickpea dough samples were determined to be in the range of 4.30-6.89 and 4.60-7.85 log CFU/g on mMRS and gM17 media, respectively. The mean, 25th, median, and 75th percentiles of the cell counts were determined as 5.94, 6.15, 5.06 and 6.86 log CFU/g on mMRS and 6.26, 6.22, 5.28 and 7.33 log CFU/g on gM17. The highest

cell densities on mMRS and gM17 agar media were found in the CD-N2 and CD-B2 samples, respectively. Among the chickpea dough samples, the lowest cell densities on mMRS and gM17 media were counted in the CD-A samples at both sampling times. The growth of presumptive LAB from chickpea liquid and dough samples is shown in Figure 4.32.

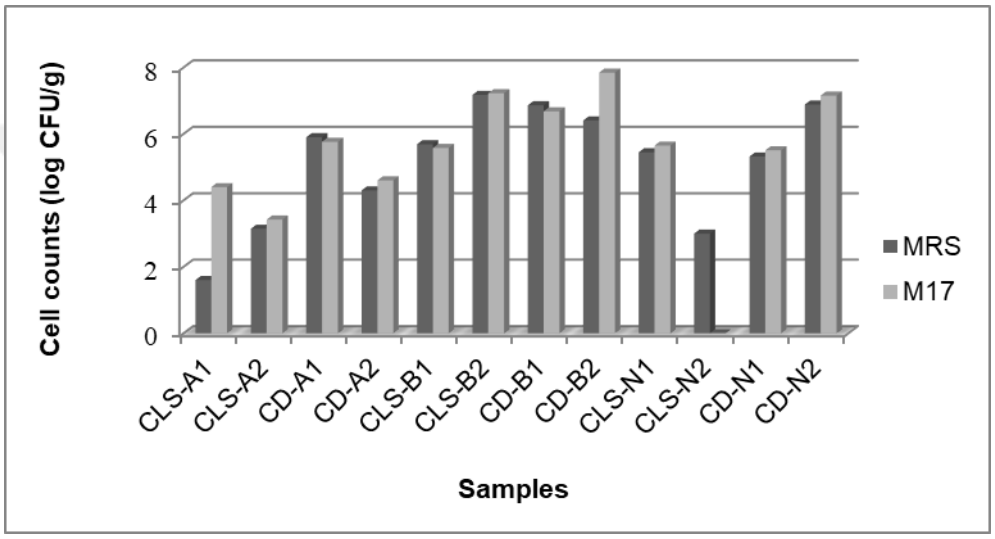

Figure 4.32. Presumptive LAB count of chickpea liquid starter and dough samples on MRS and M17 agar

Çebi (2009) reported the LAB count of chickpea liquids at the end of 16 hours fermentation conducted at 40°C as 6.43 and 6.31 log CFU/g on MRS and M17 agar media, respectively. In the same study, LAB count of chickpea dough samples were reported as 6.76 on MRS and 6.72 log CFU/g on M17 agar media. In their study, a slightly higher bacteria count was observed in the chickpea dough samples compared with chickpea liquid samples. Conversely, another study reported higher LAB counts in chickpea liquid starter fermented at 37°C on MRS agar, compared with chickpea dough fermented at the same temperature. The cell counts of LAB on MRS agar were reported as 8.07 and 5.60 log CFU/g for chickpea liquid starter and dough, respectively (Erginkaya et al., 2016). Hancıoğlu-

Hancıoglu-Sıkılı (2003) reported the LAB counts in the range of 6.85-9.45 log CFU/g for chickpea liquid and 5.32-7.49 log CFU/g for chickpea dough samples collected from the Aegen region in Turkey. Another study reported LAB counts around 7-7.5 log CFU/mL in chickpea liquid starters fermented at 42°C for 16 h (Kasım, 2014).

4.2.2.2. Presumptive Yeast Cell Counts

Presumptive yeast cells were counted on YPD and L-lysine agar media. Cell counts of chickpea liquid samples were in the range of 0-5.85 log CFU/g on two different media. The mean, 25th, median, and 75th percentiles of the cell counts were determined to be 2.72, 2.65, 0 and 5.34 log CFU/g on YPD and 2.40, 2.52, 0 and 4.50 log CFU/g on L-lysine media. The highest cell densities on YPD and L-lysine agar media were detected in the CLS-N1 and CLS-A1 samples, respectively. On the other hand, according to the enumeration results on YPD and L-lysine media, no cells grew on the plates of the second sampling of A and N chickpea liquid samples. The samples of the second sampling were collected in winter and it was observed that bakeries use very hot or boiling water in colder weather. This result is in consistent with the maximum growth temperatures of yeasts. Growth temperatures of yeasts, which varies with species, but in general, many species are unable to grow at a temperature above 35°C (Madan and Thind, 2000).

Presumptive yeast cell counts of chickpea dough samples were in the range of 2.20-6.83 and <1-3.97 log CFU/g on YPD and L-lysine media, respectively. The mean, 25th, median, and 75th percentiles of the cell counts were determined as 4.36, 3.93, 3.35 and 5.90 log CFU/g on YPD and 2.90, 3.50, 1.96 and 3.90 log CFU/g on L-lysine. The highest cell densities on both media were counted in CD-A1 and CD-N2 samples, respectively. The growth of presumptive yeasts in chickpea liquid and dough samples on two different media is shown in Figure 4.33.

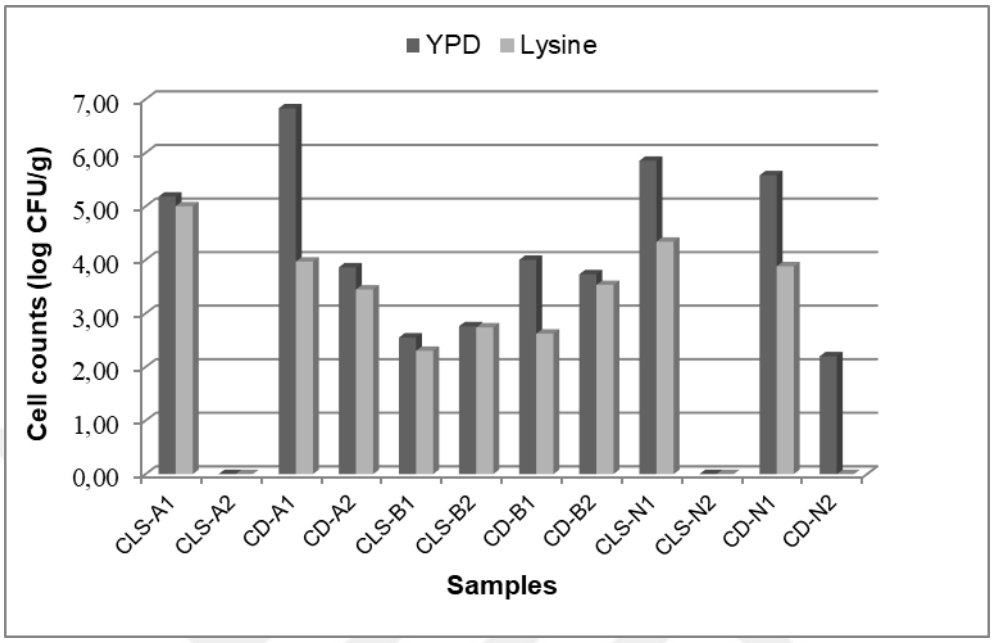

Figure 4.33. Presumptive yeast counts of chickpea liquid starter and dough samples on YPD and L-lysine agar

Erginkaya et al. (2016) reported yeast counts of approximately 4 log CFU/g for chickpea liquid and dough starter samples, which is inconsistent with the mean yeast count of the present study. Another study reported the yeast counts in the range of <1-4.90 and <1-4.23 log CFU/g for chickpea liquid and chickpea dough samples collected from the Aegean region of Turkey, respectively (Hancıoglu-Sıkılı, 2003).

4.2.2.3. Enumeration of other Microorganisms

According to the microbiological analysis results, the total bacteria counted on NA medium was in the range of 2.20-7.70 and 3.53-7.39 log CFU/g for chickpea liquid starter and dough samples, respectively. The mean, median, 25th and 75th percentiles of the cell counts were determined to be 5.28, 5.49, 4.13 and 6.41 log CFU/g for chickpea liquid starter and 5.28, 5.04, 3.91 and 6.82 log CFU/g for chickpea dough. The highest counts of chickpea liquid starter and chickpea

dough samples on NA medium were observed in the B2 sample, reaching values of 7.70 and 7.39 log CFU/g for the chickpea liquid starter and dough, respectively. Conversely, the lowest counts were observed in the first sampling of the B sample in both products, which could be related to the sampling time as *Bacillus* spp. have been reported to increase during chickpea liquid fermentation (Hatzikamari et al., 2007b). In that study, *Bacillus* spp. counts on NA agar increased during the fermentation of a chickpea liquid starter from 1.37 log CFU/mL at the beginning of the fermentation to 7.72 log CFU/mL at the end of fermentaton. During the first 8 hours, the count increased rapidly to 6.46 log CFU/mL and at the end of the fermentation it reached 7.72 log CFU/mL. The count of an adapted dough at the end of fermentation was reported to be 7.18 log CFU/mL (Hatzikamari et al., 2007b). Another study determined *Bacillus* spp. based on the aerobic growth and spore-forming properties. According to the results, aerobic spore-forming bacteria in a chickpea liquid starter and dough were less than 3 log CFU/mL and more than 2 log CFU/mL, respectively (Erginkaya et al., 2016).

In the present study, total mesophilic aerobic bacteria was enumerated on PCA and was in the range of 3.45-7.11 and 4.20-8.08 log CFU/g for chickpea liquid starter and dough samples, respectively. The mean, median, 25th and 75th percentiles of the cell counts were determined to be 5.36, 5.43, 4.83 and 5.89 log CFU/g for chickpea liquid starter and 6.05, 5.79, 5.17 and 7.24 log CFU/g for chickpea dough samples. The highest and lowest cell counts on NA medium were in the CLS-B2 and CLS-B1 samples, respectively. Among the dough samples, CD-B2 showed the highest cell counts on NA medium, whereas the lowest count was found in the CD-A2 sample. Çebi (2009) reported the mesophilic aerobic bacteria count of chickpea liquid starters and doughs on PCA media as 6.14 and 6.81 log CFU/g, respectively (Çebi, 2009). Hatzikamari et al. (2007b) determined the mesophilic aerobic bacteria count on PCA medium to be 7.94 and 7.63 log CFU/mL at the end of the chickpea liquid primary starter and adapted dough starter, respectively. According to Katsaboxakis and Mallidis (1996), regardless of

the incubation temperature of 32, 37 and 42 °C, soak water of ground chickpeas resulted in almost 8 log CFU/mL viable counts of bacteria on PCA medium at the end of the 30 hours. As expected, incubation at higher temperatures caused a significant increase. Between 16-18 hours, the counts on PCA was almost 6 log CFU/ml at 32 °C and more than 8 log cfu/mL at 37 and 42 °C (Katsaboxakis and Mallidis, 1996).

With the exception of the CLS-B1 and CLS-N1 samples, molds were not observed in chickpea liquid starter samples. Conversely, mold count in CLS-B1 and CLS-N1 samples were 1.60 and 3.58 log CFU/g, respectively. Among the chickpea dough samples, the CD-A1 sample count was <1 log CFU/g and the highest mold count was enumerated in the CD-N1 sample (4.40 log CFU/g).

Presumptive coliforms were assessed by growth in LST broth and gas production in the Durham tube. Presumptive total coliform bacteria were detected at <0.3 MPN/g in chickpea liquid samples with the exception of CLS-B2, CLS-N1 and CLS-N2 samples. Among the chickpea doughs, the first sampling of A and B samples were found to be <3 MPN/g, as shown in Table 3. The highest counts were determined in CD-N1 and CD-N2 samples as 1100 and 460 MPN/g, respectively. Development of a red color in upper the layer of the gas positive tubes after the addition of Kovacs' indole reagent was recorded as presumptive *Escherichia coli* (Halkman, 2005). As *Escherichia coli* can break down tryptophan into indole by tryptophanase enzyme resulting in red color due to reaction with *p-dimethylaminobenzaldehyde* contained in the Kovacs reagent (Macfaddin, 2000). *Escherichia coli* is indole-positive culture but for complete identification of the *Escherichia coli* isolates additional biochemical confirmation is needed (Yousef and Carlstrom, 2003). Therefore results are shown as probable *Escherichia coli* in Table 4.24.

Table 4.24. Presumptive total coliform bacteria and indol test in chickpea fermentations

Sample	Presumptive total coliform bacteria (MPN/g)	Indol test/Presumptive *Escherichia coli* (MPN/g)
CLS-A1	<0.3	-
CD-A1	<3	-
CLS-A2	<0.3	-
CD-A2	35	-
CLS-B1	<0.3	-
CD-B1	<3	-
CLS-B2	3	-
CD-B2	9.20	+/3.60
CLS-N1	0.36	-
CD-N1	1100	+/150
CLS-N2	0.36	-
CD-N2	460	+/240

Microbial cell densities were investigated for each group of microorganisms in the laboratory produced chickpea liquid starter and dough samples. Microbiological analysis was conducted at the beginning and end of the liquid and dough fermentations at 32 and 37°C and the cell counts on the mMRS, gM17, YPD, NA and PCA media are shown in Figure 4.34.

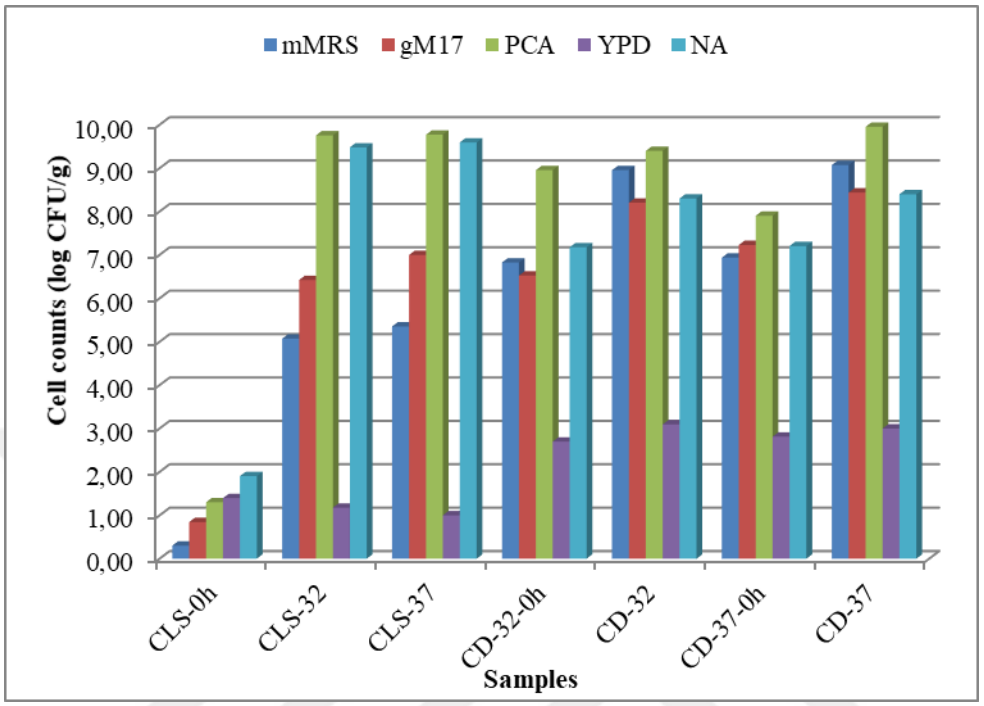

Figure 4.34. Cell counts in the laboratory scale chickpea fermentations (CLS0h: unfermented chickpea liquid, CLS32: chickpea liquid starter fermented at 32°C, CLS37: chickpea liquid starter fermented at 37°C, CD-32-0h:unfermented dough produced with chickpea liquid starter fermented at 32°C, CD-37-0h:unfermented dough produced with chickpea liquid starter fermented at 37°C, CD-32: chickpea dough fermented at 32°C, CD-37: chickpea dough fermented at 37°C).

At the beginning of the chickpea liquid fermentation, presumptive LAB counts on mMRS and gM17 were determined as 0.30 and 0.85 log CFU/g, respectively. At the end of the 18 hour fermentation, mMRS and gM17 counts were 5.70 and 6.42 log CFU/g at 32°C and 5.35 and 7.00 log CFU/g at 37°C. In the chickpea doughs, counts on mMRS and gM17 were determined to be 8.95 and 8.20 log CFU/g at 32°C and 9.07 and 8.44 log CFU/g at 37°C. The total bacteria counts on PCA and NA agar media were very high, both counts were above 9 log CFU/g in the chickpea liquid starter samples. In the chickpea doughs, PCA counts were 9.40 and 9.95 log CFU/g at 32 and 37°C, respectively. Total bacteria count on

NA medium at at 32 and 37°C was determined as 8.30 and 8.40 log CFU/g, respectively. Yeast counts were less compared with the bacteria counts. In chickpea doughs, the final yeast counts were 3.10 and 3.00 log CFU/g at 32 and 37°C, respectively.

Growth was not observed in L-lysine and MXA agar media prepared for non-*Saccharomyces* yeasts and molds, respectively. In the chickpea liquid starter, presumptive total coliform counts were <0.3 MPN/g as no gas bubbles were detected in Durham tubes. In the chickpea doughs, presumptive total coliform bacteria were 15 MPN/g in unfermented dough and 11 and 9.2 MPN/g in the doughs fermented at 32 and 37°C, respectively.

4.2.3. Multivariate Statistical Analysis of the Chickpea Liquid Starter and Dough Samples

The microbiological and chemical parameters of chickpea liquid starter and dough samples were subjected to the multivariate analysis to evaluate the differences/variabilities among the samples. AHC classified the samples in accordance to their mutual dissimilarity and relationship (Figure 4.35). This analysis basically generated two main mega-clusters. As expected, all of the chickpea doughs were gathered together with the control trial. Similarly, all of the chickpea liquid starters were included in another cluster. On the other hand, the CLS-N samples and first sampling of the A and B liquid starters were included in a different class from the second sampling of the A and B liquid starters. Laboratory produced liquid starters were in the same class with them. The chickpea dough group was more homogeneous than the other groups.

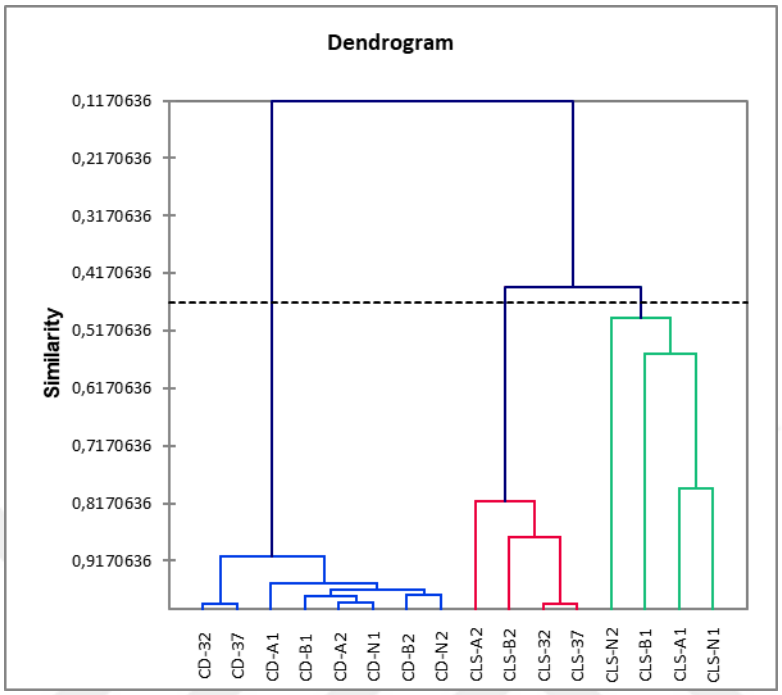

Figure 4.35. Dendrogram resulting from hierarchical cluster analysis on chickpea liquid starter and dough samples

Data of the microbiological and chemical inputs of chcikpea liquid starter and dough samples were also subjected to PCa to express the important information as principal components. Three eigen-values were higher than 1 and correspond to 79.45% of the variance. The eigenvalues and the corresponding factors by descending order with the variability they represent is shown in Figure 4.36.

Factor 1 and Factor 2 explained 36.34 and 33.76 % of variability, respectively. A large part of the variability was taken into account by the first two axes since the percentage of variability represented by these two factors was 70.10% of total variability as shown in Figure 4.36. Correlation matrix (Pearson (n)) of the variables is shown in Table 4.25.

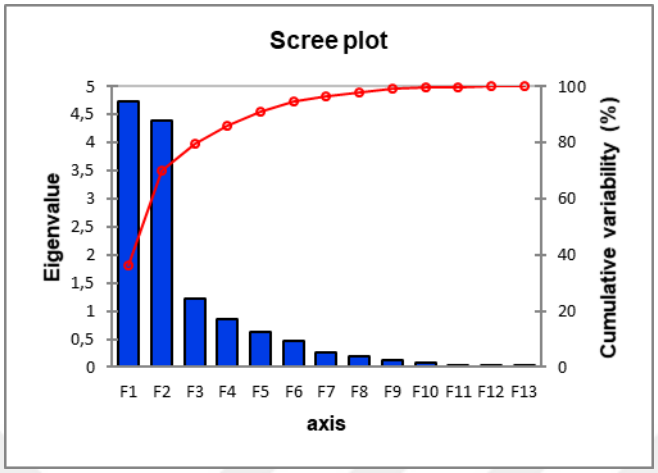

Figure 4.36. The Eigenvalues and the corresponding factors by descending order withl the variability they represent

Correlation circle (Figure 4.37A) composed of two distinct groups with regards to F1. The variables including acetic acid, ethanol, PCA and NA were in the negative correlation compared to others with regards to F1. LAB counts on MRS, M17 and TTA and lactic acid were positively correlated as expected. The pH was negatively correlated with TTA and positively correlated with YPD, fructose and glucose. The score plot (Figure 4.37B) clearly shows the far distance among the samples collected from different bakeries. Chickpea dough samples were seperated from liquid starter samples along with F1. As it can be seen, close relation was found between laboratory produed samples at two different temperatures. Chickpea liquid starter samples were characterized with acetic acid. Laboratory produced doughs were characterized with MRS, M17 counts and also lactic acid contents.

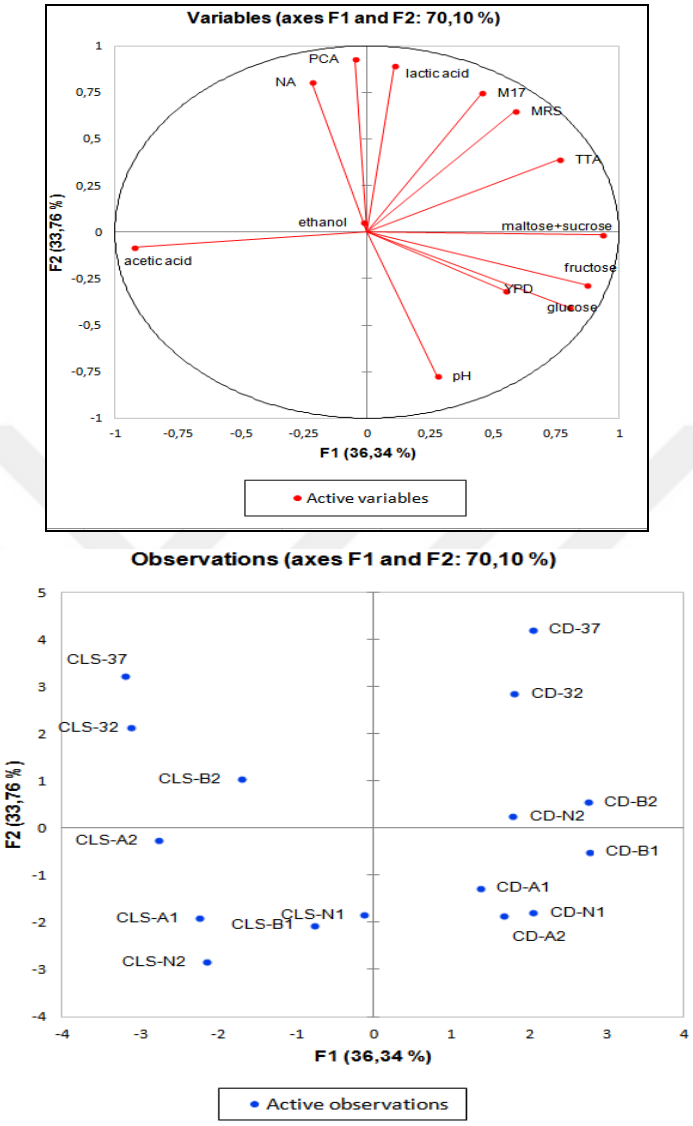

Figure 4.37. Loading plot (A) and score plot (B) resulting from principal component analysis of chickpea fermentations

Table 4.25. Correlation matrix (Pearson (n)) of the variables

Variables	MRS	NA	YPD	M17	PCA	pH	TTA	maltose+ sucrose	glucose	fructose	lactic acid	acetic acid	ethanol
MRS	**1**	0.343	0.121	0.829	0.535	-0.238	0.545	0.449	0.202	0.360	0.667	-0.568	-0.054
NA	0.343	**1**	-0.332	0.461	0.917	-0.501	0.082	-0.176	-0.458	-0.358	0.557	0.147	0.154
YPD	0.121	-0.332	**1**	0.247	-0.270	0.416	0.160	0.467	0.503	0.445	-0.282	-0.378	0.034
M17	0.829	0.461	0.247	**1**	0.627	-0.457	0.503	0.352	0.071	0.142	0.679	-0.393	0.004
PCA	0.535	0.917	-0.270	0.627	**1**	-0.590	0.300	-0.045	-0.394	-0.295	0.757	-0.046	0.114
pH	-0.238	-0.501	0.416	-0.457	-0.590	**1**	-0.268	0.239	0.553	0.512	-0.668	-0.196	0.120
TTA	0.545	0.082	0.160	0.503	0.300	-0.268	**1**	0.737	0.505	0.548	0.449	-0.744	-0.121
maltose+ sucrose	0.449	-0.176	0.467	0.352	-0.045	0.239	0.737	**1**	0.682	0.796	0.041	-0.954	0.193
glucose	0.202	-0.458	0.503	0.071	-0.394	0.553	0.505	0.682	**1**	0.808	-0.261	-0.632	-0.139
fructose	0.360	-0.358	0.445	0.142	-0.295	0.512	0.548	0.796	0.808	**1**	-0.146	-0.756	-0.148
lactic acid	0.667	0.557	-0.282	0.679	0.757	-0.668	0.449	0.041	-0.261	-0.146	**1**	-0.172	0.029
acetic acid	-0.568	0.147	-0.378	-0.393	-0.046	-0.196	-0.744	-0.954	-0.632	-0.756	-0.172	**1**	-0.115
ethanol	-0.054	0.154	0.034	0.004	0.114	0.120	-0.121	0.193	-0.139	-0.148	0.029	-0.115	**1**

4.2.4. Biodiversity of the LAB and Yeasts in Chickpea Fermentations

A total of 395 LAB and 238 yeast isolates were collected from chickpea liquid starter and dough samples, including laboratory scale production. The distribution of all the isolates is shown in Figure 4.38. No yeast was isolated from CLS-N2 and CLS-A2 samples; moreover LAB were not isolated from the CLS-N2 sample.

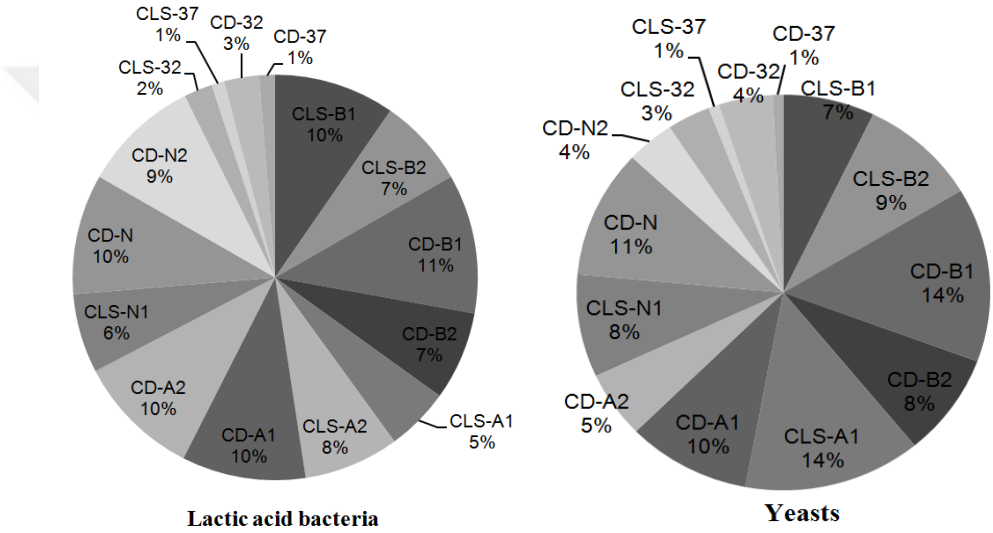

Figure 4.38. Distribution of presumptive LAB and yeasts in the chickpea fermentations

4.2.4.1. LAB Identification

A total of 366 colonies were isolated from the petri dishes of the 12 chickpea liquid starter and chickpea dough samples collected from bakeries. In addition, 29 colonies were collected from the fermentation of the chickpea liquid starter and dough samples produced under laboratory conditions. All of the 395 LAB cultures were subjected to microscopic inspection and Gram-stain and catalase tests. After Gram-stain characterization and catalase testing, 360 strains were still considered putative LAB cultures (Gram-positive and catalase-negative). All of the LAB cultures were grown in the MRS or M17 broth 12-24 hours and

subjected to DNA extraction by using Instagene matrix kit. Then genomic DNA of the isolates were subjected to RAPD analysis using M13 primer. Some strains showed weak band profile and were eliminated for further analysis. Bands were evaluated according to the DNA marker by using the Infinity gel documentation imaging system software. Band patterns of RAPD-PCR profiles of 269 strains were scored as band absent (0) or present (1) and data were entered into a binary matrix. The dissimilarity index was calculated on the basis of the Jaccard coefficient generated with the DARwin (6.0.15) software package. A dendrogram was also constructed based on the genetic distances with the UPGMA method as shown in Figure 4.12. According to the calculated genetic distance matrix, a total of 74 strains were chosen for sequence analysis that had a genetic distance at the level 0.4≤.

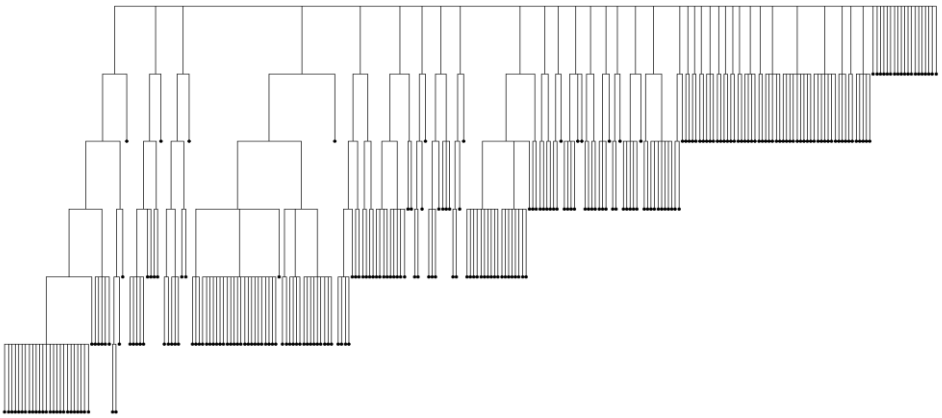

Figure 4.39. Dendrogram obtained from RAPD-PCR(M13) band profiles of LAB isolates in chickpea fermentations

Selected strains were subjected to 16s rRNA gene sequencing analysis. Obtained sequences and their ABI chromatograms were examined with Bioedit Sequence Alignment Editor 7.2.6. (Hall, 1999). The sequences more than 1400 bp were compared by BLAST with nucleotide sequences deposited at the database National Center for Biotechnology Information (NCBI) (Altschul et al., 1990).

Sequences with at least 98% identity to the sequences of the closest relative available within the NCBI database showed strains belonging to the same species. Strains with less than 98 % identity were identified at the genus (94%<) and family (86%<) level (Yarza et al., 2014).

A total of 54 strains representing 149 isolates were confirmed to be members of the LAB group with a sequence length of more than 1250 bp. Based on the 16s rRNA sequence analysis, a total of 35 strains (1400 bp≤) representing 121 isolates were identified at the species level (98%≤). The identified strains along with their accession numbers are given in Table 4.26.

Table 4.26. Identified LAB isolates (sequence length 1400 bp≤) at the species level (98%≤) in chickpea fermentations

Strain	Number of isolates	Species	Similarity % (accession number of closest relative by GenBank)	Sequence length (bp)	Accession number
RL419	1	*W. confusa*	98 (NR_113258.1)	1526	MH704144
RL425	26	*W. confusa*	98 (NR_113258.1)	1461	MH704145
RL453	1	*Pd. acidilactici*	98 (NR_042057.1)	1510	MH704146
RL458	12	*W. cibaria*	98 (NR_036924.1)	1446	MH704147
XL484	5	*E. faecium*	98 (NR_114742.1)	1412	MH704148
BL509	5	*Leu. mesenteroides*	98 (NR_074957.1)	1466	MH704149
BL512	1	*Pd. pentosaceus*	98 (NR_042058.1)	1467	MH704150
BL513	1	*Leu. mesenteroides*	98 (NR_074957.1)	1480	MH704151
RL898	1	*W. confusa*	98 (NR_113258.1)	1476	MH704152
RL899	1	*W. cibaria*	98 (NR_036924.1)	1512	MH704153
RL900	2	*W.confusa*	99 (NR_113258.1)	1523	MH704154
RL902	2	*W.confusa*	98 (NR_113258.1)	1552	MH704155
RL910	8	*W.confusa*	98 (NR_113258.1)	1455	MH704156
RL1139	12	*W.confusa*	98 (NR_113258.1)	1481	MH704157
XL1150	9	*E. faecium*	98 (NR_114742.1)	1413	MH704158
RL1165	1	*Lb. brevis*	98 (NR_116238.1)	1407	MH704159
RL1169	1	*Lb. brevis*	98 (NR_116238.1)	1482	MH704160
BL1171	3	*E. faecium*	98 (NR_114742.1)	1424	MH704161
RL1184	2	*E. faecium*	98 (NR_114742.1)	1477	MH704162
BL1196	2	*Lb. plantarum*	98 (NR_113338.1)	1420	MH704163
RL1220	1	*Pd. acidilactici*	98 (NR_042057.1)	1538	MH704164
RL1223	1	*E. faecium*	98 (NR_114742.1)	1522	MH704165
RL1227	5	*E. faecium*	98 (NR_114742.1)	1580	MH704166
BL1229	1	*E. lactis*	98 (NR_117562.1)	1559	MH704167
BL1233	2	*Lb. brevis*	98 (NR_116238.1)	1489	MH704168
RL1252	1	*W.confusa*	98 (NR_113258.1)	1487	MH704169
RL1253	1	*Leu. mesenteroides* subsp. *dextranium*	98 (NR_040817.1)	1508	MH704170
RL1346	1	*St. lutetiensis*	99 (NR_115719.1)	1496	MH704171
BL1361	1	*W. cibaria*	98 (NR_036924.1)	1540	MH704172
BL1362	1	*St.lutetiensis*	98 (NR_042051.1)	1494	MH704173
RL1386	1	*St.lutetiensis*	98 (NR_042051.1)	1591	MH704174
BL1406	1	*W.confusa*	98 (NR_113258.1)	1533	MH704175
RL1734	4	*E. faecium*	98 (NR_114742.1)	1500	MH704176
XL1742	2	*St. salivarius*	98 (NR_042776.1)	1416	MH704177
XL1747	2	*E. faecium*	98 (NR_114742.1)	1485	MH704178

19 strains representing 28 isolates were identified only at the genus (94%≤) or family (86%≤) level as shown in Table 4.27. Strains sequence length less than 1400 bp also identified as species level despite of the similarity of 98%.

Table 4.27. Identified LAB isolates at the genus (94%≤) or family level (86%≤) in chickpea fermentations

Strain	Number of isolates	Family/Genus	Similarity % (accession number of closest relative by GenBank)	Sequence length (bp)	Accession number
XL486	1	*Enterococcus* spp.	95 (NR_114742.1)	1552	MH704227
XL493	1	*Enterococcus* spp.	96 (NR_114453.1)	1490	MH704228
RL498	1	*Weissella* spp.	95 (NR_113258.1)	1525	MH704229
BL504	1	*Weissella* spp.	96 (NR_113258.1)	1465	MH704230
BL514	1	*Enterococcus* spp.	94 (NR_114453.1)	1458	MH704231
XL880	1	*Enterococcus* spp.	97 (NR_114742.1)	1478	MH704232
XL890	3	*Streptococcaceae*	94 (NR_040956.1)	1424	MH704233
RL1133	1	*Lactobacillaceae*	94 (NR_042057.1)	1453	MH704234
RL1137	1	*Enterococcus* spp.	97 (NR_114742.1)	1477	MH704235
RL1158	3	*Lactobacillus* spp.	96 (NR_114251.1)	1485	MH704236
RL1189	2	*Enterococcaceae*	86 (NR_114742.1)	1369	MH704237
XL1199	1	*Enterococcus* spp.	94 (NR_114742.1)	1562	MH704238
BL1363	1	*Lactobacillus* spp.	95 (NR_117814.1)	1487	MH704239
BL1367	3	*Streptococcus* spp.	95 (NR_115719.1)	1507	MH704240
XL1368	1	*Weissella* spp.	98 (NR_113258.1)	1335	MH704241
XL1377	1	*Streptococcus* spp.	95 (NR_115719.1)	1527	MH704242
RL1387	4	*Streptococcus* spp.	98 (NR_115719.1)	1368	MH704243
XL1400	1	*Streptococcus* spp.	95 (NR_042051.1)	1555	MH704244

In the present study, 121 strains belonging to 12 LAB species were identified at the species level as shown in Table 4.28. *W. confusa* (44.6%) was the dominant species, followed by *E. faecium* (25.6%) and *W. cibaria* (11.6%). Furthermore, *Leu. mesenteroides* (5%), *Lb. brevis* (3.3%) and *Streptococcus lutetiensis* (2.5%) were found as minor species. Conversely, *Lb. plantarum, Pd. acidilactici, St. salivarius, E. lactis, Pd. pentosaceus* and *Leu. mesenteroides* subsp. *dextranium* were only isolated from 1 or 2 samples.

Table 4.28. Percentage of the isolated species in chickpea fermentations

Species	Number of species	%
W. confusa	54	44.6
E. faecium	31	25.6
W. cibaria	14	11.6
Leu. mesenteroides	6	5.0
Lb. brevis	4	3.3
St. lutetiensis	3	2.5
Lb. plantarum	2	1.7
Pd. acidilactici	2	1.7
St. salivarius	2	1.7
E. lactis	1	0.8
Pd. pentosaceus	1	0.8
Leu. mesenteroides subsp. *dextranium*	1	0.8
Total	121	100 %

The number of *W. confusa* was 54 in a total of 121 strains and comprised almost half of the isolates. *W. confusa* was isolated from all collected chickpea liquid starter and dough samples at both sampling times, except the A2 sample. No *W. confusa* strains were isolated from the chickpea liquid starter and dough samples of Bakery A at the second sampling. *E. faecium* was commonly isolated from collected samples, except the B1 and N1 chickpea liquid starter and dough samples. This species was also identified in laboratory-scale chickpea fermentations conducted at 37°C. *W. cibaria* was identified in A and N chickpea dough samples at both sampling times. It was also identified in the CLS-B1, CD-B2 and CLS-B2 samples. Of the other isolated strains, *Leu. mesenteroides* was only identified in the CD-A1 sample. Other LAB species *Lb. brevis*, *St. lutetiensis*, *Lb. plantarum*, *Pd. acidilactici*, *St. salivarius*, *E. lactis* and *Leu. mesenteroides* subsp. *dextranium* were isolated from different bakeries. None of the strains were identified as LAB in the CLS-A1 and CLS-N2 samples. The number of LAB identified at the species level in chickpea liquid starter and dough samples is shown in Table 4.29.

The identified species in the fermentations of Bakery A are detailed in Figure 4.40. No LAB species were detected in the chickpea liquid starters at the first sampling. On the other hand, 5 species were identified in the A1 dough adapted from that liquid starter, and the flour and fermentation environment could be the source of these species. In the CD-A1 sample, *Leu. mesenteroides* and *E. faecium* co-dominated the fermentation. Other identified minor species were *W. cibaria, Pd. pentosaceus* and *W. confusa.* In the second sampling, *E. faecium* dominated the chickpea fermentation. *E. faecium* and *Lb. plantarum* were identified in both chickpea liquid starter and the dough that produced that liquid starter. On the other hand, *Lb. brevis, Pd. acidilactici, E. lactis* and *W. cibaria* were only identified in the chickpea liquid starter and dough at the second sampling of Bakery A, respectively. The chickpea liquid starter contained a more complex LAB flora than dough since only certain species dominated the chickpea dough fermentations. On the other hand, *W. cibaria* was identified in the dough sample despite not being isolated in the chickpea liquid starter; the flour and production equipment could be the source of this species.

Table 4.29. Number of LAB identified at the species level in chickpea liquid starter and dough samples

Species	CD-A1	CLS-A2	CD-A2	CLS-B1	CD-B1	CLS-B2	CD-B2	CLS-N1	CD-N1	CD-N2	CLS-32	CD-32	CLS-37	CD-37
W. confusa	1/15			11/15	15/15	2/13	6/14	1/2	5/8	13/14				
E. faecium	5/15	7/12	3/5			5/13	5/14						3/3	3/3
W. cibaria	2/15		1/5	3/15		3/13	3/14		1/8	1/14				
Leu. mesenteroides	6/15													
Lb. brevis		2/12				2/13								
St.lutetiensis								1/2	2/8					
Lb. plantarum		1/12	1/5											
Pd. acidilactici		1/12		1/15										
St. salivarius											1/1	1/1		
E. lactis		1/12												
Pd. pentosaceus	1/15													
Leu. mesenteroides subsp. *dextranium*						1/13								
Total LAB	15	12	5	15	15	13	14	2	8	14	1	1	3	3

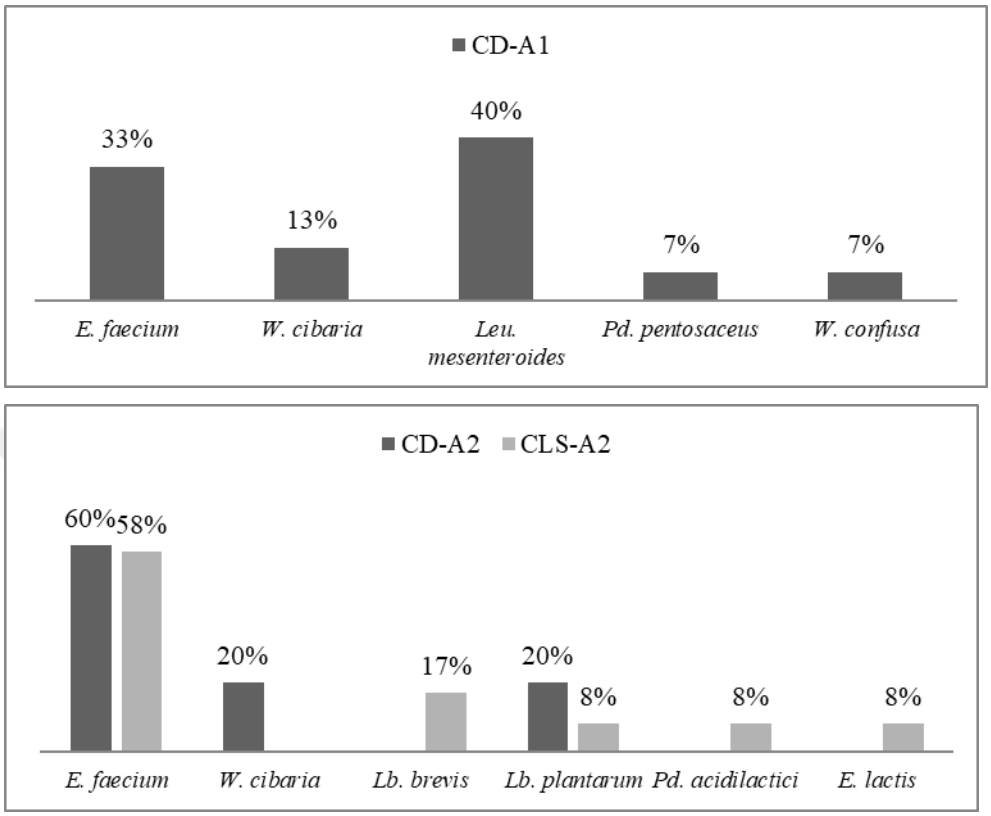

Figure 4. 40. LAB species identified in Bakery A chickpea fermentations

The identified species in the fermentations of the Bakery B are shown in Figure 4.41. *W. confusa* was isolated from all of the samples collected from Bakery B at both sampling times. *W. confusa*, *W. cibaria* and *Pd. acidilactici* were identified in the CLS-B1 sample. However, *W. confusa* alone dominated the chickpea dough fermentation adapted from that starter. The second sampling exhibited a richer diversity than the first sampling, with *E. faecium* and *W. cibaria* dominating the liquid starter fermentation. Furthermore, *W. confusa*, *Lb. brevis* and *Leu. mesenteroides* subsp. *dextranium* were identified as minor species. Certain species continue to dominate the dough fermentation. From the most dominant to

the least, *W. confusa*, *E. faecium* and *W. cibaria* were identified in the chickpea dough of Bakery B at the second sampling.

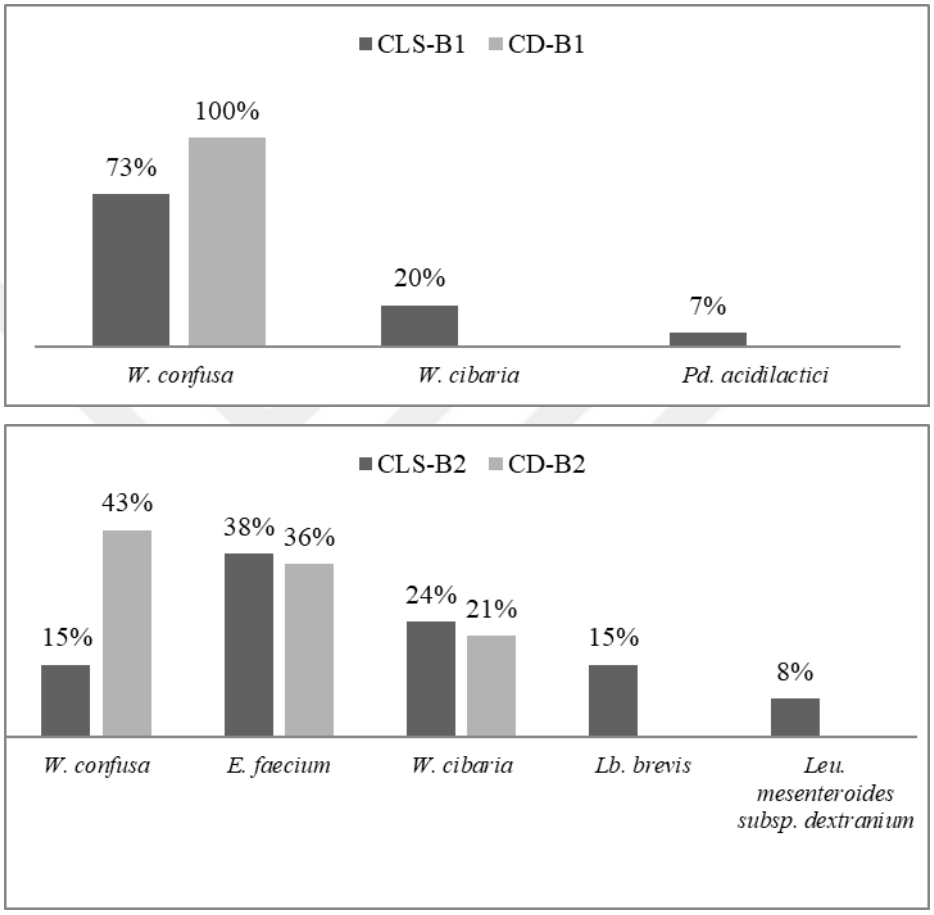

Figure 4.41. LAB species identified in Bakery B chickpea fermentations

The identified species in the fermentations of the Bakery N are given in Figure 4.42. The number of isolated species was very low in the first sampling of the Bakery N to discuss the dominant species. Only two strains belonging to *W. confusa* and *St. lutetiensis* species were isolated from the CLS-N1 sample. The

chickpea dough adapted from that liquid starter contained *W. confusa* as the dominant species. On the other hand, *St. lutetiensis* and *W. cibaria* were identified in the CD-N1 sample. No LAB species were detected in the chickpea liquid starter at the second sampling. Conversely, two species were identified in the N2 dough produced with that liquid starter. The flour and fermentation environment could be the source of these species. *W. confusa* dominated the dough fermentation at the first sampling.

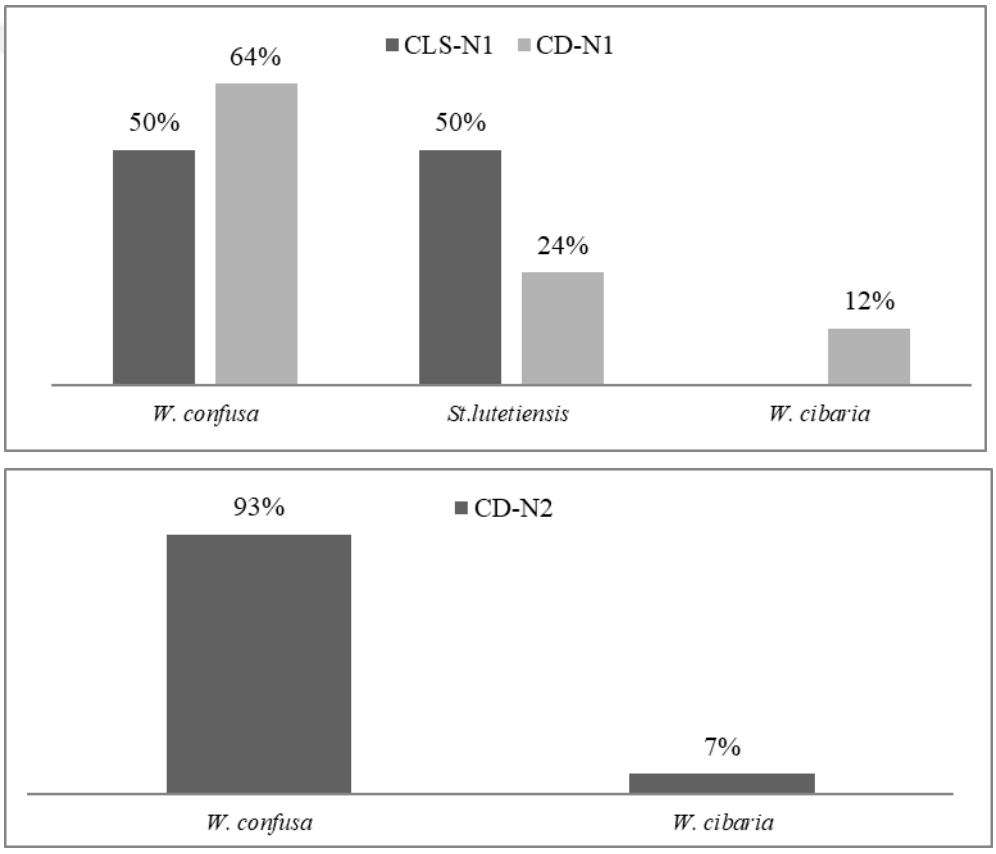

Figure 4.42. LAB species identified in Bakery N chickpea fermentations

Laboratory-scale chickpea fermentations were conducted at 32 and 37°C. A total of 8 LAB strains were isolated from the laboratory-scale chickpea fermentations. *St. salivarius* species was isolated from samples fermented at 32°C and *E. faecium species* was isolated from samples fermented at 37°C. Both species were isolated from the chickpea liquid starter and dough as shown in Figure 4.43. As production was performed under semi-sterile conditions, the flora observed in the liquid starter was seen in the dough producing that starter. However, the number of the isolated strains was too low to discuss the dominant flora.

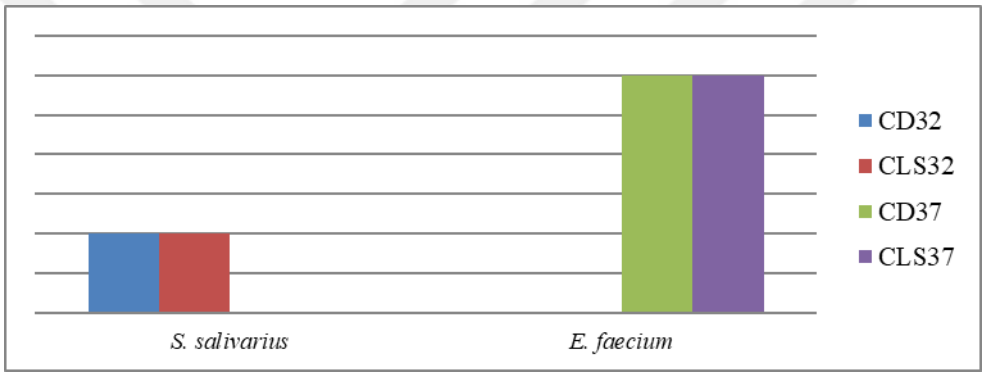

Figure 4.43. LAB species identified in the laboratory scale chickpea fermentations

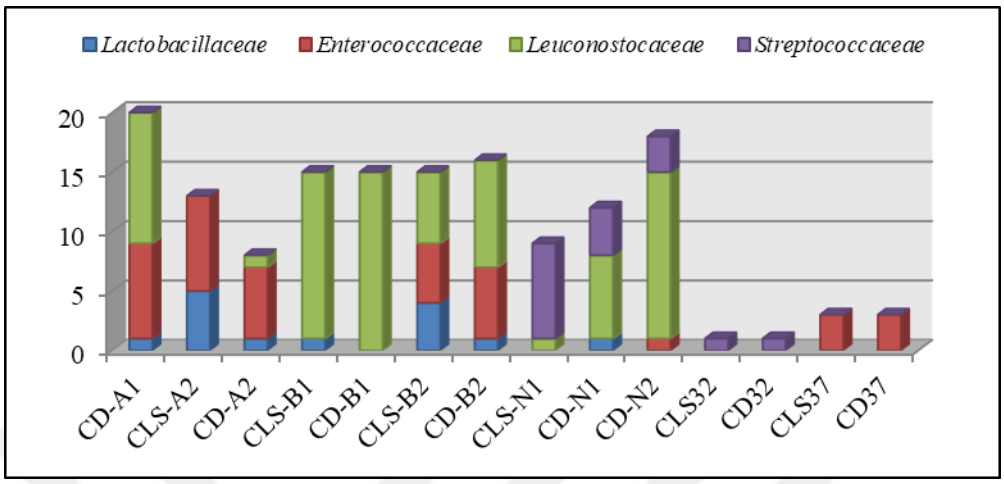

Figure 4. 44. Distribution of the LAB strains in the chickpea fermentations at the family level

LAB detected in the chickpea fermentations at the family level is shown in Figure 4.44. The isolated strains from the chickpea liquid starter and dough samples belonged to four families, i.e., *Lactobacillaceae*, *Enterococcaceae*, *Leuconostocaceae* and *Streptococcaceae*. The distribution as a percentage is shown in Figure 4.45.

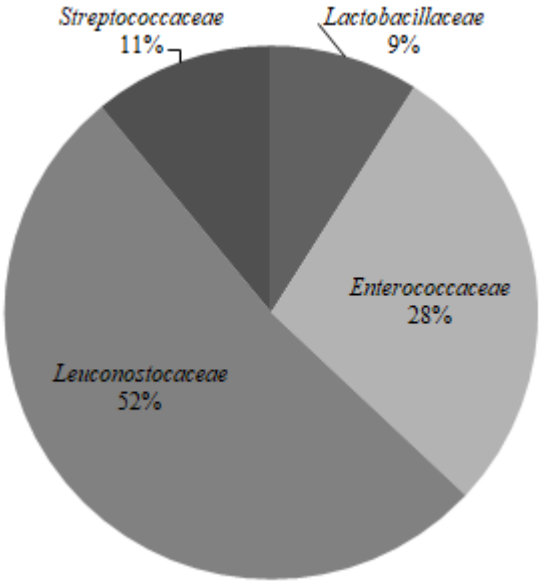

Figure 4.45. Frequency of the LAB strains in the chickpea fermentations at the family level

As it can be seen, many of the isolated strains belonged to the *Leuconostocaceae* family, followed by *Enterococcaceae*. Among 149 isolates, the number of strains in the *Leuconostocaceae, Enterococcaceae, Lactobacillaceae,* and *Streptococcaceae* families were 78, 40, 14 and 17, respectively. The *Leuconostocaceae* family was dominant in the CLS-B1, CD-B1, CD-N1 and CD-N2 samples. On the other hand, it was co-dominant with *Enterococcaceae* in the A and B2 chickpea liquid starter and dough samples.

The distribution of LAB strains detected in the chickpea fermentations at the genus level is shown in Figure 4.46. The isolated strains from sourdough samples belonged to 6 genera, i.e., *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Weissella* and *Streptococcus.* As it can be seen, many of the isolated strains belonged to *Weisella* spp. 143 strains were identified at the genus level and

the number of *Weissella*, *Enterococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus* were 71, 38, 14, 10, 7 and 3, respectively.

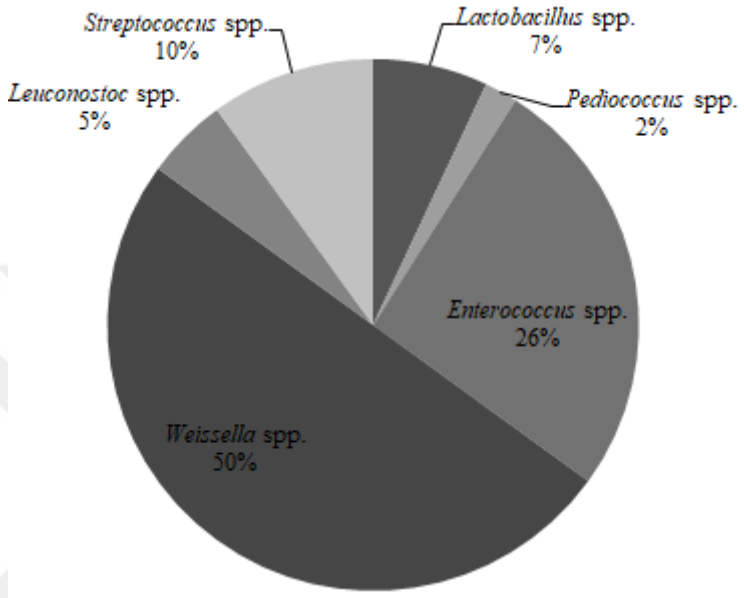

Figure 4.46. Frequency of LAB strains in the chickpea fermentations at the genus level

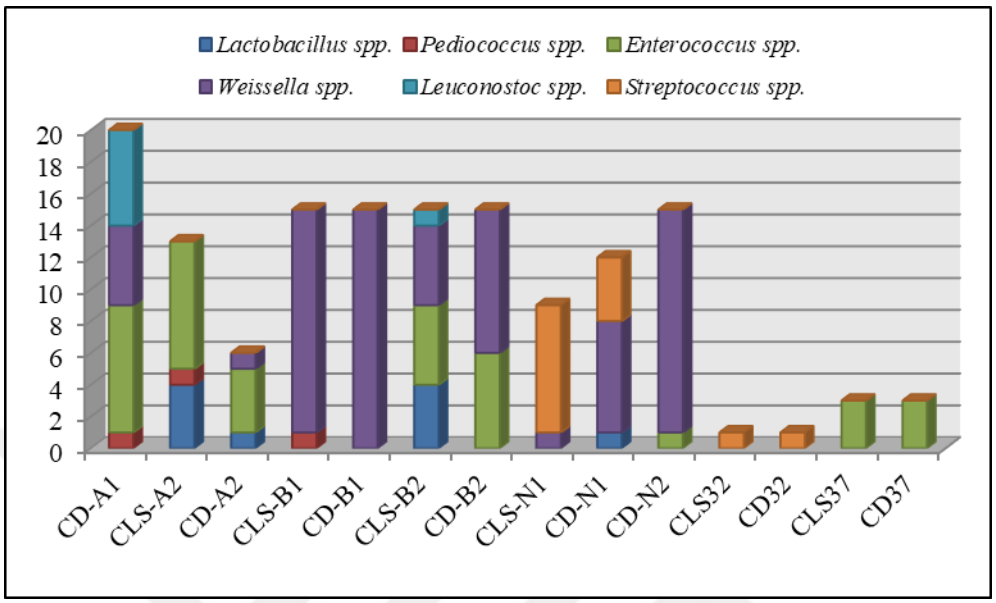

Figure 4.47. Distribution of LAB strains in the chickpea fermentations at the genus level

As it can be seen from Figure 4.47, the chickpea fermentation in Bakery B was dominated by *Weissella* spp. at the first sampling; whereas, *Weissella* spp. and *Enterococcus* spp. were co-dominant in the second sampling. *Enterococcus* spp. was frequently isolated from the chickpea fermentations of Bakery A. In the N Bakery fermentations, *Streptococcus* spp. and *Weissella* spp. were co-dominant; however, *Weissella* spp. was dominant in the second sampling.

In the present study, half of the identified strains belonged to the genus *Weissella* spp. and the most frequenly isolated species was *W. confusa*. The second most frequenly isolated species was *E. faecium.* The microbial patterns of the chickpea liquid starter and dough samples collected from different bakeries were different from each other. The processing parameters and raw materials used during production differed in the different bakeries. The production environment, fermentation conditions, type of chickpea seeds, flour and even temperature of the

water used in the production can affect the microflora of the fermentations; hence, the dominant species differed among different bakeries.

In a study conducted on the LAB flora of chickpea fermentations, *Lc. lactis*, *Lb. brevis* and *Lb. plantarum* were identified, via phenotypic methods, in the chickpea liquid starter. In the chickpea dough, the same species and also *Lb. pentosus* and *W.confusa* were detected (Çebi, 2009). In another study, *E. mundtii/E. gallinarum, E. casseliflavus, Lb. plantarum/pentosus, Lb. sanfrancisco, Lb. viridescens, Lb. bifermantans, Pd. urinea-equi, St. thermophiles* and *Lc. lactis* subsp. *cremoris* were isolated from chickpea fermentations (Hancıoglu-Sıkılı, 2003). The species *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Pediococcus* spp. were previously reported in chickpea-containing fermented foods made in India (Reddy et al., 1982)

In the present study, non-*Lactobacillus* spp. dominated the chickpea fermentations. The chickpea dough is characterized by a higher pH compared with the sourdough. Final pH values of the chickpea liquid starter and dough samples were in the range of 4.82–5.67. *Lactobacillus* spp. are more resistant to acidic conditions than other LAB and are able to grow well at pH values as low as 3-4 (Hammes and Hertel, 2009). Therefore, *Lactobacillus* spp. dominate in an acidic sourdough environment. However, other species that grow at higher pH values are commonly identified in chickpea fermentations. In addition, chickpea fermentations are conducted in a very hot environment compared with sourdough fermentations. The range of pH conditions for *Weissella* spp. growth is 5–7 and they can grow up to 42–45°C (Fusco et al., 2015). *Enterococcus* species can survive temperatures above 60°C for short periods (around 30 min), whereas the optimum temperature is 37°C for *Enterococcus* and *Streptococcus* (Švec and Franz 2014). *Leuconostoc* species are non-acidophilic and the optimal temperature for their growth is in the range of 10–37°C (Pikuta and Hoover, 2014).

4.2.4.2. Phylogenetic Relation of the LAB Strains

Phylogenetic trees were constructed based on the 16S rRNA gene sequences (1400 bp≤) of the identified strains at the species level using two possible tree reconstruction methods, minimum evolution and UPMGA, in MEGA 7 (Kumar et al., 2016).

The evolutionary history inferred using the Minimum Evolution method is shown in Figure 4.48 (Rzhetsky and Nei, 1992). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1 (Nei and Kumar, 2000). The Neighbor-joining algorithm was used to generate the initial tree (Saitou and Nei, 1987). The analysis involved 35 nucleotide sequences.

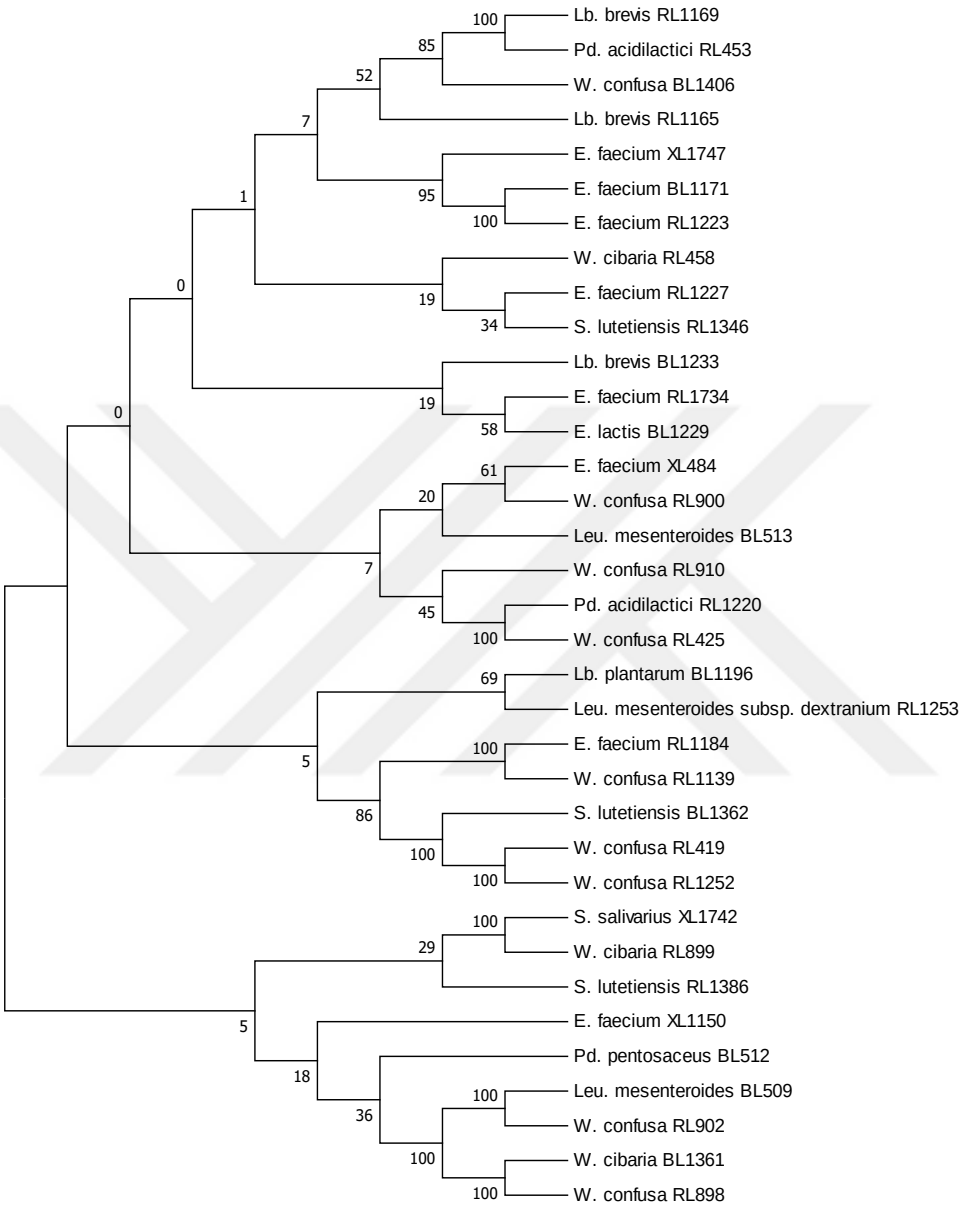

Figure 4.48. The evolutionary history inferred using the Minimum Evolution method

The evolutionary history inferred using the UPGMA method is shown in Figure 4.49 (Sneath and Sokal, 1973). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence (Nei and Kumar, 2000).

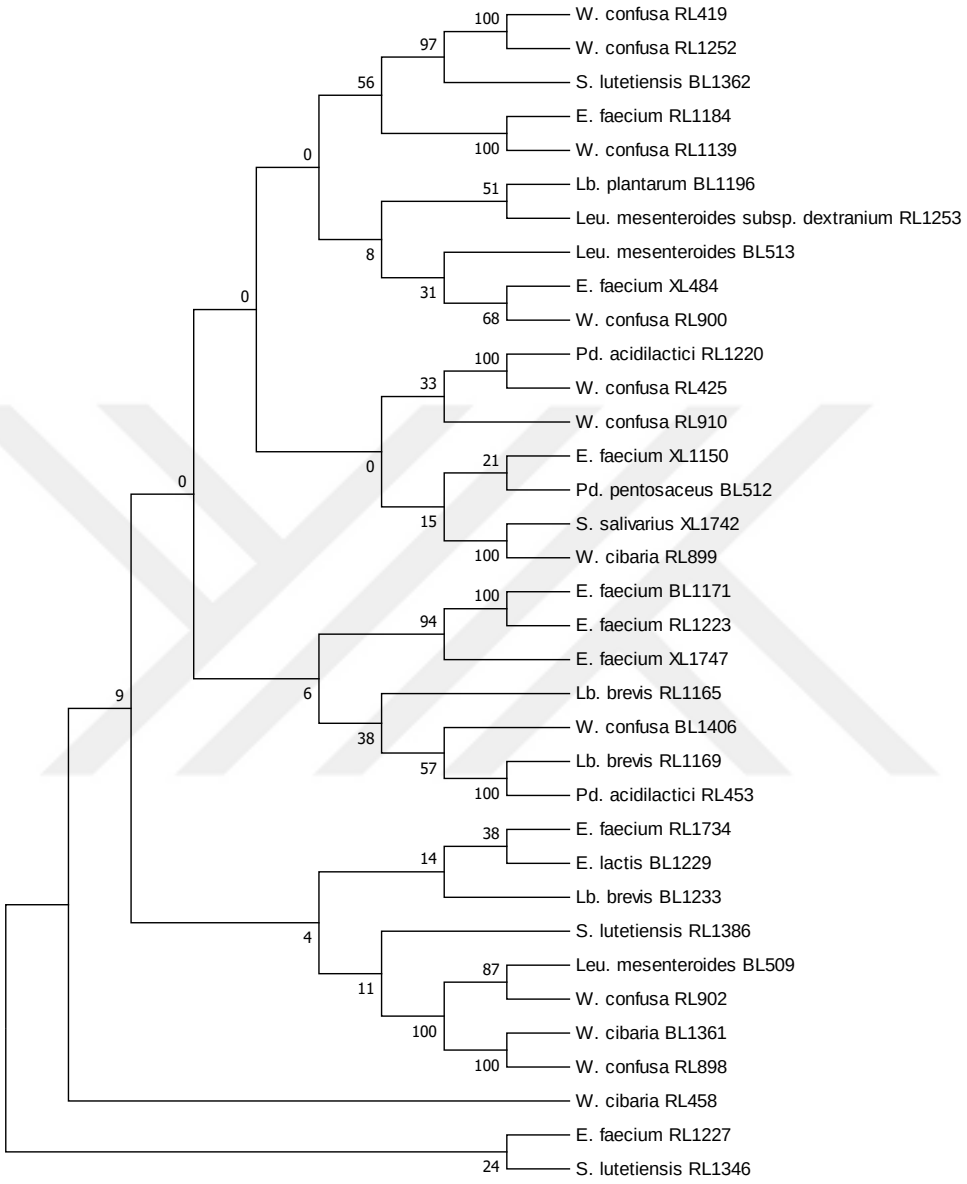

Figure 4.49. The evolutionary history inferred using the UPGMA method

4.2.4.3. Yeast Identification

A total of 216 presumptive yeast colonies were picked from YPD and L-lysine media of the 12 chickpea liquid starter and dough samples collected from bakeries. In addition, 22 colonies were collected from the laboratory-scale chickpea fermentations. All of the 238 presumptive yeast cultures were grown in YPD medium for 24-36 hours and subjected to DNA extraction using Instagene matrix kit. Before extraction, all of the yeasts isolated were treated with lyticase enzyme to degrade the cell walls. Totally 126 genomic DNA were extracted and subjected to 5.8S ITS rRNA region amplification using primers ITS1 and ITS4. PCR products showing visible bands on the agarose gel were subsequently digested using the restriction endonucleases *Hae* III, *Hha* I and *Hinf* I. For a total of 6 profiles were determined according to the restriction fragments as shown in Table 4.30. Strains showed a unique restriction pattern for each species with the three endonucleases used.

Table 4.30. Restriction fragments of the identified yeast species from chickpea fermentations

RFLP Profile	Species	PCR products (bp)	Restriction fragments (bp)		
			Hae III	*Hha* I	*Hinf* I
I	*S. cerevisiae*	880	315+240+180+145	385+365+130	390+130
II	*P. fermentans*	450	340+80+30	170+100+80	250+200
III	*C. parapsilosis*	550	420+115	300+240	270+240
IV	*M. guilliermondii*	625	400+120+50	300+250	320+300
V	*Cr. albidosimilis*	630	500	330+300	350+280
VI	*Wickerhamiella* spp.	420	420	280+200	180

A total of 59 isolates belonging to 5 species were identified by 26S rRNA gene sequencing as shown in Table 4.31. Only one isolate was identified at the genus level as *Wickerhamiella* spp.For a species-level identification identity more than 99% with the sequence length at least 400 bp was selected (Romanelli et al., 2010).

The strains belonged to the 6 genera *Saccharomyces, Candida, Meyerozyma, Pichia, Cryptococcus (Cr.)* and *Wickerhamiella* as shown in Figure 4.50.

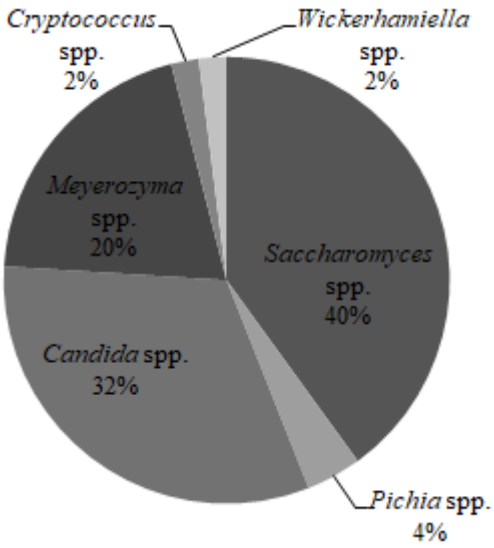

Figure 4.50. Distribution of the 6 genera in the identified yeast isolates

Table 4.31. Accession numbers of the identified yeast species with their closest relatives and type strains

RFLP Profile	Species	N[1]	Strain[2]	Accession number	bp[3]	Closest relative Accession number / Identity(%)[4]	Type strain Accession number/ Identity(%)[5]	Divergent bases[6]
I	*S. cerevisiae*	24	PM 343	MH704189	579	*S. cerevisiae* SFM45 MG017586.1/99	*S. cerevisiae* NRRL Y-12632 NG_042623.1/99	5
II	*P. fermantans*	2	NM 1088	MH704190	537	*P. fermentans* A16 KM589463.1/99	*P.fermentans* NRRL Y-1619 NG_055109.1/99	4
III	*C. parapsilosis*	20	PM 1076 PM 1124	MH704191 MH704192	601 593	*C. parapsilosis* M66 GU080053.1/99	*C. parapsilosis* ATCC 22019 NG_054833.1/100 *C. parapsilosis* ATCC 22019 NG_054833.1/99	0 4

Table 4.31. (Continued)

IV	*M. guilliermondii*	12	NM 1119 NM 322	MH704193 MH704194	569 599	*M. guilliermondii* Y2M MG478478.1/99 *M. guilliermondii* SSA1523 KX791409.1/99	*M. guilliermondii* NRRL Y-2075 NG_042640.1/99 *M. guilliermondii* NRRL Y-2075 NG_042640.1/98	3 5
V	*Cr. albidosimilis*	1	NM 1115	MH704195	622	*Cr. albidosimilis* OF-17 JQ916060.1 /99	*N. albidosimilis* CBS 7711[7] NG_057653.1 /98	12
VI	*Wickerhamiella* spp.	1	PM 331	MH704196	613	*Wi. pararugosa* QWD KF268260.1 /97	*Wi. pararugosa* NRRL Y-17089 NG_055327.1/96	18

[1]Number of species [2]26S rRNA gene sequenced strain representing each RFLP profile, [3]sequence lengh, [4]Sequence identity in the D1/D2 region of isolates with species in the GenBank, [5]Sequence identity in the D1/D2 region of isolates with type strain of the same species in the GenBank, [6]Number of the divergent bases from type strain [7]*Naganishia albidosimilis*, Synonymy ≡*Cryptococcus albidosimilis* -Vishniac & Kurtzman, International Journal of Systematic Bacteriology 42: 550 (1992).

In present study, 59 yeast strains belonging to 5 species were identified as shown in Table 4.32. *S. cerevisiae* (40.7 %) was the dominant yeast species among the identified strains. Other identified yeast species were *P. fermentans* (3.4 %), *C. parapsilosis* (33.9 %), *M. guilliermondii* (20.3 %) and *Cr. albidosimilis* (1.7 %).

Table 4.32. Percentage of the isolated yeast species in chickpea fermentations

Species	Number of the species	%
S. cerevisiae	24	40.7
C. parapsilosis	20	33.9
M. guilliermondii	12	20.3
P. fermentans	2	3.4
Cr. albidosimilis	1	1.7
Total	59	100%

Table 4.33. Number of yeasts identified at the species level in chickpea fermentations

Yeast species	CLS-A1	CD-A1	CD-A2	CLS-B1	CD-B1	CLS-B2	CD-B2	CD-N2
S. cerevisiae	4/4	9/9	4/4		1/3	3/21	2/15	1/1
C. parapsilosis						16/21	4/15	
M. guilliermondii				2/2	2/3	1/21	7/15	
P. fermentans							2/15	
Cr. albidosimilis						1/21		
Total LAB	4	9	4	2	3	21	15	1

In the present study, yeast diversity was less than that of the LAB microbiota, as 5 yeast species were identified in the collected chickpea liquid starter and dough samples. *S. cerevisiae* was the most frequently isolated yeast species. Collected chickpea doughs were produced without using baker's yeast; however, *S. cerevisiae* was isolated from all of the samples including the laboratory-produced sourdough (Table 4.33). The presence of *S. cerevisiae* in the bakery sourdoughs could be related to contamination of the bakery environment and flour. No yeasts were identified in the CLS-A2, CLS-N1, CD-N1 and CLS-N2 samples. In the N Bakery, only one *S. cerevisiae* strain was isolated from the chickpea dough at the second sampling. *S. cerevisiae* was the only identified species from the A Bakery at both sampling times. On the other hand, chickpea fermentations in Bakery B showed a rich biodiversity especially at the second sampling (Figure 4.51). At the first sampling, *M. guilliermondii* was identified from the CLS-B1 sample. Together with *M. guilliermondii*, *S. cerevisiae* was also identified in the chickpea dough sample produced from that liquid. In addition, one strain in the CLS-B1 sample was identified at the genus level and belonged to *Wickerhamiella* spp. Conversely, B2 chickpea liquid starter and dough samples showed a rich biodiversity. *C. parapsilosis* was the most identified yeast strain in the CLS-B2 sample. Minor species identified in that chickpea liquid starter were *S. cerevisiae, M. guilliermondii* and *Cr. albidosimilis*. In the chickpea dough produced from that liquid starter, *M. guilliermondii* was dominant. Furthermore, *C. parapsilosis, S. cerevisiae* and *P. fermentans* were also isolated; whereas, *P. fermentans* was only isolated from the dough sample and the source of this species could be the flour.

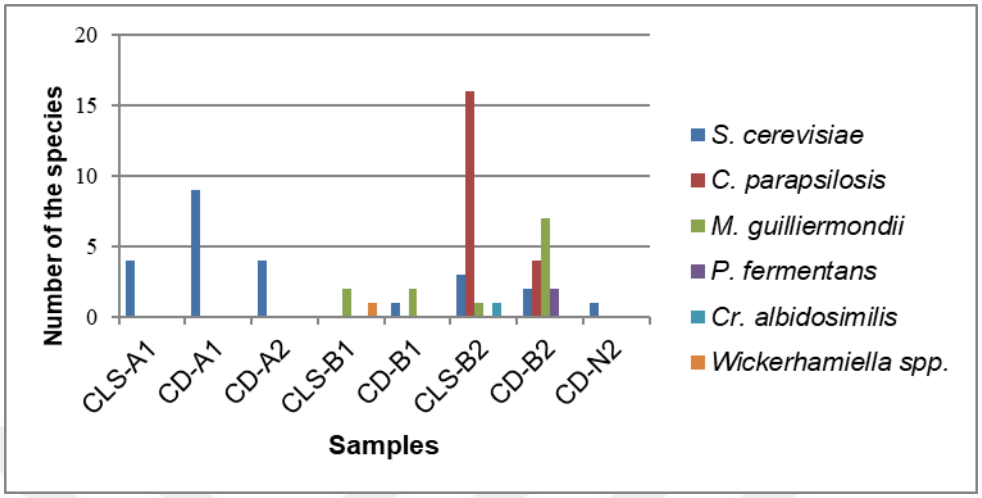

Figure 4.51. Distribution of the yeast species in chickpea fermentations

The collected samples showed different yeast diversity. The differences in bakeries could be related to the location, as samples were collected from different cities. Furthermore, the production environment, fermentation conditions, variety of the chickpeas, flour and even temperature of the water used in the production can affect the microflora of the fermentations, hence the dominant species differed among different bakeries. Since chickpea doughs were produced in different regions, the chickpeas and flour used in the production of these liquid starters and doughs were region specific. In Bakery B, wholemeal flour was used in the production of chickpea dough. Since nutrients in chickpea flour are different to that of white wheat flour, different yeast species can grow. On the other hand, A and N bakeries use boiling water, however, Bakery B uses hot water in the production of the chickpea liquid starter and high water temperatures can prevent the development of the microflora. In addition, no yeasts were identified in the laboratory-scale chickpea fermentations. The temperature of water was 50°C for the production of chickpea liquid; in addition, the fermentation was conducted under semi-sterile conditions. After hot water is added to the seeds, the lid of the

jar was closed; therefore, if the microorganisms were killed by the hot water, no microorganisms from the external environment could reach the liquid starter. Katssboxakis and Mallidis (1996) reported that yeasts were unable to grow during the fermentation of chickpea seeds (Katsaboxakis and Mallidis, 1996). However, to evaluate the relationship of processing parameters with the microflora, every processing parameter should be investigated indivdually both under bakery and laboratory conditions.

There is limited research focusing on yeasts in chickpea fermentations, with only one study reporting *S. cerevisiae* in chickpea fermentations (Hancıoglu-Sıkılı, 2003).

C. parapsilosis was previously isolated from food fermentations of pozol (a Mexican fermented maize dough), chinese steamed wheat buns, sourdough in China and also Turkish sourdoughs (Ulloa et al., 1987; Luangsakul et al., 2009; Zhang et al., 2011; Yagmur et al., 2016) However, this yeast species was recognized as potantially pathogenic fungi (Trofa et al., 2008). *M. guilliermondii* (formerly *P. guilliermondii*) was isolated from Turkish sourdough and Spanish laboratory-made wheat sourdough (Barber and Baguena, 1988; Yagmur et al., 2016; Gordún et al., 2018). Another study reported the presence of *P. fermentans* species in southern Italian sourdoughs (Succi et al., 2003). Cr. *albidosimilis* (synonym *Naganishia albidosimilis*) was only isolated from one chickpea liquid starter. Interestingly, this yeast species was first isolated from soil in Antarctica (Vishniac and Kurtzman, 1992). On the other hand, *Cr. albidosimilis* was identified during the initial stages of the processing of barley (steeping and germination) in an industrial malting facility in Finland (Laitila et al., 2006).

4.2.4.4. Phylogenetic Relation of the Yeast Strains

Phylogenetic trees were constructed based on the 26S rRNA gene sequences (400 bp≤) of the identified yeast strains using a possible tree reconstruction method UPMGA in MEGA 7 (Kumar et al., 2016).

The evolutionary relation of the isolated yeast strans from sourdough samples was inferred using the UPGMA method as shown in Figure 4.52 (Sneath and Sokal, 1973). The bootstrap consensus tree inferred from 200 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (200 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Tamura et al., 2004). The analysis involved 8 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.

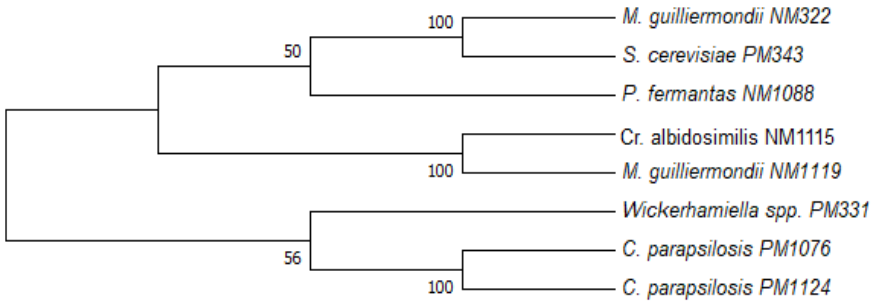

Figure 4.52. Evolutionary relationships of yeast with UPMGA method

4.3. Evaluation of the Technological Attributes of Selected LAB

4.3.1. LAB Strain Selection as Starter Culture in Sourdough and Chickpea Fermentations

The most frequently isolated species were selected for further analysis in sourdough and chickpea fermentations. For that purpose, some strains of *Lb. sanfranciscensis, Lb. plantarum* and *Lb. paralimentarius* were investigated for technological potential to be used as starter culture in sourdough fermentations. Experimental production with starter culture was planned with mono- and dual-culture. *Lb. sanfranciscensis* was the most frequent species in sourdoughs due to the good adaptation of this strain to sourdough environmental conditions. Therefore, strains of *Lb. sanfranciscensis* were chosen and investigated for their technological potential for use as a starter culture in sourdough fermentations. For strain 2, the properties of *Lb. plantarum* and *Lb. paralimentarious* species were compared. Then, depending on their technological properties, strains belong to *Lb. sanfranciscensis* and *Lb. plantarum* or *Lb. paralimentarious* were chosen for experimental sourdough production. In chickpea fermentations, *W. confusa* was the most frequent species and strains of *W. confusa* were investigated for technological potential to be used as starter culture in chickpea fermentations. Strains investigated for technological evaluation in sourdough and chickpea fermentations are shown in Table 4.34.

Table 4.34. Strains investigated for technological evaluation in sourdough and chickpea fermetations

Strain	Species/Family/Genus	Isolation source
RL658	*Lb. sanfranciscensis*	SD-K1
RL976	*Lb. sanfranciscensis*	SD-T2
BL631	*Lb. sanfranciscensis*	SD-K1
RL986	*Lb. sanfranciscensis*	SD-T2
BL1023	*Lb. sanfranciscensis*	SD-K2
RL1046	*Lb. plantarum*	SD-K2
XL24	*Lb. plantarum*	SD-M1
XL23	*Lb. plantarum*	SD-M1
RL749	*Lb. plantarum*	SD-W2
RL233	*Lb. paralimentarius*	SD-T1
RL17	*Lb. paralimentarius*	SD-M1
RL982	*Lb. paralimentarius*	SD-T2
BL740	*Lb. paralimentarius*	SD-W2
RL1639	*Lb. paralimentarius*	SD-L7
RL1628	*Lb. paralimentarius*	SD-L4
RL425	*W.confusa*	CD-B1
RL1139	*W.confusa*	CD-B2
RL898	*W.confusa*	CD-N2
RL910	*W.confusa*	CD-N2
RL1252	*W.confusa*	CLS-B2
BL1406	*W.confusa*	CLS-N1

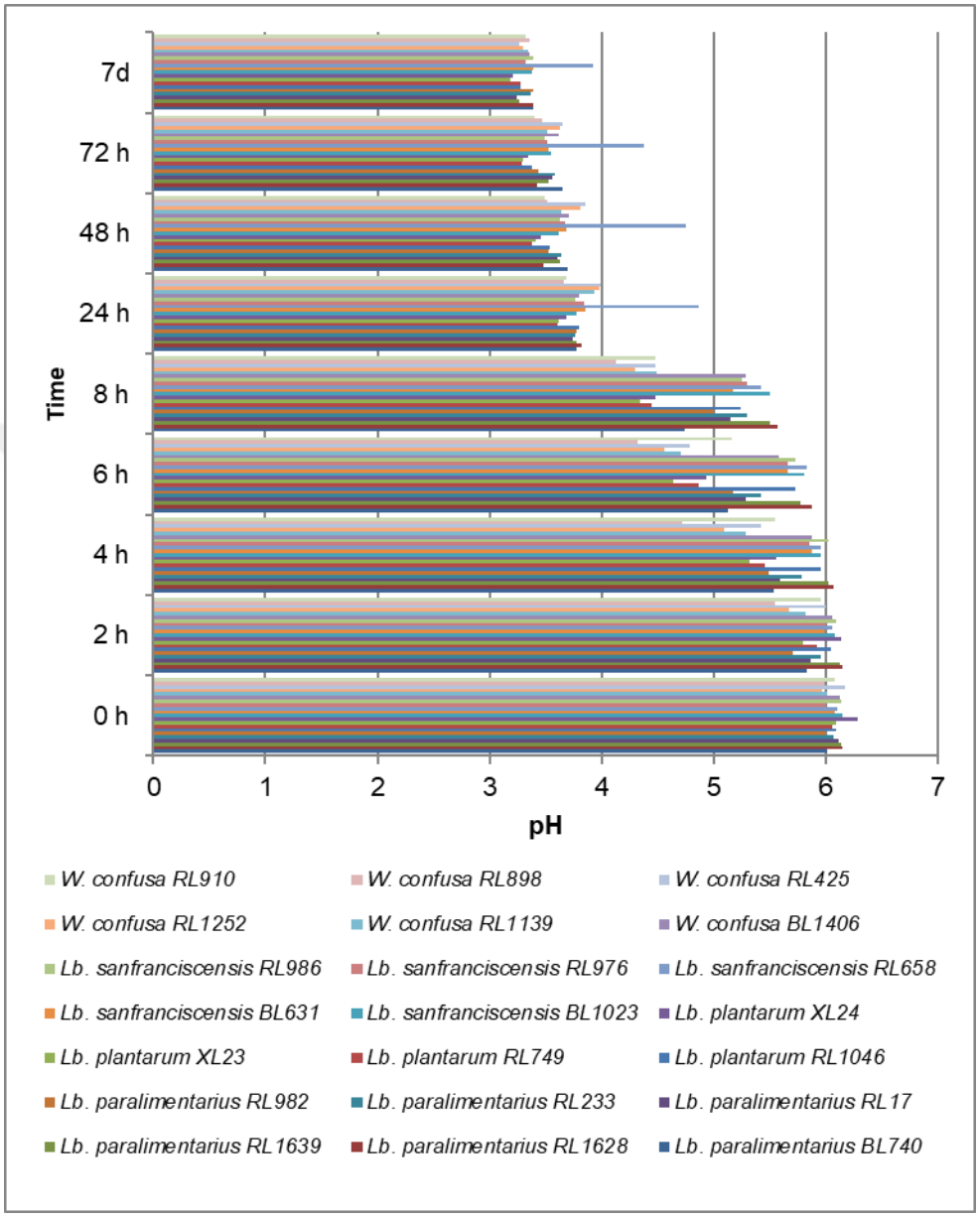

Figure 4.53. Acidification kinetics of SFE by the 21 strains

To investigate acidification activity, LAB cultures were inoculated into the SFE. The results of the acidification kinetics of SFE by the 21 strains are shown in

Figure 4.53 and Table 4.35. 10 LAB strains (*W. confusa* RL898, RL1252, RL1139, RL425, RL910; *Lb. plantarum* XL23, RL749, XL24; *Lb. paralimen*tarius BL740, RL982) were able to decrease the pH below 5.0 after 8 h. At 24 h, almost all of the strains acidified the medium to below pH 4.0. After 3 days, the lowest pH values were measured in the SFEs inoculated with *Lb. plantarum* species. *Lb. plantarum* XL23 showed the lowest pH value at the 7th day. Among the *Lb. sanfranciscensis* strains, RL976 exhibited the lowest acidity values.

As reported previously, the 11 LAB strains belonging to different species were able to decrease the SFE pH below 5.0 after 6 h and almost all of the strains acidified the medium to below pH 4.0 after 24 hours (Alfonzo et al., 2013).

Table 4.35. Acidification kinetics of SFE by LAB strains

	0 h	2 h	4 h	6 h	8 h	24 h	48 h	72 h	7d	Lactic acid (mM)	Acetic acid (mM)	FQ
Lb. paralimentarius BL740	6.0	5.83	5.54	5.13	4.74	3.77	3.70	3.65	3.40	8.00	11.13	0.72
Lb. paralimentarius RL1628	6.1	6.15	6.07	5.87	5.57	3.82	3.48	3.42	3.39	5.21	12.06	0.43
Lb. paralimentarius RL1639	6.1	6.12	6.02	5.77	5.50	3.78	3.63	3.53	3.26	5.42	13.25	0.41
Lb. paralimentarius RL17	6.1	5.87	5.59	5.28	5.15	3.74	3.60	3.57	3.25	7.26	11.63	0.62
Lb. paralimentarius RL233	6.0	5.96	5.78	5.42	5.30	3.76	3.64	3.58	3.37	7.35	11.38	0.65
Lb. paralimentarius RL982	6.0	5.70	5.49	5.17	5.00	3.78	3.53	3.44	3.39	7.16	11.36	0.63
Lb. plantarum RL1046	6.0	6.05	5.96	5.73	5.25	3.80	3.54	3.38	3.28	8.44	12.75	0.66
Lb. plantarum RL749	6.0	5.92	5.46	4.87	4.44	3.61	3.38	3.29	3.28	8.44	12.75	0.66
Lb. plantarum XL23	6.0	5.79	5.32	4.64	4.34	3.62	3.41	3.30	3.18	12.81	18.57	0.69
Lb. plantarum XL24	6.2	6.14	5.56	4.93	4.48	3.68	3.46	3.35	3.21	8.85	10.29	0.86
Lb. sanfranciscensis BL1023	6.1	6.08	5.96	5.81	5.50	3.77	3.62	3.55	3.38	7.53	17.68	0.43
Lb. sanfranciscensis BL631	6.0	5.99	5.88	5.66	5.18	3.85	3.69	3.53	3.39	6.38	11.07	0.58
Lb. sanfranciscensis RL658	6.1	6.06	5.95	5.83	5.42	4.87	4.75	4.38	3.93	11.33	12.80	0.88
Lb. sanfranciscensis RL976	6.0	6.00	5.85	5.66	5.30	3.84	3.67	3.51	3.33	7.36	10.75	0.68
Lb. sanfranciscensis RL986	6.1	6.09	6.02	5.73	5.25	3.76	3.63	3.49	3.40	8.68	17.64	0.49
W. confusa BL1406	6.1	6.06	5.87	5.58	5.29	3.80	3.71	3.62	3.36	7.26	17.39	0.42
W. confusa RL1139	6.0	5.82	5.28	4.71	4.49	3.94	3.65	3.51	3.34	10.14	26.45	0.38
W. confusa RL1252	5.9	5.67	5.09	4.56	4.30	3.98	3.81	3.63	3,30	9.04	18.26	0.50
W. confusa RL425	6,1	6,00	5,42	4.79	4.48	3.99	3.86	3.65	3,26	7.79	17.63	0.44
W. confusa RL898	5.9	5.55	4.72	4.32	4.13	3.66	3.52	3.47	3.36	8.78	14.03	0.63
W. confusa RL910	6.0	5.95	5.55	5.16	4.48	3.68	3.49	3.40	3.33	7.23	12.20	0.59

After 8 hours of fermentation, acidified SFE samples were analysed for their lactic and acetic acid content. The highest lactic acid was detected in the acidified SFE inoculated with *Lb plantarum* XL23. The lactic acid content of acidified SFE was determined to be in the range of 5.21-12.81 mM, with the lowest amount corresponding to *Lb. paralimentarius* RL1628 and the highest to *Lb plantarum* XL23. Acetic acid production was very high among strains. In terms of mass per mass, the content of lactic and acetic acids were in the range of 0.47–1.15 and 0.62–1.58 mg/g, respectively. In the present study, all of the investigated strains were heterofermentative. The acetic acid levels were also higher than those of previously reported studies, which could be related to the composition of the flour extract. In the present study, the supernantant of the flour extract was in the semi-solid form, therefore the dry matter composition could be higher than the SFE taken as liquid supernatant. Alfonzo et al. (2013) reported the highest acetic acid content as 0.11 mg/g in the SFE inocukated with a *Weisella* spp. Settanni et al. (2013) reported the lactic and acetic acid contents produced by different LAB strains in sourdoughs processed with non sterile flour in the range of 1.36-6.47 and 0.15-1.08 mg/g after 8 h of fermentation, respectively. In another study, experimental sourdoughs were produced by inoculating *Lb. plantarum* and *Lb. sanfranciscensis* and lactic and acetic acid contents of the inoculated sourdoughs were reported in the range of 1.48-4.19 and 0.33-1.05 mg/g after 8 hours fermentation, respectively (Ventimiglia et al., 2015).

In the present study, according to the acidification activity results, *Lb. paralimentarious* exhibited less acidification compared with the *Lb. plantarum* species. Therefore, *Lb. plantarum* strains were further investigated for technological potential and *Lb. paralimentarious* species were eliminated. Furthermore, it has been reported previously that, *Lb. plantarum* could be an ideal starter culture for Type I sourdoughs (Minervini et al., 2010).

Total EPS yields were determined gravimetrically. In addition, the colonies were investigated for EPS formation on agar media as mucoid colonies. Among the strains, only three *W. confusa* strains, RL1139, RL425 and RL1252, showed EPS production. Figure 4.54 shows the growth of colonies that produced EPS on agar media supplemented with sucrose (50 g/L). The EPS yield was determined as 0.00236, 0.00204 and 0.00173 g/mL for *W. confusa* RL1139, RL1252 and RL425, respectively. Dextran production from sucrose by some *W. confusa* strains has been reported previously (Collins et al., 1993; Katina et al., 2009; Björkroth et al., 2014). A study reported the significant production (11–16 g/kg DW) of polymeric dextran in wheat sourdoughs *by W. confusa* strains (Katina et al., 2009). Another study determined produced EPS in wheat broth media by *W. confusa* as 0.43 g/100 ml (Lim et al., 2018).

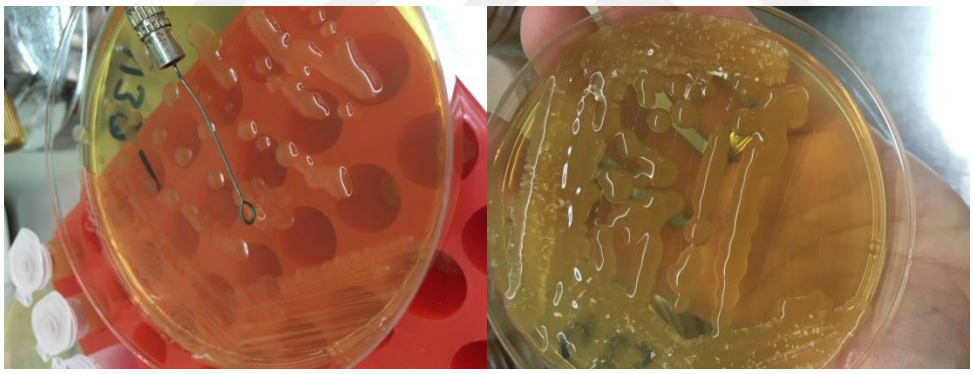

Figure 4.54. EPS production on agar media

The proteolytic activity of the strains was tested using MRS agar media supplemented with skimmed milk powder. With the exception of *Lb. sanfranciscensis* RL658, zone formation was observed by all the strains. Different strains of *Lb. sanfranciscensis* and *Lb. plantarum* have *previously* been reported to exhibit proteolytic activity during sourdough fermentation (Gobbetti et al., 1994;

Gobbetti et al., 1996a; Rollán et al., 2005). However, proteolytic activity depends on the strain and should be characterized at the strain level. In a study, the proteolytic system of *Lb. sanfranciscensis* strain DSM 20451 was characterized based on a genome-sampling approach (Vermeulen et al., 2005). In addition, by adaptation to the protein environment, proteolytic activity can be increased. A higher capacity of the *Lb. plantarum* strain, isolated from pickles, previously showed better adaptation to protein-enriched medium than other LAB species (Güler and Özcelik, 2017).

4.3.2. Investigaton of the Some Properties of the Selected LAB Strains

In the present study, sourdough strains were selected based on the acidification capability. Acid production capacity of *Lb. plantarum* XL23 was very high among *Lb. plantarum* species. After 8 hours, pH of the acidified flour extract was the lowest in the *Lb. plantarum* XL23. Also according to the final pH values at the 7th day, the lowest pH was determined in the same strain among *Lb. plantarum* strains. After 8 hours, pH values were close to each other among *Lb. sanfranciscensis* strains. On the other hand, final pH values at the 7th day was determined in the acidified flour extract inoculated with *Lb. sanfranciscensis* RL976 among other *Lb. sanfranciscensis* species. On the other hand, the FQ values were investigated for strain selection for *Lb. sanfranciscensis* and RL976 and RL658 were close to optimum value. Also, lactic acid production by *Lb. sanfranciscensis* RL658 was the highest. However, this strain exhibited a very slow acidification process and proteolytic activity was not detected. Therefore, *Lb. sanfranciscensis* RL976 was chosen for experimental sourdough production. *W. confusa* strains were selected based on the EPS production and less acidification activity during 8 hours. Because chickpea doughs exhibited higher pH values compared to sourdough fermentations. After 8 hours, *W. confusa* RL1139 and

BL1406 showed the highest pH determined in the fermented flour extract. Therefore *W. confusa* RL1139 was used as the starter culture in the experimental chickpea fermentations as the strain both producing EPS and showing less acidification.

Antimicrobial activities, growth under different conditions and enzyme profiles of the three selected strains for sourdough and chickpea fermentations were investigated.

In the present study, antimicrobial activities of three strains selected as starters were determined against *B. subtilis, B. lincheniformis*, *Escherichia coli*, *Penicillium expansum* and *Penicillium digitatum* using the dual culture overlay technique. The LAB strain *Lb. plantarum* XL23 showed inhibitory activity against *B. subtilis, B. lincheniformis*, *Escherichia coli* and *Penicillium expansum. Lb. sanfranciscensis* RL976 showed inhibitory activity only against *B. subtilis.* On the other hand, *W. confusa* RL1139 did not exhibit any antimicrobial activity.

It was reported that *Lb. plantarum* 21B showed a very broad spectrum of activity and inhibited many fungus species including *Penicillium* spp. (Lavermicocca et al., 2000). Corsetti et al. (1996) investigated the *Lactobacillus* spp. isolated from sourdoughs and reported all the strains were inhibitory to *B. subtilis* and among the strains *Lb. sanfranciscensis* and *Lb. plantarum* strains had the largest inhibitory spectrum. However, the inhibitory spectrum among strains of the same species varied.

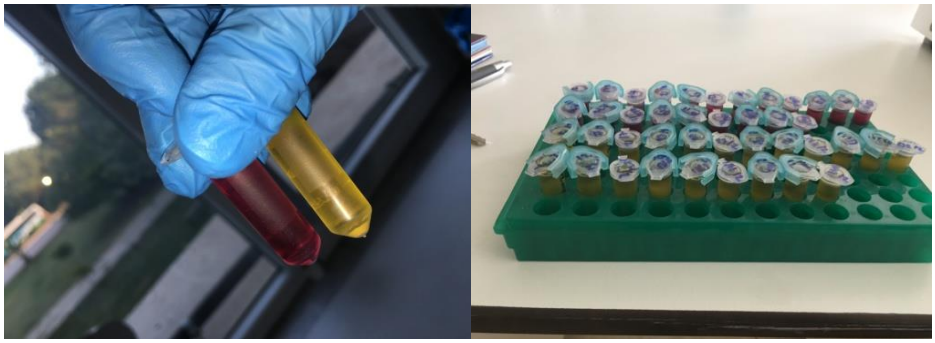

Figure 4.55. Acid production and color change with different carbohydrate sources

Growth of the selected strains at 15, 28, 37 and 45°C was investigated. *Lb. plantarum* XL23 and *Lb. sanfranciscensis* RL976 strains did not grow at 45°C, whereas *W. confusa* RL1139 strain grown at all temperatures. Growth at 45°C generally varies among strains but many of the *Lactobacillus* spp. do not grow at that temperature (Pot et al., 2014). Among *W. confusa* strains, growth at 45°C is strain dependent with some strains showing good growth at this temperature (Collins et al., 1993). *Lb. plantarum* XL23 tolerated all the conditions. On the other hand, *Lb. sanfranciscensis* RL976 did not grow in the presence of 8% NaCl and at pH 3.5. As reported previously, *Lb. sanfranciscensis* growth was inhibited at pH 4.0 (Brandt et al., 2004). *W. confusa* RL1139 did not grow in the presence of 6 and 8% NaCl and at pH 3.5. Acid was produced from glucose, fructose, sucrose, maltose and mannose in all strains. The color of the tubes changed from red to yellow as a result of low pH caused by acid production (Figure 4.55). Consumption of other sugars changed according to the strain. Raffinose and xylose were only used by *Lb. plantarum* XL23 and *W. confusa* RL1139, respectively. Acid production from xylose, but not from arabinose, lactose, and raffinose was reported for *W. confusa* strains previously (Fusco et al., 2015). None of the investigated strains used ramnose and arabinose as carbohydrate sources. Growth of the selected strains under different conditions are shown in Table 4.36.

Table 4.36. Growth of the selected strains under different conditions

Growth conditions	*Lb. plantarum* XL23	*Lb. sanfranciscensis* RL976	*W. confusa* RL1139
15°C	+	+	+
28°C	+	+	+
37°C	+	+	+
45°C	-	-	+
%4 NaCl	+	+	+
%6 NaCl	+	+	-
%8 NaCl	+	-	-
pH 3.5	+	-	-
pH 4.5	+	+	+
pH 6.5	+	+	+
Glucose	+	+	+
Fructose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Galactose	+	+	-
Lactose	+	+	-
Mannose	+	+	+
Mannitol	+	+	-
Raffinose	+	-	-
Xylose	-	-	+
Ramnose	-	-	-
Arabinose	-	-	-

The enzyme profile of the selected strains was investigated using the API ZYM enzyme testing system. An image of the color changes in the wells resulting from enzyme activity is shown in Figure 4.56. Enzyme pattern results are given in Table 4.37. *Lb. plantarum* XL23 produces enzymes as follows: leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bi-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase. Similar enzyme profiles of other *Lb. plantarum* strains have been previously reported (Park and Lim, 2015; Mikelsaar et al., 2016). *Lb. sanfranciscensis* RL976 produces enzymes as follows: leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bi-phosphohydrolase, α-glucosidase,

β-glucosidase and N-acetyl-β- glucosaminidase. In a study, positive enzyme activities were reported as only leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase in API ZYM kit (Hoang et al., 2015). *W. confusa* RL1139 produces alkaline phosphatase, acid phosphatase and naphthol-AS-Bi-phosphohydrolase.

Figure 4.56. Investigation of the enzyme activities by API ZYM kit

Table 4.37. Enzyme activities of the strains

Enzyme	Code	*Lb. plantarum* XL23	*Lb. sanfranciscensis* RL976	*W. confusa* RL1139
Control	1	-	-	-
Alkaline phosphatase	2	-	-	+
Esterase	3	-	-	-
Esterase Lipase	4	-	-	-
Lipase	5	-	-	-
Leucine arylamidase	6	+	+	-
Valine arylamidase	7	+	+	-
Cystine arylamidase	8	-	-	-
Trypsin	9	-	-	-
α-chymotrypsin	10	-	-	-
Acid phosphatase	11	+	+	+
Naphthol-AS-Bi-phosphohydrolase	12	+	+	+
α -galactosidase	13	-	-	-
β -galactosidase	14	+	-	-
β -glucuronidase	15	-	-	-
α -glucosidase	16	+	+	-
β - glucosidase	17	+	+	-
N-acetyl- β-glucosaminidase	18	+	+	-
α -mannosidase	19	-	-	-
α -fucosidase	20	-	-	-

4.3.3. Production of Experimental Sourdoughs and Evaluation of Chemical and Microbiological Properties

Based on the technological screening, *Lb. plantarum* XL23 and *Lb. sanfranciscensis* RL976 were selected to act as starter for experimental sourdough production. Experimental sourdoughs were produced using selected strains. Overnight LAB cultures with an optical density (OD) of ca.1.00, corresponds to an approximate concentration of 10^9 CFU/g, was used as inocula at the concentration 1 % (Settanni et al., 2013). The final concentration of the inoculum for each strain was approximately 10^6 CFU/g in dough. *Lb. sanfranciscensis* 976 and *Lb.*

plantarum XL23 strains were used indivually and as dual-culture in the production of experimental sourdoughs as shown below:

-Sourdough C (SD-C)-Control
-Sourdough 1 (SD-1)-*Lb. plantarum* XL23
-Sourdough 2 (SD-2)-*Lb. sanfranciscensis* RL976
-Sourdough 3 (SD-3)-*Lb. plantarum* XL23+*Lb. sanfranciscensis* RL976

Sourdough fermentations were conducted at 28 °C for 3 days with daily refreshment. Some microbiological and chemical properties of the produced sourdoughs were investigated and compared with the control sourdough. The pH and TTA values registered for the experimental sourdoughs are shown in Figure 4.57.

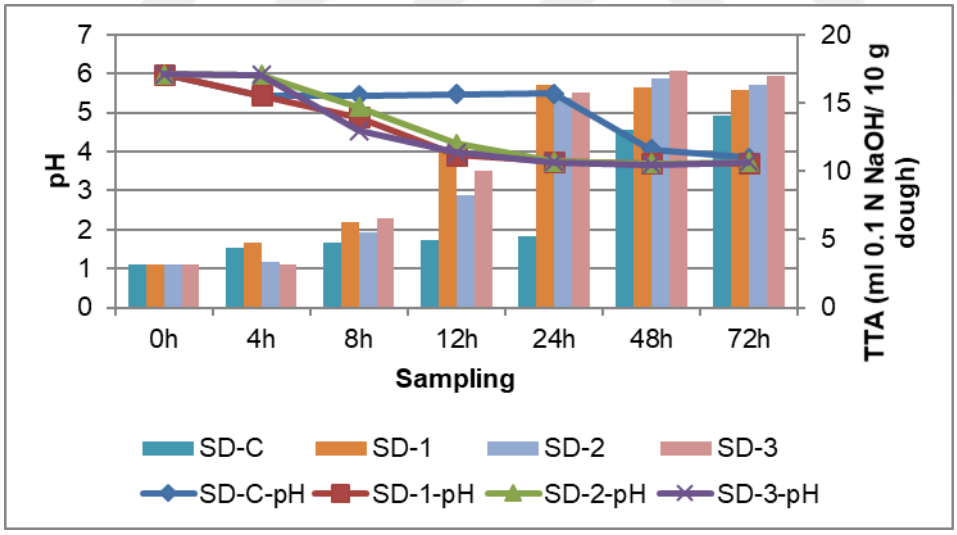

Figure 4.57. pH and TTA values of the experimental sourdoughs

The initial pH and TTA of the dough were 5.98 and 3.2 mL, respectively. Doughs inoculated with mono- or dual-culture of *Lb. plantarum* XL23 reached the pH values less than 4.0 in 12 hours. Dough inoculated with *Lb. sanfranciscensis* RL976 reached pH values less than 4.0 after 24 hours. At 24 hours, all of the inoculated sourdoughs reached pH values around 3.75 and were stable until the last refreshment. Conversely, in the control sourdough the pH decreased very slowly and reached similar pH values with the inoculated sourdoughs after 48 h. pH of the control sourdough was 3.85 at the last refreshment.

After 12 hours, the TTA of the inoculated sourdoughs was determined to be in the range of 8.25-11.75 mL 0.1 N NaOH/10 g dough. The dough inoculated with mono-culture of *Lb. plantarum* XL23 reached the highest acidity value as 11.75 mL 0.1 N NaOH/10 g dough. The dough inoculated with dual-culture of *Lb. plantarum* XL23 and with *Lb. sanfranciscensis* RL976 reached an acidity value of 10.05 mL 0.1 N NaOH/10 g dough. Among the inoculated strains, the lowest acidity at 12 hours was determined in the SD-2 dough inoculated with *Lb. sanfranciscensis* RL976 as 8.25 mL 0.1 N NaOH/10 g dough. After 24 hours, the acidity of the inoculated sourdoughs were in the range of 15.35-16.03 mL 0.1 N NaOH/10 g dough. On the other hand, acidity values of the control dough confirmed the trend showed by pH. TTA increased after 48 hours to 13.05 mL 0.1 N NaOH/10 g dough. At the last refreshment, the highest acidity was determined in the SD-3 sourdough produced with the dual-culture inoculum. The highest acidity was registered in the presence of *Lb. plantarum* XL23, alone and in combination with *Lb. sanfranciscensis* RL-976. Acidifying capacity varies among strains and *Lb. plantarum* XL23 showed good acidification in the present study. In a study, experimental sourdoughs were produced by using mono- and dual-starter culture combinations of *Lb. plantarum* and *Lb. sanfranciscensis.* At the end of the

fermentation, pH and TTA values were in the range of 3.44-4.09 and 10.10-12.60 mL (Ventimiglia et al., 2015).

Presumptive LAB, yeast, total mesophilic aerobic, mold and coliform bacteria counts of the sourdoughs were investigated. The results of the cell counts on mMRS agar are shown in Figure 4.58. Sourdoughs were inoculated with LAB cultures at 6 log CFU/g; hence, LAB counts of the inoculated strains were around 6 log CFU/g. Inoculated strains dominated the fermentations as observed on the morphological investigation of the petri dishes. Sourdoughs produced with mono-culture inoculums contained colonies with the same appearence, whereas in the multi-culture there were 2 colonies of different appearance. Figure 4.59 shows the mMRS petri dishes of the mono- and dual-culture inoculums at the beginning of the fermentations. On the first day, the lowest count was determined in the control sourdough (8 log CFU/g). On the other hand, the counts of the sourdoughs inoculated with starter cultures were close to each other. After 2 days, LAB counts reached more than 11 log CFU/g in all sourdoughs.

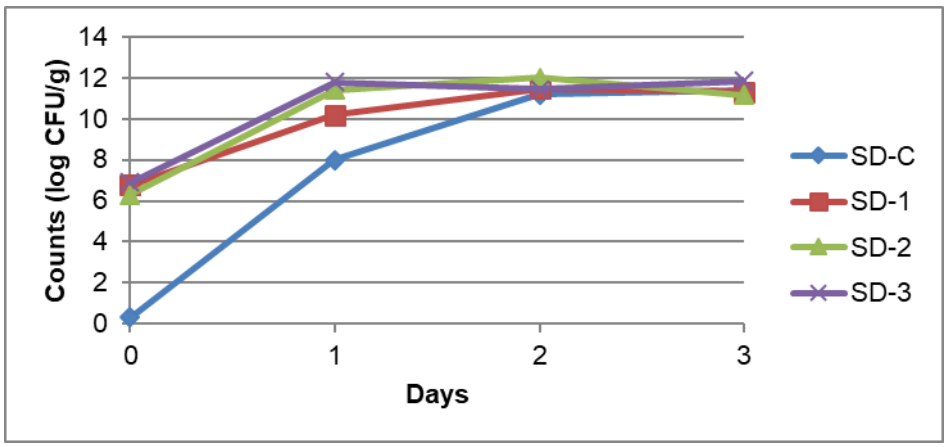

Figure 4.58. Cell counts of LAB on the mMRS agar
(SD-C: control, SD-1: *Lb. plantarum* XL23, SD-2: *Lb. sanfranciscensis*, SD-3:Lb. *plantarum*+*Lb. sanfranciscensis*)

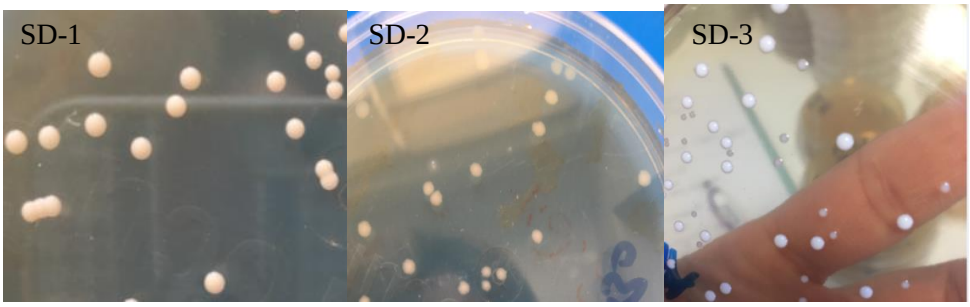

Figure 4.59. mMRS petri dishes of the mono- and dual-culture inoculums at the beginning of the fermentations

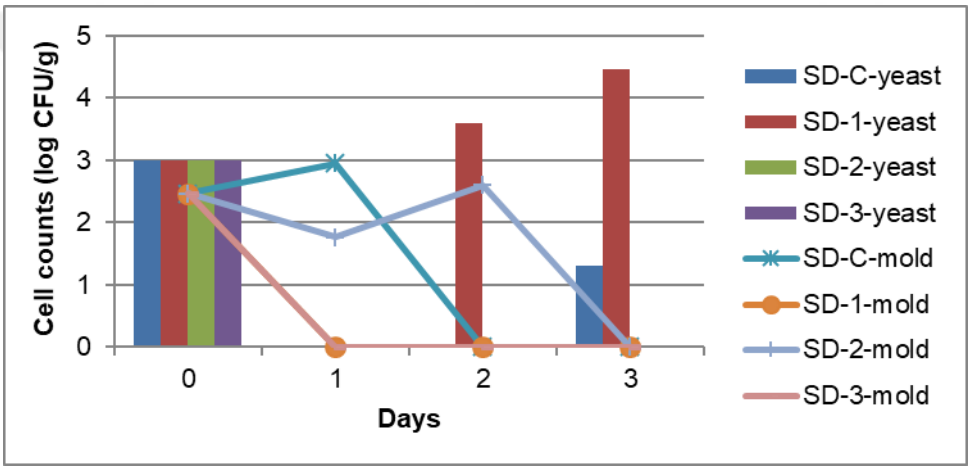

Figure 4.60. Cell counts of yeasts and molds
(SD-C: control, SD-1: *Lb. plantarum* XL23, SD-2: *Lb. sanfranciscensis*, SD-3:*Lb. plantarum*+*Lb. sanfranciscensis*)

In the unfermented doughs, yeast and mold counts were 3 and 2.47 log CFU/g, respectively (Figure 4.60). Mold counts were <1 log CFU/g in the sourdoughs produced with *Lb. plantarum* XL23 on the first day. At the 3rd day of refreshment, no colonies were not detected on agar media. Yeast counts exhibited variations. On the first day, yeast growth was not observed on agar media; however, some of the sourdoughs showed different patterns every refreshment.

Presumptive yeast counts were 1.30 and 4.46 log CFU/g in the control and

SD-1 sourdough samples at the last back-slopping stage, respectively. As it can be seen, every flour addition changed the flora in the doughs.

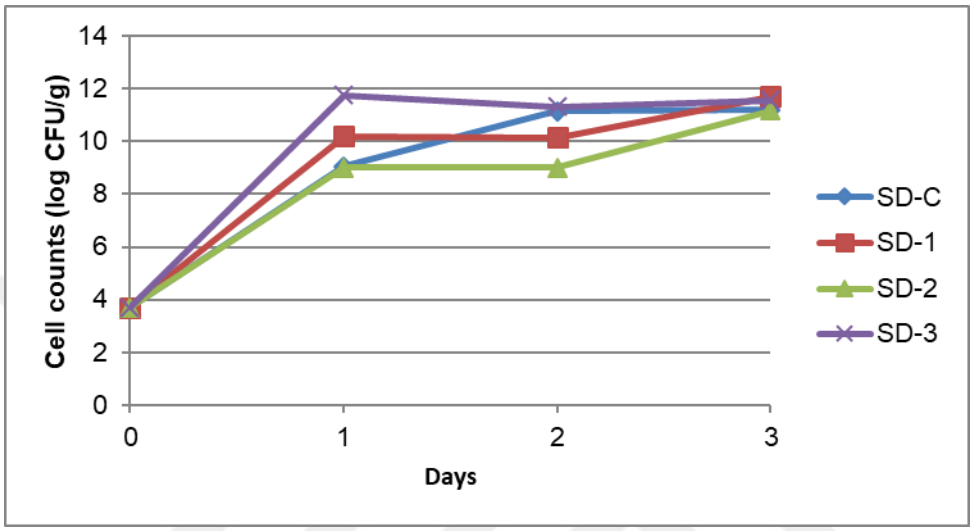

Figure 4.61. Cell counts of total mesophilic aerobic counts
(SD-C: control, SD-1: *Lb. plantarum* XL23, SD-2: *Lb. sanfranciscensis*, SD-3:*Lb. plantarum*+*Lb. sanfranciscensis*)

In the unfermented doughs, total mesophilic aerobic bacteria counts were determined as 3.7 log CFU/g. All of the TMAB counts were increased on the first day. At the last refreshment, TMAB counts were in the range of 11.17-11.69 log CFU/g in the sourdoughs as shown in Figure 4.61.

Presumptive total coliform bacteria counts of the sourdoughs were 120 MPN/g at the begining of the fermentation. At the 1st day of refreshment, no gas bubbles were observed in any of the LST broth tubes of the sourdoughs inoculated with starter culture, hence the presumptive coliform group bacteria was <3 MPN/g. In the control sourdough, presumptive total coliform bacteria counts were 75 MPN/g on the first day of refreshment and then decreased after 2 days. As it can be seen, control sourdough reached the characteristics of the inoculated sourdough

after 48 hours. Disappearance of the presumptive coliform bacteria can be related to the pH decrease. In the inoculated sourdoughs, acidification was faster than in control dough. Inoculated sourdoughs were characterized with high LAB counts, fast acidification and low pH values. At the first refreshment, pH values of the inoculated doughs were decreased below 4.0. On the other hand, sourdough sample produced without starter culture addition reached this pH at the 2nd refreshment. Acidity values and LAB counts of the samples confirmed the trend showed by pH. After two days the control sourdough exhibited the same patterns with the inoculated sourdoughs.

Carbohydrate, ethanol and organic acid content of the experimental sourdoughs were also investigated and the results are given in Table 4.38. At the final refreshment, the maltose+sucrose content was in the range of 9.19-13.34 g/kg and was less in inoculated sourdoughs than the control sourdough. Differences among the inoculated sourdough samples were not significant ($p>0.05$). At the final refreshment, the glucose content was in the range of 6.17-10.51 g/kg. The highest glucose content was detected in the control sourdough. Differences between the glucose content of sourdoughs inoculated with mono and dual-culture of *Lb. plantarum* XL23 were not significant ($p>0.05$). The final fructose contents were in the range of 2.17-3.80 g/kg. The fructose content was less in inoculated sourdoughs than the control sourdough. Differences in fructose content among the inoculated sourdough samples were not significant ($p>0.05$). Lactic acid production at the last refreshment was determined to be in the range of 8.87-11.53 g/kg. Among the inoculated sourdoughs, the lowest lactic acid production was determined in the SD-1 sourdough, however, differences among the inoculated sourdoughs were not significant ($p>0.05$). The acetic acid content was the highest in the control dough, followed by the SD-S sourdough inoculated with *Lb.*

sanfranciscensis RL976 as a mono-culture. Differences in ethanol content among the sourdough samples were not significant ($p>0.05$).

Table 4.38. Carbohydrate, ethanol and organic acid contents of the experimental sourdoughs at the last refreshment

Compounds (g/kg)		Experimental sourdoughs			
Refreshment day		SD-C	SD-1	SD-2	SD-3
Maltose+sucrose					
	0	12.03 ^{a} ±0.098	12.03 ^{a} ±0.098	12.03 ^{a} ±0.098	12.03 ^{a} ±0.098
	1	13.25 ^{a} ±0.09	11.11 ^{b} ±0.38	13.15 ^{a} ±1.15	13.92 ^{a} ±0.41
	2	14.48 ^{a} ±0.68	9.84 ^{b} ±0.042	9.87^{b}±0.29	10.07^{b}±0.44
	3	13.34^{a}±0.47	9.19^{b}±0.89	9.76^{b}±0.14	9.87^{b}±0.90
Glucose					
	0	7.41^{a}±0.27	7.41^{a}±0.27	7.41^{a}±0.27	7.41^{a}±0.27
	1	7.08^{ab}±1.09	9.64^{b}±0.49	8.18^{ab}±0.04	6.15^{a}±0.41
	2	10.03^{a}±1.03	9.36 ^{ab}±0.12	8.89 ^{a}±0.50	7.81^{b}±0.46
	3	10.51^{c}±0.90	8.45^{a}±0.28	10.03^{bc}±0.50	6.17^{a}±0.74
Fructose					
	0	6.21^{a}±0.66	6.21^{a}±0.66	6.21^{a}±0.66	6.21^{a}±0.66
	1	4.08 ^{b}±0.60	2.36 ^{a}±0.17	4.50 ^{b}±0.11	2.76 ^{a}±0.06
	2	3.17^{b}±0.24	2.31 ^{a}±0.04	3.01^{bc}±0.02	2.56 ^{ab}±0.31
	3	3.80 ^{b}±0.89	2.17 ^{a}±0.25	2.52 ^{ab}±0.44	2.47 ^{ab}±0.19
Ethanol					
	0	<LOQ	<LOQ	<LOQ	<LOQ
	1	2.70^{b}±0.00	2.44 ^{a}±0.12	2.52^{a}±0.02	<LOQ
	2	3.01^{b}±0.04	2.42^{a}±0.00	2.64^{a}±0.06	<LOQ
	3	2.54 ^{a}±0.02	2.40 ^{a}±0.00	2.62 ^{a}±0.06	<LOQ
Lactic acid					
	0	<LOQ	<LOQ	<LOQ	<LOQ
	1	1.41^{a}±0.40	11.34^{b}±1.02	12.38^{b}±0.09	10.80^{b}±0.36
	2	6.15^{a}±0.79	10.74^{b}±0.11	11.77^{b}±0.97	11.38^{b}±0.17
	3	8.87 ^{a}±0.62	10.85 ^{b}±0.37	11.05^{b}±0.85	11.53 ^{b}±0.95
Acetic acid					
	0	<LOQ	<LOQ	<LOQ	<LOQ
	1	1.70^{a}±0.57	1.07^{a}±0.20	2.10^{a}±0.44	1.85^{a}±0.17
	2	2.48^{b}±0.13	1.66^{ab}±0.18	1.63^{ab}±0.38	1.47^{a}±0.54
	3	1.76^{b}±0.12	1.20^{ab}±0.62	1.56^{b}±0.28	0.75^{a}±0.26

$^{a-c}$Different superscript letters within same line indicate significant difference (Duncan $p<0.05$) Results are given mean±SD (SD-C: control, SD-1: *Lb. plantarum* XL23, SD-2: *Lb. sanfranciscensis*, SD-3:*Lb. plantarum+Lb. sanfranciscensis*)

As a result of the activities in sourdoughs, some VOC compounds are generated. The SPME-GC-MS chromatographic analysis of the experimental sourdoughs revealed the presence of 37 VOC compounds belonged to different chemical groups as shown Table 4.39. VOC compounds were determined based on the relative peak area. A GC-MS chromatogram image of VOCs is shown in Appendix 11. In control dough at 0h, lower number of chemicals was detected (n=13). At the end of the third refreshment, number of the VOC compounds detected in the SD-C, SD-1, SD-2 and SD-3 sourdoughs were 22, 20, 19 and 21, respectively. According to the relative peak area, formamide was the most detected in the unfermented dough. Ethyl acetate and D-limonene were the most found in SD-C and SD-1 sample. Besides these VOCs, heptenal and acetic acid in SD-C and pentane and formamide in SD-1 was found. In SD-2, formamide, D-limonene, ethenyl acetate, hexanal, heptenal and pentane and in SD-3 D-limonene, acetic acid, ethenyl acetate, formamide, (1-methylbutyl)-oxirane, 1-hexanol, 3-methyl-1-butanol and pentane were the most determined VOC compounds. In the present study, D-limonene was detected in all sourdoughs. On the other hand, 3-methyl-1-butanol and 1-hexanol was only determined in the SD-3 sourdough. In another study, these alcohol comounds, 3-methyl-1-butanol and 1-hexanol, were detected in the control, *Lb. plantarum* and *Lb. sanfranciscensis* inoculated sourdoughs (Ventimiglia et al., 2015). Some VOC compounds as the metabolite of *Lb. plantarum* were reviewd and among them ethyl acetate, acetaldehyde, 3-methyl-1-butanal and heptenal were also detected in our study (Salim Ur et al., 2006).

Table 4.39. VOCs in the experimental sourdough samples as relative peak area (%)

VOC compounds[a]	D-0[b]	SD-C[c]	SD-1[c]	SD-2[c]	SD-3[c]
2-Octen-1-ol (E)	5.42	n.d.	0.14	n.d.	n.d.
(1-methylbutyl)-Oxirane	n.d.	1.78	1.2	n.d.	9.12
(E-E)-2, 4-nonadienal	0.28	0.88	n.d.	0.59	n.d.
1-Hexanol	n.d.	n.d.	n.d.	n.d.	7.96
1-Pentanol	n.d.	n.d.	n.d.	n.d.	4.44
2-Penten-1-ol	n.d.	0.78	n.d.	0.28	n.d.
2-pentyl-furan	n.d.	0.47	1.29	0.57	0.84
3-methyl-1-Butanol	n.d.	n.d.	n.d.	n.d.	6.42
3-methyl-butanal	3.87	n.d.	2.135	n.d.	n.d.
4-amino-1-Pentanol	1.25	0.43	n.d.	n.d.	n.d.
4-methyl- trans-Cyclohexanol	n.d.	n.d.	0.295	n.d.	n.d.
5-(pentyloxy)-1-Pentene	n.d.	n.d.	0.59	0.75	n.d.
5-ethyl-4-methyl-3-Heptanone	n.d.	n.d.	0.28	n.d.	n.d.
Acetaldehyde	2.84	0.29	1.27	5.14	4.31
Acetic acid	n.d.	6.50	n.d.	n.d.	13.69
Butyl acetate	n.d.	1.00	n.d.	n.d.	n.d.
Ethenyl acetate	n.d.	n.d.	n.d.	14.41	11.18
Hexyl acetate	n.d.	n.d.	0.25	n.d.	n.d.
Pentyl acetate	n.d.	n.d.	0.52	n.d.	n.d.
Benzene, 1,3-dichloro-	1.02	n.d.	n.d.	0.26	0.22
Cyclobutanol	1.42	0.44	n.d.	0.39	0.21
Cyclopentanol	1.88	0.19	0.41	1.45	0.95
D-Limonene	n.d.	12.32	11.03	17.92	13.96
Ethyl Acetate	n.d.	52.59	59.70	n.d.	n.d.
Formamide	40.87	3.88	6.14	25.11	12.62
Ethenyl formate	2.58	n.d.	n.d.	0.89	0.50
Ɣ-Terpinene	n.d.	0.19	0.50	0.33	0.35
Heptanal	1.14	6.75	2.67	8.41	n.d.
Hexanal	29.20	2.01	1.62	14.41	0.04
Humulene	n.d.	0.15	0.32	0.48	1.41
l-Menthone	n.d.	n.d.	n.d.	n.d.	0.23
o-Cymene	n.d.	0.33	0.66	1.08	0.99
Pentanal	2.55	n.d.	n.d.	n.d.	n.d.
Pentane	n.d.	3.93	8.82	6.22	7.91
propyl-Propanedioic acid	n.d.	0.69	n.d.	n.d.	n.d.
tert-butyl-benzene	n.d.	0.59	n.d.	n.d.	n.d.
trans-1,2-Cyclopentanediol	n.d.	2.1	n.d.	0.9	1.74

Results indicate mean values of two measurements and are expressed as relative peak areas (peak area of each compound/total area) × l00 ±SD.n.d., not detected. [a] The chemicals are shown alphabetically.[b]D-0:unfermented dough[c]Sourdoughs at the final refreshment (3rd day) SD-C: control sourdough, SD-1: fermented dough with *Lb. plantarum* XL23, SD-2: inoculated dough with *Lb. sanfranciscensis* RL976, SD-1: inoculated dough with *Lb. plantarum* XL23+ *Lb. sanfranciscensis* RL976

4.3.4. Multivariate Statistical Analysis of Experimental Sourdoughs

The microbiological and chemical parameters of sourdough samples were subjected to the multivariate analysis to evaluate the differences/variabilities among the samples. Data of the sourdough samples were subjected to PCa and a total of 47 variables were investigated. They were grouped as microbiological, chemical and VOC compounds and coded as M, C and V letters, respectively. The loading and score plots of PCa analysis in Figure 4.62 shows that an overall 77.04% of variance was explained by the first component (F1 of 43.20%) and second component (F2 of 33.84%).

As it can be seen, control sourdough, SD-C, differed from the inoculated sourdoughs along Factor 1. SD-C sourdough was explained by the higher pH, maltose+sucrose, glucose and acetic acid contents than other sourdoughs. Acidity was the lowest in that sample as it can be seen in the negative correlation of pH and TTA. Also control sourdough was characterized by the VOC compounds including 4-amino-1-Pentanol, (E-E)-2, 4-nonadienal, 2-Penten-1-ol, butyl acetate, tert-butyl-benzene and propyl-Propanedioic acid. Among the inoculated sourdoughs, SD-1 differed from SD-2 and SD-3 sourdoughs along Factor 2. Especially, YPD and PCA counts were the highest in that sample and relation was observed in the bi-plot. SD-1 sourdough was characterized the VOC compounds especially 2-Octen-1-ol (E), 5-ethyl-4-methyl-3-Heptanone, hexyl acetate, pentyl acetate, 3-methyl-butanal, 4-methyl- trans-cyclohexanol and also Ɣ-Terpinene, 2-pentyl-furan, pentane. SD-2 and SD-3 sourdoughs, inoculated with mono- and dual-culture of *Lb. sanfranciscensis* RL976, were characterized with many VOC compounds and acidity. SD-3 was mostly characterized with the high MRS counts, TTA, lactic acid and VOC compounds.

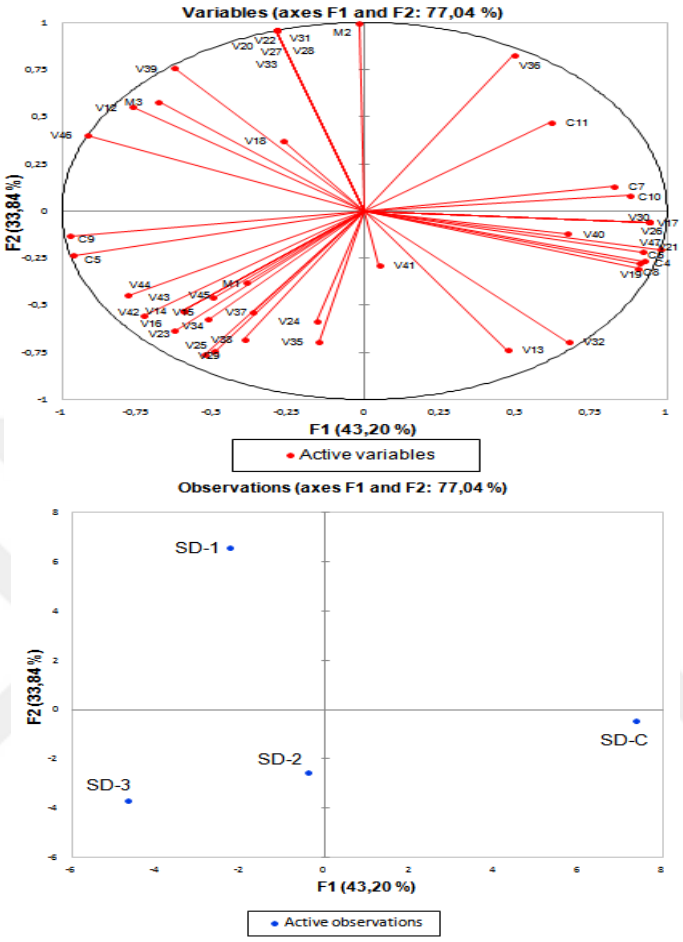

Figure 4.62. Loading plot (A) and score plot (B) resulting from principal component analysis of variables determined on sourdoughs

(M1:MRS, M2:YPD, M3:PCA, C4:pH, C5:TTA, C6:maltose+sucrose, C7: glucose, C8:fructose, C9:lactic acid, C10:acetic acid, C11:ethanol,V12:Ɣ-Terpinene, V13:trans-1,2-Cyclopentanediol, V14:3-methyl-1-Butanol, V15:1-Hexanol, V16:1-Pentanol, V17:4-amino-1-Pentanol, V18:5-(pentyloxy)-1-Pentene, V19:(E-E)-2, 4-nonadienal, V20:2-Octen-1-ol (E), V21:2-Penten-1-ol, V22:5-ethyl-4-methyl-3-Heptanone, V23:Acetaldehyde, V24:Acetic acid, V25:Ethenyl acetate, V26:Butyl acetate, V27:Hexyl acetate, V28:Pentyl acetate, V29: 1,3-dichloro Benzene, V30: tert-butyl-benzene, V31:3-methyl-butanal, V32:Cyclobutanol, V33:4-methyl- trans-Cyclohexanol, V34:Cyclopentanol, V35:D-Limonene, V36:Ethyl Acetate, V37:Formamide, V38:Ethenyl formate, V39:2-pentyl-furan, V40:Heptanal, V41:Hexanal, V42:Humulene, V43:l-Menthone, V44:o-Cymene, V45:(1-methylbutyl)-Oxirane, V46:Pentane, V47:propyl Propanedioic acid)

4.3.5. Production of Experimental Chickpea Liquid Starter and Dough Samples and Evaluation of Chemical and Microbiological Properties

Experimental chickpea liquid starter and dough samples were produced using *W. confusa* RL1139. Overnight LAB cultures with an optical density (OD) of ca.1.00, corresponds to an approximate concentration of 10^9 CFU/g, was used as inocula at the concentration 1 % (Settanni et al., 2013). The final concentration of the inoculum was approximately 10^6 CFU/mL in chickpea liquid. *W. confusa* RL1139 strain was used as mono-culture in the production of experimental chickpea liquid starters as shown below:

- Chickpea liquid without starter addition (Control): CLS-C and CD-C
- Chickpea liquid with *W. confusa* RL1139: CLS-W and CD-W

Chickpea liquid was prepared by mixing ground chickpeas with boiled and cooled water (37 °C). *W. confusa* RL1139 strain was inoculated to the chickpea liquid at the beginning. Fermentations were conducted at 37 °C for 18 hours. Then chickpea liquid starters were used in the production of adapted chickpea dough. Some microbiological and chemical properties of the produced liquid starter and dough samples were investigated and compared with control samples.

The pH and TTA values registered for the chickpea liquid starter and doughs are shown in Figure 4.63.

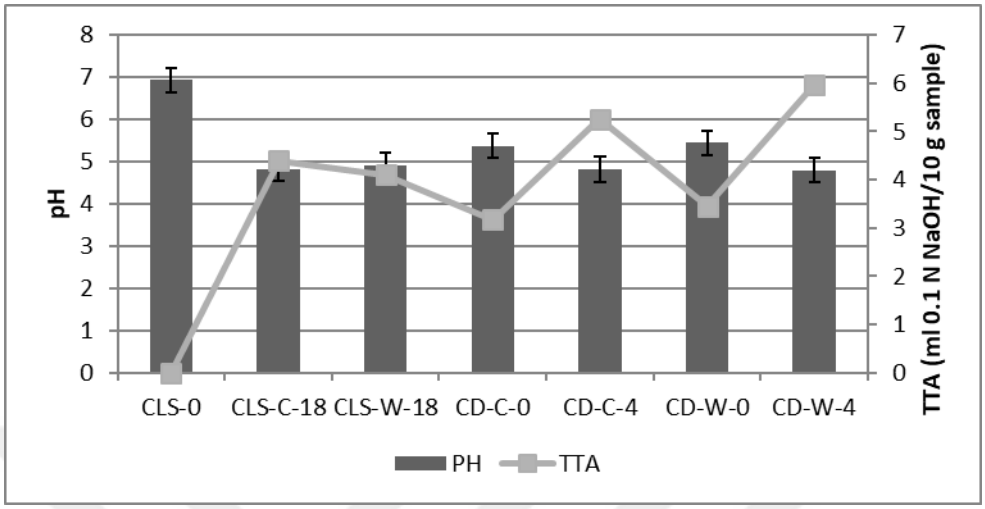

Figure 4.63. pH and TTA values of the experimental chickpea liquid starter and dough samples

The initial pH of the chickpea liquid was 6.93. The pH values of the control and inoculated chickpea liquid starters were 4.92 and 4.82 at the end of the fermentation, respectively. The final pH values of the control and inoculated chickpea doughs were 4.82 and 4.79, respectively.

The pH of chickpea liquid fermentation was monitored in the first 10 hours of the fermentation. According to the results, pH started to decrease after 6 hours in the control liquid. Conversely, the pH of the inoculated liquid decresased after 2 hours, as shown in Figure 4.64.

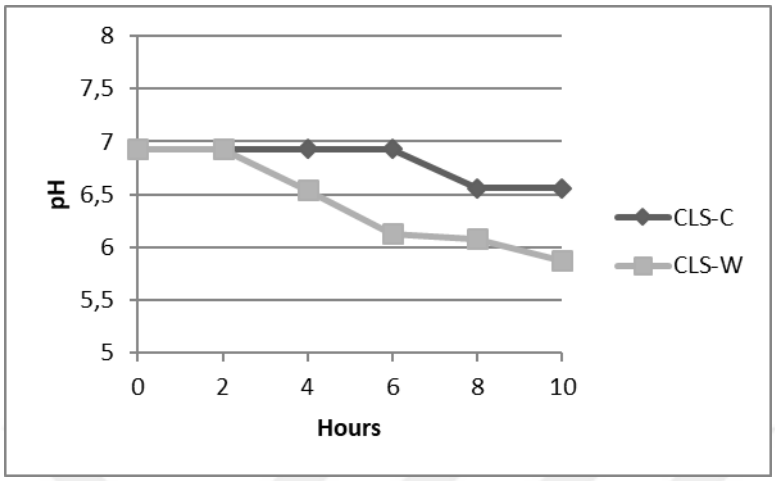

Figure 4.64. pH values monitored during 10 hours

After 18 hours, the TTA of the control and inoculated liquid starter samples were 4.4 and 4.1 mL 0.1 N NaOH/10 g sample, respectively. Final TTA values of the control and inoculated doughs produced were 5.26 and 5.97 mL 0.1 N NaOH/10 g sample, respectively. TTA was determined as 0.47% in the control dough. On the other hand, acidity in the chickpea dough produced with inoculated chickpea liquid starter was 0.54%. Lower acidity values were determined in the chikpea fermentations compared with sourdough fermentations. Chickpea dough is generally referred as “sweet dough” in many regions. In order to reach the desired level of acidification in chickpea dough, strains showing strong acidification should not be used as starter culture.

Hancıoglu-Sıkılı (2003) used three different starter cultures, *Lc*. *lactis* subsp. *cremoris*, *Lb. bifermantas* and *Lb*. *viridescens,* as mono-cultures for the production of chickpea liquid starter. Higher acidification was detected in the dough samples produced by *Lactabacillus* spp. than produced *Lactoccocus* spp. Final pH and TTA values were in the range of 4.91-5.25 and 0.407-0.740%, respectively (Hancıoglu-Sıkılı, 2003). In another study, chickpea fermentations

were conducted with three different LAB cultures as *Lb. brevis* FK2, *Lc. lactis* FK5 and *Lb. plantarum* FK25 and the pH values of the chickpea doughs were determined in the range of 4.83-4.92. It was reported that differences in the pH values of the chickpea doughs were not significant and spontanous flora in the chickpea fermentations could affect the final pH values (Çebi, 2014). Reported acidity values were in accordance with the present study.

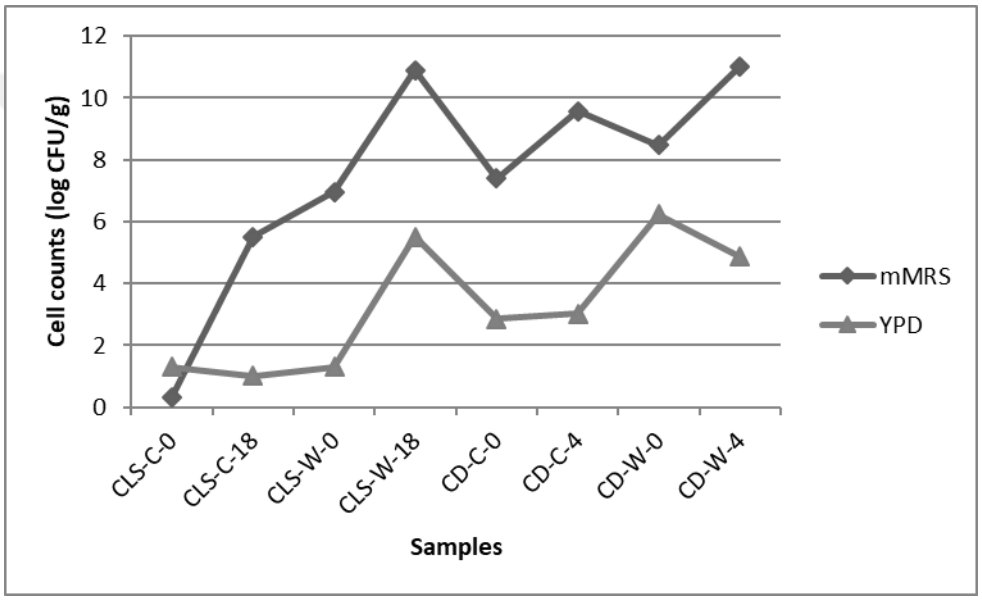

Figure 4.65. Cell counts on mMRS and YPD media

Presumptive LAB, yeast, total mesophilic aerobic, mold and coliform bacteria counts of the chickpea liquid starter and dough samples were investigated. The results of the cell counts on the mMRS and YPD agar are shown in Figure 4.65. Chickpea liquid starter was inoculated with LAB culture at 6 log CFU/g; hence, LAB counts of the inoculated sample was around 6-7 log CFU/g. On the other hand, presumptive LAB counts of the control liquid was very low. The cells counts of coth liquid starters on the mMRS were increased at the end of the 18 hour

fermentation, however, increase in the inoculated liquid starter was higher than the control liquid starter. Cell counts on mMRS agar were higher in the chickpea dough produced with the inoculated chickpea liquid starter compared with control dough. Final LAB counts were 9.56 and 11.01 log CFU/g in the CD-C and CD-W, respectively. Presumptive yeast counts varied during fermentations. Counts were 1 log CFU/g at the begining of the fermentation and then increased in the inoculated chickpea liquid starter to 5.5 log CFU/g at the end of the fermentation. Yeast counts were 3.02 and 4.86 log CFU/g in the dough samples at the end of the fermentation, respectively. In the present study, yeast counts were determined less than LAB counts. In a study, used three different starter cultures, *Lc*. *lactis* subsp. *cremoris, Lb*. *bifermantas* and *Lb*. *viridescens,* as mono-cultures for the production of chickpea liquid starter and reported the final LAB and yeast counts to be in the range of 7.33-8.99 and 4.06-5.61 log CFU/g in the adapted chickpea dough samples, respectively (Hancıoglu-Sıkılı, 2003).

In the present study, total bacteria counts during chickpea fermentations are given in Figure 4.66. Total bacteria were enumerated on PCA and NA agar media incubated at 30 and 37 °C, respectively. Also spore-forming bacteria were investigated during the experimental chickpea fermentations. Spore-forming bacteria counts were given as presumptive *Bacillus* spp. Total bacteria counts on NA and PCA agar media showed similar patterns, but, number of the colonies enumerated on PCA agar were higher than the colonies counted on NA agar. *Bacillus* spp. were less than the total bacteria counts. At the end of the fermentations, *Bacillus* spp. were 4.60, 3.65, 5.0 and 4.7 log CFU/g in the CLS-C, CLS-W, CD-C and CD-W, respectively.

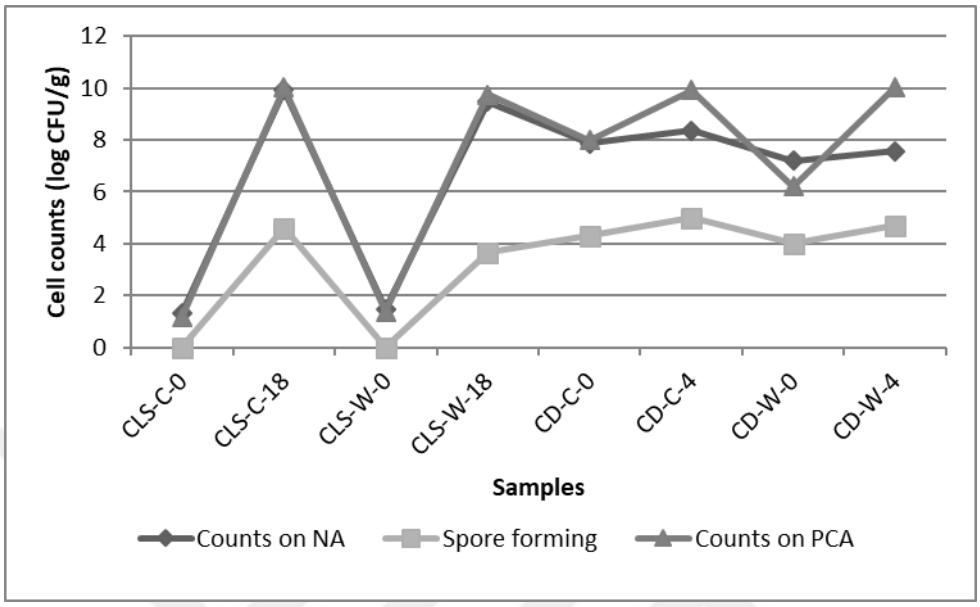

Figure 4.66. Total bacteria counts on PCA and NA media

Bacillus spp. are reported as the dominant microflora in chickpea liquids previously (Hatzikamari et al., 2007b). In the present study, total bacteria and *Bacillus* spp. counts on NA agar of chickpea liquid samples were monitored during 10 hours. Figure 4.67 shows the total and spore forming bacteria enumerated on NA agar incubated at 37 °C for 10 hours. In the control liquid starters, *Bacillus* spp. was counted as 4.69, 4.00 and 1.30 log CFU/g at the 2, 4 and 6 hours, respectively. Any colony was not observed in the inoculated liquid starter plates. Total bacteria counts were increased in both liquid samples during 10 hours.

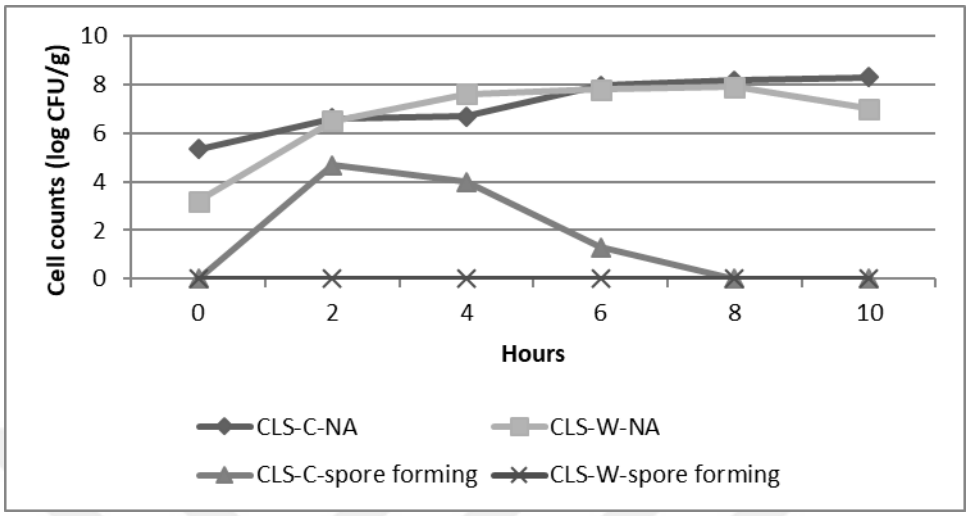

Figure 4.67. Total and spore forming bacteria during the first 10 hours of chickpea liquid fermentation

Table 4.40 shows the mold, coliform counts and indole test. Mold was observed only in the unfermented dough samples. Presumptive total coliform bacteria were <0.3 MPN/g in the liquid starter samples.

Table 4. 40. Mold and coliform counts during chickpea fermentations

Samples	Mold (log CFU/g)	Total coliform bacteria (MPN/g)	Indole test / Presumptive *Escherichia coli* (MPN/g)
CLS-C-0	0	<0.3	-
CLS-C-18	0	<0.3	-
CLS-W-0	0	<0.3	-
CLS-W-18	0	<0.3	-
CD-C-0	2	11	3.6
CD-C-4	<1	3.6	-
CD-W-0	0.3	11	3.6
CD-W-4	<1	3.6	-

Table 4.41. Carbohydrate, ethanol and organic acid contents (g/kg) of the experimental chickpea liquid starter and dough samples

Samples	Maltose+ sucrose	Glucose	Fructose	Lactic acid	Acetic acid
CLS- 0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CLS-C-18	0.30^{a}±0.07	0.75^{ab}±0.08	0.34^{a}±0.00	1.05^{bc}±0.14	1.29^{a}±0.01
CLS-W-18	0.32^{a}±0.07	0.81^{ab}±0.01	<LOQ	0.70^{a}±0.02	1.57^{a}±0.33
CD-C-0	11.83^{ab}±0.29	2.31^{bc}±0.19	4.71^{b}±0.99	0.99^{ab}±0.29	2.23^{b}±0.07
CD-C-4	13.29^{c}±0.25	3.69^{c}±0.98	5.42^{b}±0.49	0.74^{a}±0.03	2.74^{c}±0.13
CD-W-0	14.76^{d}±0.95	3.11^{c}±0.33	4.79^{b}±0.65	1.72^{d}±0.63	1.96^{b}±0.09
CD-W-4	14.60^{d}±0.58	3.51^{c}±0.02	5.33^{b}±0.27	1.84^{d}±0.05	3.43^{d}±0.09

[a-c]Different superscript letters within same column indicate significant difference (Duncan $p<0.05$) Results are given mean±SD

The contents of carbohydrate, ethanol and organic acid in the experimental chickpea liquid starter and dough samples are given in Table 4.41. Carbohydrate contents of the chickpea liquid starters were below the quantification limit at the beginning of the fermentation. Sample representing the beginning of the fermentation is taken directly from water after mixed with chickpeas; hence, compounds in the chickpeas could not passed into water yet. Therefore, all of the detected compounds were <LOQ in the liquid at the beginning of the fermentation. At the end of the chickpea liquid fermentation, differences in the maltose+sucrose and glucose contents were not significant between the liquid starters produced with and without starter culture ($p<0.05$). Lactic acid production was higher in the control liquid starter than inoculated liquid. This can be related to the spontanous flora present in the chickpea liquid since fermentations were conducted under semi-sterile conditions. Differences in the maltose+sucrose, lactic and acetic acid contents were significant but glucose and fructose contents were not signigicant between the liquid starters produced with and without starter culture ($p<0.05$). Acetic acid was produced more than lactic acid in the doughs. Lactic acid content in the chickpea dough produced with inoculated liquid starter was higher than the control dough. Ethanol contents of the all samples determined <LOQ. The possible

explanation can be low yeast counts in the samples or evaporation of ethanol during the sampling.

The SPME-GC-MS chromatographic analysis revealed the presence of 32 VOC compounds in experimental chickpea fermentations (Table 4.42). VOC compounds were determined based on the relative peak area. In control liquid starter at 0 h, lower number of chemicals was detected (n=8). In the control and inoculated chickpea liquid starters, number of the VOC compounds were 14 and 15, respectively. The number of VOC compounds in the chickpea doughs were in the range of 16-19. According to the relative peak area, formamid, hexanal and acetaldehyde were the most detected VOC compounds in the unfermented chickpea liquid. Butanoic acid (synonym butyric acid) was found in all of the fermented chickpea liquid starter and dough samples. In the fermented chickpea liquid starters, butanoic acid had the biggest relative area and followed with 2,3,4-trimethyloxetane, butyl butanoate and formamide. Relative peak area of butanoic acid were 59.30 and 72.81%, in the control and adapted dough produced with inoculated chickpea liquid starter, respectively. In chickpea fermentations, production of butyric acid can be related to the presence of *Clostridium* species as reported previously (Katsaboxakis and Mallidis, 1996). Because some strains of *Clostridium* spp. produce butyric acid (He et al., 2005; Yang et al., 2011). Hancıoglu-Sıkılı (2003) reported the occurence of butanoic and acetic acid acid in the chickpea liquid starter and dough samples produced with various starter cultures. Çebi (2014) investigated the volatile profile of the chickpea dough and bread samples produced with different starter cultures and 1% baker's yeast and determined alcohols including ethanol, 1-butanol, 1-hexanol, 1-octan-3-ol, aldehydes including hexanal and acetaldehyde, esters including ethyl acetate and hexyl butanoate more than other compounds in chickpea dough samples.

Table 4.42. VOCs in the experimental chickpea fermentations as relative peak area (%)

VOC compounds[a]	CLS-0[b]	CLS-C-18[c]	CLS-W-18[c]	CD-C-0[c]	CD-W-0[c]	CD-C-4[c]	CD-W-4[c]
1-fluoro-butane	n.d.	n.d.	0.19	n.d.	n.d.	n.d.	n.d.
3-hydroxy-butanal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09
3-methyl-butanal	n.d.	n.d.	0.14	n.d.	n.d.	n.d.	n.d.
Propyl-propanedioic acid	n.d.	n.d.	0.04	5.27	0.31	0.14	0.07
1,3-dichloro-benzene	0.55	n.d.	n.d.	0.05	0.09	n.d.	n.d.
Methoxyacetone	n.d.	1.1	n.d.	n.d.	3.14	n.d.	0.22
2,3,4-Trimethyloxetane	n.d.	25.61	41.36	31.72	42.24	20.87	7.32
2,3-Butanedione	n.d.	n.d.	n.d.	6.24	n.d.	n.d.	n.d.
2-amino-, (S)-1-propanol	n.d.	0.05	n.d.	n.d.	n.d.	0.05	n.d.
2-Nonynoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	0.06	n.d.
2-Octynoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04
3-methyl-pentanal	n.d.	0.35	1.17	0.29	n.d.	n.d.	n.d.
5-Methyloxazolidine	n.d.	n.d.	n.d.	n.d.	0.51	0.48	n.d.
Acetaldehyde	11.23	n.d.	0.09	0.04	n.d.	n.d.	n.d.
Acetic acid	n.d.	n.d.	n.d.	n.d.	1.65	3.34	4.01
Acetone	n.d.	5.08	4.9	4.58	3.86	3.6	n.d.
Butanoic acid	n.d.	41.04	30.45	16.43	21.14	59.3	72.81
Butyl acetate	n.d.	1.82	n.d.	1.26	n.d.	2.29	2.05
Butyl butanoate	n.d.	13.5	5.7	2.99	0.37	3.49	0.78
Cyclobutanol	n.d.	0.28	0.4	0.14	0.43	0.18	0.22
Ethenyl acetate	n.d.	n.d.	n.d.	4.32	1.57	0.97	n.d.
Ethenyl formate	1.17	0.14	1.73	n.d.	n.d.	n.d.	0.02
Ethyl acetate	n.d.	0.44	0.62	7.47	7.18	n.d.	7.26
Ethyl butanoate	n.d.	4.28	5.31	1.32	0.61	0.64	1.9
Ethylene oxide	1.8	n.d.	n.d.	0.76	0.02	0.28	0.01
Formamide	43.59	5.15	7.78	11.94	6.05	3.37	3.1
Formic acid	n.d.	n.d.	n.d.	n.d.	6.3	n.d.	n.d.
Formyl acetate	n.d.	1.15	n.d.	2.41	2.41	n.d.	n.d.
Heptanal	n.d.	n.d.	n.d.	0.61	n.d.	0.05	n.d.
Hexanal	39.36	n.d.	0.12	2.09	2.17	0.94	0.15
Pentanal	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-Terpinyl acetate	0.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Results indicate mean values of two measurements and are expressed as relative peak areas (peak area of each compound/total area) × l00 ±SD., n.d., not detected., [a] The chemicals are shown alphabetically., [b] CLS-0:unfermented chickpea liquid, [c]CLS-C-18: Fermented chickpea liquid starter without inoculation, CLS-W-18: Fermented chickpea liquid starter inoculated with *W.confusa* RL1139, CD-C-0:Unfermented control chickpea dough, CD-W-0:Unfermented chickpea dough produced with inoculated chickpea liquid starter, CD-C-4:Fermented control chickpea dough, CD-W-4:Fermented chickpea dough produced with inoculated chickpea

4.3.6. Multivariate Statistical Analysis of Experimental Chickpea Fermentations

The microbiological and chemical parameters of chickpea liquid starter and dough samples were subjected to the multivariate analysis to evaluate the differences/variabilities among the samples. Data of the chickepea fermentations

were subjected to PCa and a total of 44 variables were investigated. They were grouped as microbiological, chemical and VOC compounds and coded as M, C and V letters, respectively. The loading and score plots of PCa analysis in Figure 4.68 shows that an overall 60.27% of variance was explained by the first component (F1 of 40.48%) and second component (F2 of 19.79%).

As it can be seen, unfermented chickpea liquid differed from the other samples with regards to F1. Along with F2, chickpea liquid starters and dough samples were seperated from each other. Control and inoculated samples were close to each other but characterized with different VOC compounds. CLS-W-18 sample was characterized by VOC compounds including 3-methyl-pentanal, 3-methyl-butanal and also butane1-fluoro. Chickpea dough samples were characterized with many VOC compounds. Among the VOCs, butanoic acid and also acetic acid are positively correlated with fermented chickpea dough samples.

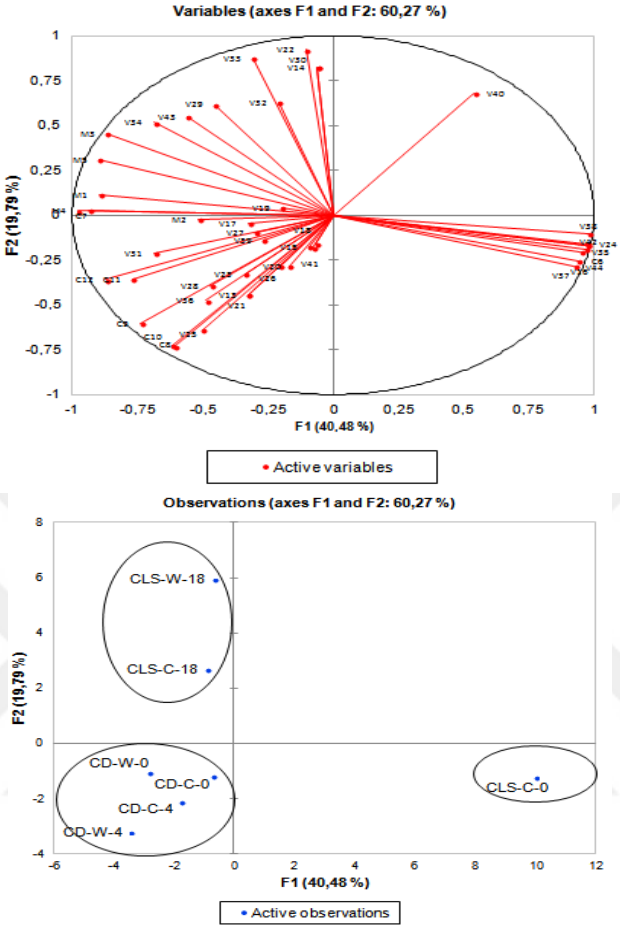

Figure 4.68. Loading plot (A) and score plot (B) resulting from principal component analysis of variables determined on chickpea fermentations

(M1:MRS, M2:YPD, M3:NA, M4:NA-spore forming bacteria, M5:PCA, C6:pH, C7:TTA, C8:maltose+sucrose, C9:glucose, C10:fructose, C11:lactic acid, C12: acetic acid, V13:3-hydroxy-butanal, V14: 3-methyl-butanal, V15: Propyl-propanedioic acid, V16:1,3dichloro-benzene, V17: Methoxyacetone, V18:2,3-Butanedione, V19:2-amino-,(S)-1-propanol, V20:2-Nonynoic acid, V21:2-Octynoic acid, V22:3-methyl-pentanal, V23:5-Methyloxazolidine, V24:Acetaldehyde, V25:Acetic acid, V26:Ethenyl acetate, V27: Formyl acetate, V28:Butyl acetate, V29:Acetone, V30: 1-fluoro-Butane, V31:Butanoic acid, V32:Butyl butanoate, V33: Ethyl butanoate, V34:Cyclobutanol, V35: β-Terpinyl acetate, V36:Ethyl Acetate, V37:Ethylene oxide, V38:Formamide, V39:Formic acid, V40: Ethenyl formate, V41:Heptanal, V42:Hexanal, V43: 2,3,4-Trimethyloxetane, V44:Pentanal)

5. CONCLUSION

In this study, microbiological and chemical characteristics and LAB and yeasts biodiversity of the sourdough and chickpea fermentations were investigated in the collected samples and laboratory scale productions. Technological potential of some LAB strains were investigated and used in the sourdough and chickpea liquid starter productions.

Results of the sourdough fermentations:

- Sourdough samples, produced by traditional method without baker's yeast addition, were collected from the bakeries in three different cities of Turkey at two different times.
- The pH levels of the collected sourdough samples ranged from 3.71 to 3.96 and the pH exhibited a mean value of 3.87. The highest pH value was measured in the rye sourdough sample. pH values showed differences among sourdoughs and sampling times.
- TTA levels of the collected sourdough samples ranged from 6.78 to 23.93 mL 0.1 N NaOH /10 g dough. TTA values exhibited differences among sourdoughs and sampling times and the differences between the samples were significant ($p<0.05$).
- According to the statistical results, the differences in sugar content between the samples collected from the different bakeries were significant ($p<0.05$).
- In the collected sourdoughs, lactic and acetic acids varied from 57 to 156 and 9 to 39 mM, respectively. The differences between the lactic and acetic acid contents in the collected samples from different bakeries were significant ($p<0.05$).

- The highest cell density on mMRS agar was in the rye sourdough.
- The highest cell density on YPD agar was in the rye sourdough.
- For presumptive total coliform bacteria, all of the tubes gave negative results (<3MPN/g).
- Under laboratory conditions, sourdough was produced at 28°C by propagating over a period of 7 days using the daily back-slopping (refreshment) procedure.
- The pH of the prepared sourdough did not change during the first 12 h of fermentation, but decreased to 4.58 after 24 hours. TTA was stable during the first 12 hours, but then increased. During the following days, the acidity continued to increase, but not greatly, reaching at final value of 17.56 mL 0.1 N NaOH.
- In the present study, FQ levels were determined in the range of 2.48-5.90. The FQ of the laboratory produced sourdough was high and determined as 10.84. Favored conditions such as propagation ratio, fermentation time and temperature provided the growth of LAB and metabolite production increased.
- According to the multivariate statistical analysis of the sourdoughs, acetic acid, TTA, lactic acid and SDB were positively correlated. Samples collected from the same bakery at two different times were included in a group except SD-T sample.
- Properties of the SD-T sample was different in the second sampling since processing conditions changed. Changes in the processing conditions at two different times, result in sourdoughs with different characteristics.

- *Lb. sanfranciscensis* was the dominant species in sourdough fermentations and *Lb. plantarum* and *Lb. paralimentarious* were other frequently isolated species.
- *Lb. paracasei*, *Leu. mesenteroides*, *W. confusa*, *Lb. curvatus* and *Lb. brevis* were found to be minor species. Furthermore, *Lb. pentosus, Leu. citreum, Lb. paraplantarum, Lb. acidophilus, E. faecium, Pd. inopinatus, Lb. parabrevis, Lc. lactis* subsp. *cremoris, W. cibaria* and *Pd. pentosaceus* were only isolated from 1 or 2 samples.
- In the SD-M sample, *Lb. plantarum* was detected as the predominant species at the first sampling and *Lb. paralimentarious* and *W. confusa* were co-dominant species at the second sampling.
- In the SD-T1 sample, *Lb. paralimentarious* and *Lb. paracasei* were detected as the predominant species and *Lb. sanfranciscensis* was the dominant species at the second sampling.
- The SD-K sample contained *Lb. sanfranciscensis* as the dominant species at the first sampling and at the second sampling *Lb. plantarum* was the dominant species.
- Rye sourdough contained *Lb. paralimentarious* as the dominant species at both sampling.
- In the laboratory produced sourdough, *Lb. sanfranciscensis* was not isolated during 7 days of fermentation.
- *S. cerevisiae* (72.5%) was the dominant yeast species in sourdoughs. Collected sourdoughs were produced without using baker's yeast, however, the presence of *S. cerevisiae* in the bakery sourdoughs can be related to contamination of the bakery environment with baker's yeast.

- Other isolated yeast species were *K. bulderi* (7.2%), *P. fermentans* (5.9%), *P. membranifaciens* (5.2%), *K. servazzii* (4.6%), *K. unispora* (2.6%) and *H'spora valbyensis* (2%).
- In the present study, *H'spora valbyensis* was isolated from sourdoughs for the first time. To my knowledge, the presence of this yeast species in sourdough fermentations has not been documented previously.

Results of the chickpea fermentations:

- Chickpea liquid starter and dough samples were collected from the bakeries that are well-known in their regions in Turkey and have been producing chickpea bread for many years.
- The pH levels of the chickpea liquid starter samples ranged from 4.82 to 5.67. There was a wide variation among pH levels of the chickpea liquid samples and sampling times. According to the statistical results, the differences between the samples collected from different bakeries were significant ($p<0.05$).
- The pH levels of the chickpea dough samples ranged from 5.12 to 5.53. According to the statistical results, the differences between the samples collected from different bakeries were significant ($p<0.05$).
- Acidity levels of the collected chickpea liquid samples ranged from 1.65 to 3.20 ml 0.1 N NaOH/10 g sample. The acidity content was significantly ($p<0.05$) different between chickpea liquid samples.
- The acidity levels of the collected chickpea dough samples ranged from 3.03 to 5.40 mL 0.1 N NaOH/10 g sample. The acidity content was significantly ($p<0.05$) different among chickpea dough samples. The values also showed a significant difference among some samples collected on two different sampling times.

- The levels of the acetic acid in the dough samples were below the LOQ.
- The highest cell densities on mMRS and gM17 agar media were counted in the CLS-B2 sample.
- The highest cell densities on YPD and L-lysine agar media were detected in the CLS-N1 and CLS-A1 samples, respectively.
- The control chickpea liquid starter and dough samples were produced in duplicate under laboratory conditions at 32 and 37°C. The total titratable acidity value was higher in the chickpea liquid starter fermented at 37°C (2.95 mL 0.1 N NaOH/10 g sample) compared with 32°C (1.95 mL 0.1 N NaOH/10 g sample).
- According to the multivariate statistical analysis of the chickpea fermentations, all of the chickpea doughs were gathered together with the control trial. Similarly, all of the chickpea liquid starters were included in another cluster.
- LAB counts on MRS, M17 and TTA and lactic acid contents were positively correlated as expected. The pH was negatively correlated with TTA and positively correlated with YPD, fructose and glucose.
- Chickpea dough samples were seperated from liquid starter samples in the dendogram constructed according to the PCa analysis.
- *W. confusa* was the dominant species, followed by *E. faecium* and *W. cibaria*.
- *Leu. mesenteroides*, *Lb. brevis* and *St. lutetiensis* were found as minor species. Conversely, *Lb. plantarum*, *Pd. acidilactici*, *St. salivarius*, *E. lactis* and *Leu. mesenteroides* subsp. *dextranium* were only isolated from 1 or 2 samples.

- In the CD-A1 sample, *Leu. mesenteroides* and *E. faecium* co-dominated the fermentation. In the second sampling, *E. faecium* dominated the chickpea fermentation.
- *W. confusa* was isolated from all of the samples collected from Bakery B at both sampling times. The second sampling exhibited a richer diversity than the first sampling, with *E. faecium* and *W. cibaria* dominating the liquid starter fermentation. From the most dominant to the least, *W. confusa*, *E. faecium* and *W. cibaria* were identified in the chickpea dough of Bakery B at the second sampling.
- *W. confusa* dominated the fermentations in N Bakery.
- Non-*Lactobacillus* spp. dominated the chickpea fermentations. *Lactobacillus* spp. are more resistant to acidic conditions than other LAB and therefore, *Lactobacillus* spp. dominate in an acidic sourdough environment. However, other species that grow at higher pH values were commonly identified in chickpea fermentations.
- *S. cerevisiae* was the dominant yeast species.
- Other isolated yeast species were *P. fermentans*, *C. parapsilosis*, *M. guilliermondii* and *Cr. albidosimilis*. All of them were reported for the first time in chickpea fermentations.
- Also *Bacillus* spp. are found in chickpea fermentations. Some of the sequences showed high identity with *Bacillus* spp. (data not shown)

Results of the experimental sourdough and chickpea fermentations:

- Strains of *Lb. sanfranciscensis*, *Lb. plantarum* and *Lb. paralimentarius* were investigated for technological potential to be used as starter culture in sourdough fermentations.

- In chickpea fermentations, *W. confusa* was the most frequent species and strains of *W. confusa* were investigated for technological potential to be used as starter culture in chickpea fermentations.
- The lowest pH values were measured in the sterile flour extract inoculated with *Lb. plantarum* species.
- Among the strains, only three *W. confusa* strains, RL1139, RL425 and RL1252, showed EPS production.
- Based on the technological screening, *Lb. plantarum* XL23 and *Lb. sanfranciscensis* RL976 were selected to act as starter culture for experimental sourdough production.
- Doughs inoculated with mono- or dual-culture of *Lb. plantarum* XL23 reached the pH values less than 4.0 in 12 hours. However, dough inoculated with *Lb. sanfranciscensis* RL976 reached pH values less than 4.0 after 24 hours.
- In the control sourdough the pH decreased very slowly and reached similar pH values with the inoculated sourdoughs after 48 h.
- Inoculated sourdoughs were characterized with high LAB counts, fast acidification and low pH values. At the first refreshment, pH of the inoculated doughs were decreased below 4.0. On the other hand, sourdough sample produced without starter culture addition reached this pH at the 2nd refreshment. Acidity values and LAB counts of the samples confirmed the trend showed by pH. After two days the control sourdough exhibited the same patterns with the inoculated sourdoughs.
- Ethyl acetate and D-limonene were the most found in SD-C and SD-1 sample. In SD-2, formamide, D-limonene, ethenyl acetate, hexanal, heptenal and pentane and in SD-3, D-limonene, acetic acid, ethenyl

acetate, formamide, (1-methylbutyl)-oxirane, 1-hexanol, 3-methyl-1-butanol and pentane were the most determined VOC compounds.

- D-limonene was detected in all sourdoughs.
- SD-2 and SD-3 sourdoughs, inoculated with mono- and dual-culture of *Lb. sanfranciscensis* RL976, were characterized with many VOC compounds and acidity. SD-3 was mostly characterized with the high MRS counts, TTA, lactic acid content and VOC compounds.
- Experimental chickpea liquid starter and dough samples were produced using *W. confusa* RL1139.
- Lower acidity values were determined in the chikpea fermentations compared with sourdough fermentations. Chickpea dough is generally referred as “sweet dough” in many regions. In order to reach the desired level of acidification in chickpea dough, strains showing strong acidification should not be used as starter culture.
- Total bacteria and *Bacillus* spp. counts on NA agar of chickpea liquid samples were monitored during 10 hours. Total bacteria counts were increased in both liquid samples during 10 hours.
- Butanoic acid was found in all of the fermented chickpea liquid starter and dough samples.
- Chickpea fermentations were characterized with butanoic acid.

The following suggestions can be given for further studies based on the results of this thesis:

- Traditional bread production has gained importance due to increasing demand by consumers for more organic and healthy foods. Sourdough and chickpea breads are produced with fermentation without baker's

yeast addition. Therefore, potentiality of the industrial production of sourdough and chickpea breads should be investigated.

- For the industrial production, starter fermentations should be examined. In future studies, starter culture characteristics of the identified microorganisms can be investigated to develop starter culture combinations for sourdough and chickpea fermentations. To obtain a final product with same characteristics, same conditions should be applied during fermentations. Starter culture addition and providing same conditions will result in doughs with same characteristics. Therefore, by starter culture addition, industrial production of standard sourdough and chickpea breads will be possible everytime at the same quality.
- The quality parameters of the breads should be examined by using combinations of LAB strains to improve the quality parameters.
- In chickpea fermentations, besides LAB, some *Bacillus* spp. can be investigated to be used as starter cultures.

REFERENCES

Abbad Andaloussi, S., Talbaoui, H., Marczak, R. and Bonaly, R., 1995. Isolation and characterization of exocellular polysaccharides produced by *Bifidobacterium* longum. Applied Microbiology and Biotechnology, 43 (6): 995-1000.

Abdelnasser, S. M., Sm, M. Y., Mohamed, W. F., Asker, M. M., Abu Shady, H. M., Mahmoud, M. G. and Gadallah, M. A., 2017. Antitumor Exopolysaccharides Derived from Novel Marine *Bacillus*: Isolation, Characterization Aspect and Biological Activity. Asian Pacific Journal of Cancer Prevention, 18 (7): 1847-1854.

Adam, A., Leenhardt, F., Lopez, H. W., Leuillet, M. and Re´Me´Sy, C., 2003. Les possibilite´s d'ame´lioration de la valeur nutritionnelle du pain (Possibilities for improvement of the nutritional value of bread). Cahiers de Nutrition et Die´te´tique, 38: 316-322.

Adams, M. R. and Nicolaides, L., 1997. Review of the sensitivity of different foodborne pathogens to fermentation. Food Control, 8 (5): 227-239.

Alfonzo, A., Miceli, C., Nasca, A., Franciosi, E., Ventimiglia, G., Di Gerlando, R., Tuohy, K., Francesca, N., Moschetti, G. and Settanni, L., 2017. Monitoring of wheat lactic acid bacteria from the field until the first step of dough fermentation. Food Microbiology, 62: 256-269.

Alfonzo, A., Ventimiglia, G., Corona, O., Di Gerlando, R., Gaglio, R., Francesca, N., Moschetti, G. and Settanni, L., 2013. Diversity and technological potential of lactic acid bacteria of wheat flours. Food Microbiology, 36: 343-354.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J., 1990. Basic local alignment search tool. Journal of Molecular Biology, 215 (3): 403-410.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25 (17): 3389-3402.

Alvarez-Sieiro, P., Redruello, B., Ladero, V., Martín, M. C., Fernández, M. and Alvarez, M. A., 2016. Screening sourdough samples for gliadin-degrading activity revealed *Lactobacillus casei* strains able to individually metabolize the coeliac-disease-related 33-mer peptide. Canadian Journal of Microbiology, 62 (5): 422-430.

Amari, M., Arango, L. F., Gabriel, V., Robert, H., Morel, S., Moulis, C., Gabriel, B., Remaud-Simeon, M. and Fontagne-Faucher, C., 2013. Characterization of a novel dextransucrase from *Weissella confusa* isolated from sourdough. Applied Microbiology and Biotechnology, 97 (12): 5413-5422.

Anonymous, 2013. Tahıl raporu (*Cereal Report).* Gaputaem Güncel, Gıda, Tarım ve Hayvancılık Bakanlığı, Gap Uluslararası Tarımsal Araştırma ve Eğitim Merkezi, Tarımsal Ekonomi ve Politika Araştırmaları Bölüm Başkanlığı, 1 (1) ISSN:2148-1962 *(in Turkish).*

Anonymous, 2013 Baklagil Raporu (*Legume Report)*. Gaputaem Güncel, Nüsha 3, Gıda, Tarım ve Hayvancılık Bakanlığı, Gap Uluslararası Tarımsal Araştırma Ve Eğitim Merkezi, Tarımsal Ekonomi ve Politika Araştırmaları Bölüm Başkanlığı, 1 (1) ISSN:2148-1962 *(in Turkish).*

Arendt, E. K., Ryan, L. A. and Dal Bello, F., 2007. Impact of sourdough on the texture of bread. Food Microbiology, 24 (2): 165-174.

Axelsson, L., 2004. Lactic Acid Bacteria: Classification and Physiology (S. Salminen, A. Von Wright and A. Ouwehand Editors). Lactic Acid Bacteria Microbiological and Functional Aspects Third Edition, Marcel Dekker, Inc., New York, NY, p 1-66.

Barber, S. and Baguena, R., 1988. Microflora of the sourdough of wheat flour bread. V. Isolation, identification and evaluation of functional properties of sourdough's microorganisms. Revista de Agroquímica y Tecnología de Alimentos, 28: 67-78.

Bartkiene, E., Bartkevics, V., Krungleviciute, V., Pugajeva, I., Zadeike, D. and 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, 82 (10): 2371-2378.

Baykara, P., 2006. Geleneksel Nohut Mayasının Endüstriyel Beyaz Buğday Unu Ekmeği Üretiminde Kullanımı *(Using of Traditional Chickpea Leavener in the Manufacture of Industrial White Wheat Bread).* Msc Thesis, Trakya University, Graduate School of Natural and Applied Sciences, Main Science Branch of Food Engineering, Tekirdağ, Turkey, 90 p *(in Turkish).*

Behera, S. S. and Ray, R. C., 2015. Sourdough Bread (C. M. Rosell, J. Bajerska, A. F. El Sheikha Editors). Bread and Its Fortification: Nutrition and Health, CRC Press, Boca Raton, p 53–67.

Berghofer, L. K., Hocking, A. D., Miskelly, D. and Jansson, E., 2003. Microbiology of wheat and flour milling in Australia. International Journal of Food Microbiology, 85 (1): 137-149.

Bhardwaj, H. L., Rangappa, M. and Hamama, A. A., 1999. Chickpea, faba bean, lupin, mungbean, and pigeonpea: potential new crops for the Mid-Atlantic Region of the United States (J. Janick Editor). Perspectives on new crops and new uses, ASHS Press, Alexandria, VA, p 202.

Bidyarani, N., Prasanna, R., Babu, S., Hossain, F. and Saxena, A. K., 2016. Enhancement of plant growth and yields in Chickpea (*Cicer arietinum* L.) through novel cyanobacterial and biofilmed inoculants. Microbiological Research, 188-189: 97-105.

Björkroth, J., Dicks, L. M. T. and Endo, A., 2014. The genus *Weissella* (W. H. Holzapfel and B. J. B. Wood Editors). Lactic Acid Bacteria: Biodiversity and Taxonomy, First Edition, John Wiley & Sons, Ltd**.,** West Sussex, UK, p 417-428.

Böcker, G., Stolz, P. and Hammes, W. P., 1995. New results on the eco system sourdough and on the physiology of sourdough specific of *Lactobacillus sanfrancisco* and *Lactobacillus pontis*. Getreide, Mehl und Brot 49: 370-374.

Brandt, M. J., 2005. Geschichte des Sauerteiges (M. J. Brandt and M. G. Ganzle Editors). Handbuch Sauerteig. Hamburg, Behr's Verlag, p 1-5.

Brandt, M. J., Hammes, W. P. and Gänzle, M. G., 2004. Effects of process parameters on growth and metabolism of Lactobacillus sanfranciscensis and *Candida humilis* during rye sourdough fermentation. European Food Research and Technology, 218 (4): 333-338.

Brandt, M. J., 2007. Sourdough products for convenient use in baking. Food Microbiology, 24 (2): 161-164.

Butler, M., Sparling, R. and Court, D., 2010. Energy Metabolism of Cells Used for Industrial Production (M. C. Flickinger Editor). Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, Wiley, p 5248.

Cai, Y., Okada, H., Mori, H., Benno, Y. and Nakase, T., 1999. *Lactobacillus paralimentarius* sp. nov., isolated from sourdough. International Journal of Systematic Bacteriology, 49 (4): 1451-1455.

Caplice, E. and Fitzgerald, G. F., 1999. Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology, 50 (1): 131-149.

Cappelle, S., Guylaine, L., Gänzle, M. G. and Gobbetti, M., 2013. History and Social Aspects of Sourdough (M. Gobbetti and M. G. Gänzle Editors). Handbook on Sourdough Biotechnology, Springer Science+Business Media, New York, p 1-9.

Catzeddu, P., 2011. Sourdough Breads (V. Preedy, R. Watson and V. Patel Editors). Flour and Breads and their Fortification in Health and Disease Prevention, Academic Press, San Diego, p 37-46.

Catzeddu, P., Mura, E., Parente, E., Sanna, M. and Farris, G. A., 2006. Molecular characterization of lactic acid bacteria from sourdough breads produced in Sardinia (Italy) and multivariate statistical analyses of results. Systematic and Applied Microbiology, 29 (2): 138-144.

Cauvain, S. P., 1998. Other cereals in breadmaking (S. P. Cauvain and L. S. Young Editors). Technology of breadmaking, Blackie Academic and Professional, London, p 330-346.

Cauvain, S. P., 2001. Breadmaking (G. Owens Editor). Cereals processing technology, CRC Press, Boca Raton, p 204-230.

Cerning, J., 1990. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiology Letters, 87 (1): 113-130.

Cerning, J., 1995. Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. Lait, 75 (4-5): 463-472.

Chaoui, A., Faid, M. and Belhcen, R., 2003. Effect of natural starters used for sourdough bread in Morocco on phytate biodegradation. Eastern Mediterranean Health Journal, 9 (1-2): 141-147.

Chavan, R. S. and 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.

Clesceri, L. S., Greenberg, A. E. and Eaton, A. D., 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, DC.

Coda, R., Cassone, A., Rizzello, C. G., Nionelli, L., Cardinali, G. and Gobbetti, M., 2011. Antifungal Activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during Sourdough Fermentation: Identification of Novel Compounds and Long-Term Effect during Storage of Wheat Bread. Applied and Environmental Microbiology, 77 (10): 3484-3492.

Coda, R., Kianjam, M., Pontonio, E., Verni, M., Di Cagno, R., Katina, K., Rizzello, C. G. and Gobbetti, M., 2017. Sourdough-type propagation of faba bean flour: Dynamics of microbial consortia and biochemical implications. International Journal of Food Microbiology, 248: 10-21.

Codina, G., Mironeasa, S., V. Voica, D. and Mironeasa, C., 2013. Multivariate Analysis of Wheat Flour Dough Sugars, Gas Production, and Dough Development at Different Fermentation Times. Czech Journal of Food Sciences, 31(3): 222-229.

Collins, M. D., Samelis, J., Metaxopoulos, J. and Wallbanks, S., 1993. Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. The Journal of Applied Bacteriology, 75 (6): 595-603.

Corona, O., Alfonzo, A., Ventimiglia, G., Nasca, A., Francesca, N., Martorana, A., Moschetti, G. and Settanni, L., 2016. Industrial application of selected

lactic acid bacteria isolated from local semolinas for typical sourdough bread production. Food Microbiology, 59: 43-56.

Corsetti, A., 2013. Technology of Sourdough Fermentation and Sourdough Applications (M. Gobbetti and M. G. Gänzle Editors). Handbook on Sourdough Biotechnology, Springer US, Boston, MA, p 85-103.

Corsetti, A., Gobbetti, M., Rossi, J. and Damiani, P., 1998. Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. Applied Microbiology and Biotechnology, 50 (2): 253-256.

Corsetti, A., Gobbetti, M. and Smacchi, E., 1996. Antibacterial activity of sourdough lactic acid bacteria: isolation of a bacteriocin-like inhibitory substance from *Lactobacillus sanfrancisco* C57. Food Microbiology, 13 (6): 447-456.

Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F., Tosti, N. and Gobbetti, M., 2001. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. International Journal of Food Microbiology, 64 (1-2): 95-104.

Corsetti, A. and Settanni, L., 2007. Lactobacilli in sourdough fermentation. Food Research International, 40 (5): 539-558.

Corsetti, A., Settanni, L., Lopez, C. C., Felis, G. E., Mastrangelo, M. and Suzzia, G., 2007a. A taxonomic survey of lactic acid bacteria isolated from wheat (*Triticum durum*) kernels and non-conventional flours. Systematic and Applied Microbiology, 30: 561-571.

Corsetti, A., Settanni, L., Valmorri, S., Mastrangelo, M. and Suzzi, G., 2007b. Identification of subdominant sourdough lactic acid bacteria and their

evolution during laboratory-scale fermentations. Food Microbiology, 24 (6): 592-600.

Corsetti, A., Settanni, L. and Sinderen, D. V., 2004. Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their in vitro and in situ activity. Journal of Applied Microbiology, 96 (3): 521-534.

Coton, E., Coton, M. and Guichard, H., 2016. Cider (Cyder; Hard Cider): The Product and Its Manufacture. Reference Module in Food Science Encyclopedia of Food and Health, p 119-128.

Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., Fall, A., Daube, G. and Coton, E., 2017. Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. FEMS Microbiology Ecology, 93 (5): fix048.

Cotton, R. H. and Ponte, J. G., 1973. Baking industry (G. E. Inglett Editor). Wheat: Production and Utilization. Avi Publishing Company, Westport, p 192-332.

Çebi, K., 2009. Nohut Mayası ve Hamurundan Laktik Asit Bakterilerinin İzolasyonu ve İdentifikasyonu *(Isolation and Identification of Lactic Acid Bacteria From Chickpea Yeast And Dough).* Msc Thesis, Atatürk University, Graduate School of Natural and Applied Sciences, Department of Food Engineering, Erzurum, Turkey, 72 p *(in Turkish).*

Çebi, K., 2014. Nohut Mayası/Hamurundan İzole Edilen Laktik Asit Bakteri Suşlarının Ekmeğin Uçucu Profili Ve Diğer Bazı Kalite Parametreleri Üzerine Etkileri *(The Effects Of Lactıc Acıd Bacterıa Straıns Isolated From Chıckpea Fermentatıon/Dough Products On Volatıle Profile And Other Qualıty Parameters Of Bread).* PhD Thesis, Atatürk University, Graduate

School of Natural and Applied Sciences, Department of Food Engineering, Erzurum, Turkey, 95 p *(in Turkish).*

De Angelis, M., Damiano, N., Rizzello, C. G., Cassone, A., Di Cagno, R. and Gobbetti, M., 2009. Sourdough fermentation as a tool for the manufacture of low-glycemic index white wheat bread enriched in dietary fibre. European Food Research and Technology, 229 (4): 593-601.

De Angelis, M., Gallo, G., Corbo, M. R., Mcsweeney, P. L., Faccia, M., Giovine, M. and Gobbetti, M., 2003. Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. International Journal of Food Microbiology, 87 (3): 259-270.

De Angelis, M., Rizzello, C. G., Alfonsi, G., Arnault, P., Cappelle, S., Di Cagno, R. and Gobbetti, M., 2007. Use of sourdough lactobacilli and oat fibre to decrease the glycaemic index of white wheat bread. British Journal of Nutrition, 98 (6): 1196-1205.

De Vuyst, L. and Degeest, B., 1999. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiology Reviews, 23 (2): 153-177.

De Vuyst, L., Harth, H., Van Kerrebroeck, S. and Leroy, F., 2016. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. International Journal of Food Microbiology, 239: 26-34.

De Vuyst, L. and Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions. Trends in Food Science & Technology, 16 (1): 43-56.

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H. M. and Weckx, S., 2014. Microbial ecology of sourdough fermentations: Diverse or uniform? Food Microbiology, 37: 11-29.

De Vuyst, L., Van Kerrebroeck, S. and Leroy, F., 2017. Microbial Ecology and Process Technology of Sourdough Fermentation. Advances in Applied Microbiology, 100: 49-160.

Dertli, E., Mercan, E., Arıcı, M., Yılmaz, M. T. and Sağdıç, O., 2016. Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. LWT - Food Science and Technology, 71: 116-124.

Deshpande, S. S. and Damodaran, S., 1990. Food legumes: chemistry and technology (Y. Pomeranz Editor). Advances in Cereal Science and Technology, Vol. X. American Association of Cereal Chemists, St. Paul, MN, p 147-241.

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., Gaenzle, M., Ciati, R. and Gobbetti, M., 2006. Glucan and fructan production by sourdough *Weissella cibaria* and *Lactobacillus plantarum*. The Journal of Agricultural and Food Chemistry, 54 (26): 9873-9881.

Di Cagno, R., Pontonio, E., Buchin, S., De Angelis, M., Lattanzi, A., Valerio, F., Gobbetti, M. and Calasso, M., 2014. Diversity of the Lactic Acid Bacterium and Yeast Microbiota in the Switch from Firm- to Liquid-Sourdough Fermentation. Applied and Environmental Microbiology, 80 (10): 3161-3172.

Dufour, N., Swana, J. and Rao, R. P., 2011. Fermentation organisms for 5- and 6-carbon sugars (E. E. Hood, P. Nelson and R. Powell Editors). Plant Biomass Conversion, John Wiley & Sons**.,** Chichester, U.K., p 157-198.

Endo, A. and Dicks, L. M. T., 2014. Physiology of the LAB (W. H. Holzapfel and B. J. B. Wood Editors). Lactic Acid Bacteria Biodiversity and Taxonomy. John Wiley & Sons. Ltd., West Sussex, p 13-30.

Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Storia, A., Gobbetti, M. and Di Cagno, R., 2013. Microbial Ecology Dynamics during Rye and Wheat Sourdough Preparation. Applied and Environmental Microbiology, 79 (24): 7827-7836.

Erginkaya, Z., Unal Turhan, E. and Ozer, E. A., 2016. Nohut Mayalı Ekmek Üretimi ve Hakim Mikroflora *(The Production of Bread with Chickpea Ferment and Dominant Microflora).* Journal of Agricultural Faculty of Uludag University, 30 (1): 89-99 *(in Turkish).*

Erkmen, O. and Bozoglu, T. F., 2016. Food Microbiology Principles into Practice, Volume 1- Microorganisms related to foods, foodborne diseasesand food spoilage. West Sussex, UK, Wiley, 458 p.

Erten, H., Agirman, B., Boyaci Gunduz, C. P. and Ben Ghorbal, A., 2017. Regional Fermented Vegetables and Fruits in Europe (S. Paramithiotis Editor). Lactic Acid Fermentation of Fruits and Vegetables, CRC Press, Boca Raton, p 206-235.

Erten, H., Boyacı-Gündüz, C. P., Agirman, B. and Cabaroglu, T., 2016. Fermentation, pickling and Turkish table olives (Y. H. Hui and E. Özgül Evranuz Editors). Handbook of Vegetable Preservation and Processing, Second Edition. CRC Press, Boca Raton, p 209-230.

Erten, H. and Tangüler, H., 2014. Plant-based fermented products (N. Aran Editor). Food Biotechnology, Nobel Yayınevi. Ankara, p 244-274 *(in Turkish).*

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A., 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. International Journal of Systematic Bacteriology, 49 (1): 329-337.

Estevez, A. M., Figuerola, F., Vasquez, M., Castillo, E. and Yanez, E., 1987. Supplementation of wheat flour with chickpea (Cicer arietinum) flour. II.

Chemical composition and biological quality of breads made with blends of the same. Archivos Latinoamericanos de Nutrición, 37 (3): 515-524.

Fardet, A., Leenhardt, F., Lioger, D., Scalbert, A. and Rémésy, C., 2006. Parameters controlling the glycaemic response to breads. Nutrition Research Reviews, 19 (1): 18-25.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791.

Feng, P., Weagant , S. D., Grant , M. A. and Burkhardt, W., 2002. BAM 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. FDA Laboratory-method. https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm064948.htm (Accession date:20/05/2018).

Fincher, G. B. and Stone, B. A., 1986. Cell wall and their components in cereal grain technology (Y. Pomeranz Editor). Advances in cereal science and technology. American Association Cereal Chemists, St. Paul, MN, p 207-295.

Fob, 2011. History of bread. Consumer Factsheet No. 9. https://www.fob.uk.com/wp-content/uploads/2017/01/FS-9-History-of-Bread.pdf (Accession date:11/05/2018).

Francesca, N., Carvalho, C., Sannino, C., Guerreiro, M. A., Almeida, P. M., Settanni, L., Massa, B., Sampaio, J. P. and Moschetti, G., 2014. Yeasts vectored by migratory birds collected in the Mediterranean island of Ustica and description of *Phaffomyces usticensis* f.a. sp. nov., a new species related to the cactus ecoclade. FEMS Yeast Research, 14 (6): 910-921.

Frengova, G. I., Simova, E. D., Beshkova, D. M. and Simov, Z. I., 2000. Production and monomer composition of exopolysaccharides by yogurt starter cultures. Canadian Journal of Microbiology, 46 (12): 1123-1127.

Fretzdorff, B. and Brummer, J. M., 1992. Reduction of Phytic Acid During Breadmaking of Whole-Meal Breads. Cereal Chemistry, 69 (3): 266-270.

Fujimoto, A., Ito, K., Itou, M., Narushima, N., Ito, T., Yamamoto, A., Hirayama, S., Furukawa, S., Morinaga, Y. and Miyamoto, T., 2018. Microbial behavior and changes in food constituents during fermentation of Japanese sourdoughs with different rye and wheat starting materials. Journal of Bioscience and Bioengineering, 125 (1): 97-104.

Fusco, V., Quero, G. M., Cho, G.-S., Kabisch, J., Meske, D., Neve, H., Bockelmann, W. and Franz, C. M. a. P., 2015. The genus *Weissella*: taxonomy, ecology and biotechnological potential. Frontiers in Microbiology, 6: 155.

Gaggia, F., Di Gioia, D., Baffoni, L. and Biavati, B., 2011. The role of protective and probiotic cultures in food and feed and their impact in food safety. Trends in Food Science & Technology, 22: S58-S66.

Gaglio, R., Alfonzo, A. and Francesca, N., 2017. Combined Approach for the Investigation of Dominant Fermenting Microbiota in Two Traditional Sourdoughs Produced in Sicily. Carpathian Journal of Food Science and Technology, 9 (2): 5-15.

Galle, S., 2013. Sourdough: A Tool to Improve Bread Structure (M. Gobbetti and M. Gänzle Editors). Handbook on Sourdough Biotechnology. Springer, Boston, MA, p 217-228.

Galle, S., Schwab, C., Arendt, E. and Ganzle, M., 2010. Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. The Journal of Agricultural and Food Chemistry, 58 (9): 5834-5841.

Gänzle, M. and Gobbetti, M., 2013. Physiology and Biochemistry of Lactic Acid Bacteria (M. Gobbetti and M. Gänzle Editors). Handbook on Sourdough Biotechnology, Springer US, Boston, MA, p 183-216.

Ganzle, M. G., 1998. Useful Properties of Lactobacilli for Application as Protective Cultures in Food. PhD thesis, University of Hohenheim, Germany.

Ganzle, M. G., 2014a. Enzymatic and bacterial conversions during sourdough fermentation. Food Microbiology, 37: 2-10.

Ganzle, M. G., 2014b. Sourdough bread (C. A. Batt and M. L. Tortorello Editors). Encyclopedia of Food Microbiology Second Edition, Elsevier, USA, p 309-315.

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

Garrity, G. M. and Holt, J. G., 2001. The Road Map to the Manual (D. R. Boone, R. W. Castenholz and G. M. Garrity Editors). Bergey's Manual® of Systematic Bacteriology Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria, Springer, New York, NY, p 119-166.

Gerez, C. L., Rollan, G. C. and De Valdez, G. F., 2006. Gluten breakdown by lactobacilli and pediococci strains isolated from sourdough. Letters in Applied Microbiology, 42 (5): 459-464.

Gobbetti, M., 1998. The sourdough microflora: Interactions of lactic acid bacteria and yeasts. Trends in Food Science & Technology, 9 (7): 267-274.

Gobbetti, M. and Corsetti, A., 1997. *Lactobacillus sanfrancisco* a key sourdough lactic acid bacterium:a review. Food Microbiology, 14 (2): 175-187.

Gobbetti, M., De Angelis, M., Arnaut, P., Tossut, P., Corsetti, A. and Lavermicocca, P., 1999. Added pentosans in breadmaking: fermentations

of derived pentoses by sourdough lactic acid bacteria. Food Microbiology, 16 (4): 409-418.

Gobbetti, M., Rizzello, C. G., Di Cagno, R. and De Angelis, M., 2014. How the sourdough may affect the functional features of leavened baked goods. Food Microbiology, 37: 30-40.

Gobbetti, M., Simonetti, M. S., Rossi, J., Cossignani, L., Corsetti, A. and Damiani, P., 1994. Free D- and L-Amino Acid Evolution During Sourdough Fermentation and Baking. Journal of Food Science, 59 (4): 881-884.

Gobbetti, M., Smacchi, E. and Corsetti, A., 1996a. The proteolytic system of *Lactobacillus sanfrancisco* CB1: purification and characterization of a proteinase, a dipeptidase, and an aminopeptidase. Applied and Environmental Microbiology, 62 (9): 3220-3226.

Gobbetti, M., Smacchi, E., Fox, P., Stepaniak, L. and Corsetti, A., 1996b. The Sourdough Microflora. Cellular Localization and Characterization of Proteolytic Enzymes in Lactic Acid Bacteria. LWT - Food Science and Technology, 29 (5): 561-569.

Gonzalez, R., 2006. Metabolic Engineering of Bacteria for Food Ingredients (K. Shetty, G. Paliyath, A. Pometto and R. E. Levin Editors). Food Biotechnology Second edition, Taylor & Francis Group, Boca Raton, FL, p 133-152.

Gordún, E., Puig, A., Piñol and Carbó, R., 2018. Identification of yeast isolated from laboratory sourdoughs prepared with grape, apple and yogurt. The Journal of Microbiology, Biotechnology and Food Sciences, 7 (4): 399-403.

Green , P. H. R. and Cellier , C., 2007. Celiac Disease. New England Journal of Medicine, 357 (17): 1731-1743.

Gül, H., Özçelik, S., Sağdıç, O. and Certel, M., 2005. Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. Process Biochemistry, 40 (2): 691-697.

Güler, F. and Özcelik, F., 2017. An adaptation strategy to improve the proteolytic activities of lactic acid bacteia isolated from pickles. GIDA, 42 (5): 561-567.

Halkman, K., 2005. Merck Gıda Mikrobiyolojisi Uygulamaları, Başak Matbaacılık Ltd. Şti., Ankara, Turkey, 358 sayfa *(in Turkish).*

Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 41: 95-98.

Hamad, S. H., Böcker, G., Vogel, R. F. and Hammes, W. P., 1992. Microbiological and chemical analysis of fermented sorghum dough for Kisra production. Applied Microbiology and Biotechnology, 37 (6): 728-731.

Hames, B. D. and Hooper, N. M., 2000. Instant Notes Biochemistry, Second Edition. BIOS Scientific Publishers Limited, Oxford, UK, 422 p.

Hammes, W. P., 1991. Fermentation of non-dairy foods. Food Biotechnology, 5 (3): 293-303.

Hammes, W. P. and Ganzle, M., 1998. Sourdough breads and related products (B. J. B. Woods Editor). Microbiology of Fermented Foods, Blackie Academic and Professional, London, UK, p 199-216.

Hammes, W. P. and Hertel, C., 2009. Genus I. *Lactobacillus*. Beijerink 1901 (P. Devos, Garrity, G.M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K-H., Whitman, W. B. Editors). Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 3 The Firmicutes. Springer Science+Business Media, New York, USA, p 465–510.

Hammes, W. P., Weiss, N. and Holzapfel, W. P., 1991. The genera *Lactobacillus* and *Carnobacterium* (A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K. H. Schleifer Editors). The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation Identification, Applications, Springer, New York, p 1535-1594.

Hancıoglu-Sıkılı, O., 2003. Nohut Mayasının Mikrobiyolojik ve Lezzet Karakteristiklerinin Araştırılması *(Investigation of microbiological and flavour characteristics of chickpea sweet dough).*PhD Thesis, Ege University, İzmir, Turkey, 203 p *(in Turkish).*

Hannan, R., Açıkgöz, N. and Robertson, L. D., 2001. Chickpeas (*Cicer* L.) (N. Maxted and S. J. Bennett Editors). Plant Genetic Resources of Legumes in the Mediterranean Current Plant Science and Biotechnology in Agriculture, vol 39, Springer, Dordrecht, p 115-124.

Hansen, Å. and Hansen, B., 1996. Flavour of sourdough wheat bread crumb. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 202 (3): 244-249.

Hansen, Å. S., 2012. Sourdough Bread (E. Ö. Evranuz, Y. H. Hui, F. N. Arroyo-López, L. Fan, A. S. Hansen , M. E. Jaramillo-Flores, M. Rakin, R. F. Schwan, W. Zhou Editors). Handbook of Plant-Based Fermented Food and Beverage Technology, CRC Press, Boca Raton, p 493-515.

Hardin, J., Bertoni, G. and Kleinsmith, L. J., 2012. Becker's World of the Cell. Pearson Education, Inc., San Francisco, 914 p.

Hassan, R., El-Kadi, S. and Sand, M., 2015. Effect of some organic acids on some fungal growth and their toxins production. International Journal of Advances in Biology, 2 (1): 1-11.

Hatzikamari, M., Kyriakidis, D. A., Tzanetakis, N., Biliaderis, C. G. and Litopoulou-Tzanetaki, E., 2007a. Biochemical changes during a submerged

chickpea fermentation used as a leavening agent for bread production. European Food Research and Technology, 224 (6): 715.

Hatzikamari, M., Yiangou, M., Tzanetakis, N. and Litopoulou-Tzanetaki, E., 2007b. Changes in numbers and kinds of bacteria during a chickpea submerged fermentation used as a leavening agent for bread production. International Journal of Food Microbiology, 116 (1): 37-43.

He, G.-Q., Kong, Q., Chen, Q.-H. and Ruan, H., 2005. Batch and fed-batch production of butyric acid by *Clostridium butyricum* ZJUCB. Journal of Zhejiang University, Science B, 6 (11): 1076-1080.

Helbaek, H., 1970. The plant husbandry at Hacillar. (J. Mellaart Editor). Excavation at Hacillar, Edinburgh University Press, Edinburgh , p 189-244.

Hoang, V. A., Kim, Y. J., Nguyen, N. L., Kim, S. K. and Yang, D. C., 2015. *Lactobacillus vespulae* sp. nov., isolated from gut of a queen wasp (*Vespula vulgaris*). International Journal of Systematic and Evolutionary Microbiology, 65 (10): 3326-3332.

Holzapfel, W. H. and Wood, B. J. B., 2014. Introduction to the LAB (W. H. Holzapfel and B. J. B. Wood Editors). Lactic Acid Bacteria Biodiversity and Taxonomy, John Wiley & Sons, Ltd., West Sussex, p 1-12.

Hoseney, R. C., 1994. Yeast-leavened products, Principles of Cereal Science and Technology, 2nd ed, AACC, St. Paul, MN, Inc. p 229-274.

Hoseney, R. C., Wade, P. and W, F. J., 1988. Soft wheat products (Y. Pomeranz Editor). Wheat chemistry and technology, American Association of Cereal Chemists, St. Paul, Minn, p 407-456.

Huang, J. Y., Kao, C. Y., Liu, W. S. and Fang, T. J., 2017. Characterization of high exopolysaccharide-producing *Lactobacillus* strains isolated from mustard

pickles for potential probiotic applications. International Microbiology: the Official Journal of the Spanish Society for Microbiology, 20 (2): 75-84.

Hutkins, R. W., 2006. Microbiology and Technology of Fermented Foods. Blackwell Publishing, Iowa, 475 p.

Iacumin, L., Cecchini, F., Manzano, M., Osualdini, M., Boscolo, D., Orlic, S. and Comi, G., 2009. Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. Food Microbiology, 26 (2): 128-135.

Jay, J. M., Loessner, M. J. and Golden, D. A., 2005. Modern Food Microbiology, Springer, New York, 782 p.

Johnston, M., 1999. Feasting, fasting and fermenting: glucose sensing in yeast and other cells. Trends in Genetics, 15 (1): 29-33.

Jolly, L., Vincent, S. J. F., Duboc, P. and Neeser, J.-R., 2002. Exploiting exopolysaccharides from lactic acid bacteria. Antonie van Leeuwenhoek, 82 (1): 367-374.

Joshi, S. R. and Koijam, K., 2014. Exopolysaccharide Production by a Lactic Acid Bacteria, *Leuconostoc lactis* Isolated from Ethnically Fermented Beverage. National Academy Science Letters, 37 (1): 59-64.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N., 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. British Journal of Nutrition, 108 (S1): S11-S26.

Kang, H. C., Park, Y. H. and Go, S. J., 2003. Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. Microbiological Research, 158 (4): 321-326.

Kasım, S., 2014. Ege Bölgesi'nde Geleneksel Olarak Nohut Mayası İle Üretilen Simit Ekmeği Yapımının Optimizasyonu. Msc Thesis, Celal Bayar University, Manisa, Turkey 95 p *(in Turkish).*

Katina, K., Laitila, A., Juvonen, R., Liukkonen, K. H., Kariluoto, S., Piironen, V., Landberg, R., Åman, P. and Poutanen, K., 2007. Bran fermentation as a means to enhance technological properties and bioactivity of rye. Food Microbiology, 24 (2): 175-186.

Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M. and Laitila, A., 2009. In situ production and analysis of *Weissella confusa* dextran in wheat sourdough. Food Microbiology, 26 (7): 734-743.

Katina, K. and Poutanen, K., 2013. Nutritional Aspects of Cereal Fermentation with Lactic Acid Bacteria and Yeast (M. Gobbetti and M. Gänzle Editors). Handbook on Sourdough Biotechnology, Springer US, Boston, MA, p 229-244.

Katsaboxakis, K. and Mallidis, K., 1996. The microflora of soak water during natural fermentation of coarsely ground chickpea (*Cicer arietinum*) seeds. Letters in Applied Microbiology, 23 (4): 261-265.

Kleerebezem, M. and Hugenholtz, J., 2003. Metabolic pathway engineering in lactic acid bacteria. Current Opinion in Biotechnology, 14 (2): 232-237.

Kline, L. and Sugihara, T. F., 1971. Microorganisms of the San Francisco Sour Dough Bread Process: II. Isolation and Characterization of Undescribed Bacterial Species Responsible for the Souring Activity. Applied Microbiology, 21 (3): 459-465.

Knudsen, S., 1924. Milchsäurenbakteries des Sauerteiges und ihre Bedeutung für die Sauerteiggärung. Den Kongelige Veterinær-og Landbohøjskole, Årsskrift. Copenhagen, Kandrup and Wunsch. 133-186.

Korakli, M., Rossmann, A., Ganzle, M. G. and Vogel, R. F., 2001. Sucrose metabolism and exopolysaccharide production in wheat and rye

sourdoughs by *Lactobacillus sanfranciscensis*. Journal of Agricultural and Food Chemistry, 49 (11): 5194-5200.

Kotzekidou, P. and Tsakalidou, E., 2006. Fermentation Biotechnology of Plant Based Traditional Foods of the Middle East and Mediterranean Region (K. Shetty, G. Paliyath, A. Pometto and R. E. Levin Editors). Food Biotechnology, Second Edition. CRC Press, Boca Raton, FL, p 1780-1813.

Köksel, H., Sivri, D., Scanlon, M. G. and Bushuk, W., 1998. Comparison of physical properties of raw and roasted chickpeas (leblebi). Food Research International, 31 (9): 659-665.

Krauß, I., 1994. Heute back' ich, morgen brau' ich. Eiselen Stiftung Ulm, Ulm.

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

Kurtzman, C. P. and Robnett, C. J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek, 73 (4): 331-371.

Kusmiati, Fe, K. and F, A., 2016. Exopolysaccharide (EPS) activity test of lactic acid bacteria (LAB) as immunomodulatory. JITV 21 (3): 182-189.

Lacaze, G., Wick, M. and Cappelle, S., 2007. Emerging fermentation technologies: Development of novel sourdoughs. Food Microbiology, 24 (2): 155-160.

Ladizinsky, G., 1975. A new cicer from Turkey. Notes from the Royal Botanical Garden.

Lai, H. M. and Lin, T. C., 2006. Bakery Products: Science and Technology (Y. H. Hui, H. Corke, I. D. Leyn, W.-K. Nip and N. Cross Editors). Bakery Products: Science and Technology. Blackwell Publishing, Iowa, p 3-68.

Laitila, A., Wilhelmson, A., Kotaviita, E., Olkku, J., Home, S. and Juvonen, R., 2006. Yeasts in an industrial malting ecosystem. Journal of Industrial Microbiology & Biotechnology, 33 (11): 953-966.

Larsen, A. G., Vogensen, F. K. and Josephsen, J., 1993. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401. The Journal of Applied Bacteriology, 75 (2): 113-122.

Lattanzi, A., Minervini, F., Di Cagno, R., Diviccaro, A., Antonielli, L., Cardinali, G., Cappelle, S., De Angelis, M. and Gobbetti, M., 2013. The lactic acid bacteria and yeast microbiota of eighteen sourdoughs used for the manufacture of traditional Italian sweet leavened baked goods. International Journal of Food Microbiology, 163 (2-3): 71-79.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A. and Gobbetti, M., 2000. Purification and Characterization of Novel Antifungal Compounds from the Sourdough *Lactobacillus plantarum* Strain 21B. Applied and Environmental Microbiology, 66 (9): 4084-4090.

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E. and Remesy, C., 2005. Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. Journal of Agricultural and Food Chemistry, 53 (1): 98-102.

León Peláez, A. M., Serna Cataño, C. A., Quintero Yepes, E. A., Gamba Villarroel, R. R., De Antoni, G. L. and Giannuzzi, L., 2012. Inhibitory activity of lactic and acetic acid on Aspergillus flavus growth for food preservation. Food Control, 1 (2): 177-183.

Leroy, F. and De Vuyst, L., 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science & Technology, 15: 67-78.

Lhomme, E., Lattanzi, A., Dousset, X., Minervini, F., De Angelis, M., Lacaze, G., Onno, B. and Gobbetti, M., 2015. Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. International Journal of Food Microbiology, 215: 161-170.

Lhomme, E., Urien, C., Legrand, J., Dousset, X., Onno, B. and Sicard, D., 2016. Sourdough microbial community dynamics: An analysis during French organic bread-making processes. Food Microbiology, 53: 41-50.

Liljeberg, H. G., Lonner, C. H. and Bjorck, I. M., 1995. Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. The Journal of Nutrition, 125 (6): 1503-1511.

Lim, S. B., Tingirikari, J. M. R., Seo, J. S., Li, L., Shim, S., Seo, J. H. and Han, N. S., 2018. Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. The Food Science and Biotechnology, 27 (1): 73-78.

Liu, T., Li, Y., Sadiq, F. A., Yang, H., Gu, J., Yuan, L., Lee, Y. K. and He, G., 2018. Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread. Food Chemistry, 242: 404-411.

Lopez, H. W., Krespine, V., Guy, C., Messager, A., Demigne, C. and Remesy, C., 2001. Prolonged fermentation of whole wheat sourdough reduces phytate level and increases soluble magnesium. Journal of Agricultural and Food Chemistry, 49 (5): 2657-2662.

Lopez, H. W., Leenhardt, F., Coudray, C. and Remesy, C., 2002. Minerals and phytic acid interactions: is it a real problem for human nutrition? International Journal of Food Science & Technology, 37 (7): 727-739.

Loponen, J., 2006. Prolamin degradation in sourdoughs. Dissertation, University of Helsinki, Department of Food Technology, Helsinki, Finland, 78 p.

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T. and Salovaara, H., 2004. Degradation of HMW Glutenins During Wheat Sourdough Fermentations. Cereal Chemistry, 81 (1): 87-93.

Luangsakul, N., Keeratipibul, S., Jindamorakot, S. and Tanasupawat, S., 2009. Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. LWT - Food Science and Technology, 42 (8): 1404-1412.

Lule, V., Singh, R., Behare, P. and Tomar, S. K., 2015. Comparison of exopolysaccharide production by indigenous *Leuconostoc mesenteroides* strains in whey medium. Asian Journal of Dairy And Food Research, 34 (1): 8-12.

Macfaddin, J. F., 2000. Biochemical Tests for Identification of Medical Bacteria. Williams and Wilkins, Philadelphia.

Madan, M. and Thind, K. S., 2000. Physiology of fungi, APH Publishing Corporation, New Delhi, India, p 52-54.

Madigan, M. T., Martinko, J. M., Stahl, D. A. and Clark, D. P., 2012. Biology of Microorganisms, 13th edn., Pearson Education Inc., . New York, p 1155.

Maioli, M., Pes, G. M., Sanna, M., Cherchi, S., Dettori, M., Manca, E. and Farris, G. A., 2008. Sourdough-leavened bread improves postprandial glucose and insulin plasma levels in subjects with impaired glucose tolerance. Acta Diabetologica, 45 (2): 91-96.

Malik, A., Sheilla, S., Firdausi, W., Handayani, T. and Saepudin, E., 2015. Sucrase Activity and Exopolysaccharide Partial Characterization From Three *Weissella confusa* Strains. HAYATI Journal of Biosciences, 22: 130-135.

Manley, D., 2000. Technology of biscuits, crackers and cookies, Woodhead Publishing, Cambridge, England, 528 p.

Mariotti, M., Garofalo, C., Aquilanti, L., Osimani, A., Fongaro, L., Tavoletti, S., Hager, A.-S. and Clementi, F., 2014. Barley flour exploitation in sourdough bread-making: A technological, nutritional and sensory evaluation. LWT - Food Science and Technology, 59 (2): 973-980.

Martinez-Anaya, M. A. and Devesa, A., 2000. Influence of enzymes in sourdough wheat breadmaking. Changes in pentosans. Food Science and Technology International, 6: 109-116.

Mayo, B., Aleksandrzak-Piekarczyk, T., Fernández, M., Kowalczyk, M., Álvarez-Martín, P. and Bardowski, J., 2010. Updates in the Metabolism of Lactic Acid Bacteria (F. Mozzi, R. R. Raya, G. M. Vignolo Editors). Biotechnology of Lactic Acid Bacteria, Wiley-Blackwell, Iowa, USA, p 3-33.

Mckay, M., Buglass, A. J. and Lee, C. G., 2011. Fermented beverages: Beers, ciders, wines and related drinks (A. J. Buglass Editor). Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects, John Wiley & Sons., Chichester, U.K., p 63-454.

Menteş, Ö., Akçelik, M. and Ercan, R., 2004. Türkiyede Üretilen Ekşi Hamurlardan *Lactobacillus* Suşlarının İzolasyonu, İdentifikasyonu ve Bu Suşların Temel Endüstriyel Özellikleri. Gıda, 29 (5): 347-355 *(in Turkish).*

Menteş, Ö., Ercan, R. and Akçelik, M., 2005. Türkiye'de üretilen ekşi hamurlardan elde edilen *Lactobasillus* suşlarının anti-bakteriyel aktivitelerinin belirlenmesi.. Gıda, 3: 155–164 *(in Turkish).*

Menteş, Ö., Ercan, R. and Akçelik, M., 2007. Inhibitor activities of two *Lactobacillus* strains, isolated from sourdough, against rope-forming *Bacillus* strains. Food Control, 18 (4): 359-363.

Meroth, C. B., Hammes, W. P. and Hertel, C., 2004. Characterisation of the Microbiota of Rice Sourdoughs and Description of *Lactobacillus spicheri* sp. nov. Systematic and Applied Microbiology, 27 (2): 151-159.

Meroth, C. B., Walter, J., Hertel, C., Brandt, M. J. and Hammes, W. P., 2003. Monitoring the Bacterial Population Dynamics in Sourdough Fermentation Processes by Using PCR-Denaturing Gradient Gel Electrophoresis. Applied and Environmental Microbiology, 69 (1): 475-482.

Messens, W. and De, V. L., 2002. Inhibitory substances produced by *Lactobacilli* isolated from sourdoughs--a review. International Journal of Food Microbiology, 72 (1-2): 31-43.

Middelhoven, W. J., Kurtzman, C. P. and Vaughan-Martini, A., 2000. *Saccharomyces bulderi* sp. nov., a yeast that ferments gluconolactone. Antonie Van Leeuwenhoek, 77 (3): 223-228.

Mikelsaar, M., Sepp, E., Stsepetova, J., Songisepp, E. and Mandar, R., 2016. Biodiversity of Intestinal Lactic Acid Bacteria in the Healthy Population. Advances in Experimental Medicine and Biology, 932: 1-64.

Minervini, F., De Angelis, M., Di Cagno, R., Pinto, D., Siragusa, S., Rizzello, C. G. and Gobbetti, M., 2010. Robustness of *Lactobacillus plantarum* starters during daily propagation of wheat flour sourdough type I. Food Microbiology, 27 (7): 897-908.

Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappellec, S. and Gobbettia, M., 2012a. 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.

Minervini, F., Lattanzi, A., De Angelis, M., Celano, G. and Gobbetti, M., 2015. House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs. Food Microbiology, 52: 66-76.

Minervini, F., Lattanzi, A., De Angelis, M., Di Cagno, R. and Gobbetti, M., 2012b. Influence of artisan bakery- or laboratory-propagated sourdoughs on the diversity of lactic acid bacterium and yeast microbiotas. Applied and Environmental Microbiology, 78 (15): 5328-5340.

Mohammed, I., Ahmed, A. R. and Senge, B., 2012. Dough rheology and bread quality of wheat–chickpea flour blends. Industrial Crops and Products, 36 (1): 196-202.

Moroni, A. V., Dal Bello, F. and Arendt, E. K., 2009. Sourdough in gluten-free bread-making: An ancient technology to solve a novel issue? Food Microbiology, 26 (7): 676-684.

Morris, P. C. and Bryce, J. H., 2000. Introduction (P. C. Morris and J. H. Bryce Editors). Cereal biotechnology, Woodhead Publishing, Cambridge, p 10-24.

Muehlbauer, F. J. and Sarker, A., 2017. Economic Importance of Chickpea: Production, Value, and World Trade (R. K. Varshney, M. Thudi and F. Muehlbauer Editors). The Chickpea Genome. Springer International Publishing, Switzerland, p 5-12.

Müller, M. R. A., Wolfrum, G., Stolz, P., Ehrmann, M. A. and Vogel, R. F., 2001. Monitoring the growth of *Lactobacillus* species during a rye flour fermentation. Food Microbiology, 18 (2): 217-227.

Narlıoglu, G., 2013. Geleneksel Nohut Mayasının ve Üretiminde Kullanıldığı Poğaçaların Bazı Özelliklerinin İncelenmesi *(Investigation of some Properties of Traditional Chickpea Yeast And it's use in Pastry)*. Msc

Thesis, Kahramanmaraş Sütçü İmam University Natural and Applied Sciences, Kahramanmaraş, Turkey, 70 p *(in Turkish).*

Nei, M. and Kumar, S., 2000. Molecular Evolution and Phylogenetics, Oxford University Press, New York, 333 p.

Nilsson, M., Holst, J. J. and Bjorck, I. M., 2007. Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. The American Journal of Clinical Nutrition, 85 (4): 996-1004.

Obis, D., Guillot, A. and Mistou, M. Y., 2001. Tolerance to high osmolality of *Lactococcus lactis* subsp. *lactis* and *cremoris* is related to the activity of a betaine transport system. FEMS Microbiology Letters, 202 (1): 39-44.

Oguntoyinbo, F. A. and Franz, C. M. A. P., 2017. Regional Fermented Fruits and Vegetables in Africa (S. Paramithiotis Editor). Lactic Acid Fermentation of Fruits and Vegetables, CRC Press, Boca Raton, p 237-266.

Okafor, N., 2007. Modern Industrial Microbiology and Bitechnology, Science Publishers, New Hampshire, 551 p.

Onbasli, D. and Aslim, B., 2008. Determination of antimicrobial activity and production of some metabolites by *Pseudomonas aeruginosa* B1 and B2 in sugar beet molasses. African Journal of Biotechnology 7(24): 4614-4619.

Osborne, T. B., 1907. The protein of the wheat kernel, Publication No. 84. Washington, DC, Carnegie Institute of Washington.

Osińska-Jaroszuk, M., Wlizło, K., Szałapata, K. and Jarosz-Wilkołazka, A., 2014. Correlation between the production of exopolysaccharides and oxalic acid secretion by *Ganoderma applanatum* and *Tyromyces palustris*. World Journal of Microbiology & Biotechnology, 30 (12): 3065-3074.

Ostman, E., 2003. Fermentation as a Means of Optimizing the Glycaemic Index-Food Mechanisms and Metabolic Merits with Emphasis on Lactic Acid in

Cereal Products. PhD thesis, Lund University, Department of Food Technology, Sweden, 120 p.

Özkaya, B., 1992. Starter Kültür Olarak Paket Mayası, Ekşi Hamur Mayası ve Nohut Mayasının Hamurun Reolojik Özellikleri ve Ekmeğin Kalitesine Etkileri. PhD Thesis, Atatürk University, Erzurum, Turkey *(in Turkish).*

Palla, M., Cristani, C., Giovannetti, M. and Agnolucci, M., 2017. Identification and characterization of lactic acid bacteria and yeasts of PDO Tuscan bread sourdough by culture dependent and independent methods. International Journal of Food Microbiology, 250: 19-26.

Paramithiotis, S., Chouliaras, Y., Tsakalidou, E. and Kalantzopoulos, G., 2005. Application of selected starter cultures for the production of wheat sourdough bread using a traditional three-stage procedure. Process Biochemistry, 40 (8): 2813-2819.

Paramithiotis, S. and Drosinos, E. H., 2017. The sourdough Micro-ecosystem An update (R. C. Ray and D. Montet Editors). Fermented Foods, Part II: Technological Interventions. CRC Press, Boca Raton.

Paramithiotis, S., Gioulatos, S., Tsakalidou, E. and Kalantzopoulos, G., 2006. Interactions between *Saccharomyces cerevisiae* and lactic acid bacteria in sourdough. Process Biochemistry, 41 (12): 2429-2433.

Paramithiotis, S., Müller, M. R. A., Ehrmann, M. A., Tsakalidou, E., Seiler, H., Vogel, R. and Kalantzopoulos, G., 2000. Polyphasic Identification of Wild Yeast Strains Isolated from Greek Sourdoughs. Systematic and Applied Microbiology, 23 (1): 156-164.

Park, S.-Y. and Lim, S.-D., 2015. Probiotic Characteristics of *Lactobacillus plantarum* FH185 Isolated from Human Feces. Korean Journal for Food Science of Animal Resources, 35 (5): 615-621.

Pathania, S., Kaur, A. and Sachdev, P. A., 2017. Chickpea flour supplemented high protein composite formulation for flatbreads: Effect of packaging materials and storage temperature on the ready mix. Food Packaging and Shelf Life, 11: 125-132.

Patra, J. K., Das, G. and Paramithiotis, S., 2017. Kimchi: A Well-known Korean Traditional Fermented Food (S. Paramithiotis Editor). Lactic Acid Fermentation of Fruits and Vegetables. CRC Press, Boca Raton, p 84-105.

Payne, P., Holt, L. M., Jackson, E. A., Law, C. and Damania, A., 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 304 (1120): 359-371.

Pepe, O., Blaiotta, G., Moschetti, G., Greco, T. and Villani, F., 2003. Rope-Producing Strains of *Bacillus* spp. from Wheat Bread and Strategy for Their Control by Lactic Acid Bacteria. Applied and Environmental Microbiology, 69 (4): 2321-2329.

Pessione, E., 2012. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. Frontiers in Cellular and Infection Microbiology, 2: 86.

Pikuta, E. V. and Hoover, R. B., 2014. The genus Trichococcus (W. H. Holzapfel and B. J. B. Wood Editors). Lactic Acid Bacteria: Biodiversity and Taxonomy, John Wiley & Sons, West Sussex, UK, p 135-146.

Pot, B., Felis, G. E., De Bruyne, K. D., Tsakalidou, E., Papadimitriou, K., Leisner, J. and Vandamme, P., 2014. The genus *Lactobacillus* (W. H. Holzapfel and B. J. B. Wood Editors). Lactic Acid Bacteria: Biodiversity and Taxonomy, First Edition, John Wiley & Sons, West Sussex, UK, p 249-353.

Poutanen, K., Flander, L. and Katina, K., 2009. Sourdough and cereal fermentation in a nutritional perspective. Food Microbiology, 26 (7): 693-699.

Prajapati, J. B. and Nair, B. M., 2008. The history of fermented foods (E. R. Farnworth Editor). Handbook of Fermented Functional Foods, CRC Press, Boca Raton, p 1-24.

Pronk, J. T., Yde Steensma, H. and Van Dijken, J. P., 1996. Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast, 12 (16): 1607-1633.

Pulvirenti, A., Solieri, L., Gullo, M., De Vero, L. and Giudici, P., 2004. Occurrence and dominance of yeast species in sourdough. Letters in Applied Microbiology, 38 (2): 113-117.

Randazzo, C. L., Heilig, H., Restuccia, C., Giudici, P. and Caggia, C., 2005. Bacterial population in traditional sourdough evaluated by molecular methods. Journal of Applied Microbiology, 99 (2): 251-258.

Ray, R. and Joshi, V. K., 2015. Fermented Foods: Past, Present and Future (R. C. Ray and D. Montet Editors). Microorganisms and Fermentation of Traditional Foods, CRC Press, Boca Raton, p 1-36.

Ray, R. C. and Montet, D., 2015. Microorganisms and Fermentation of Traditional Foods, CRC Press, Boca Raton, 390 p.

Reale, A., Mannina, L., Tremonte, P., Sobolev, A. P., Succi, M., Sorrentino, E. and Coppola, R., 2004. Phytate degradation by lactic acid bacteria and yeasts during the wholemeal dough fermentation: a 31P NMR study. The Journal of Agricultural and Food Chemistry, 52 (20): 6300-6305.

Reddy, N. R., Pierson, M. D., Sathe, S. K. and Salunkhe, D. K., 1982. Legume-based fermented foods: their preparation and nutritional quality. Critical Reviews in Food Science and Nutrition, 17 (4): 335-370.

Reed, G. and Nagodawithana, T. W., 1990. Brewer's Yeast (G. Reed and T. W. Nagodawithana Editors). Yeast Technology, Springer, Netherlands, p 89-149.

Ricciardi, A., Parente, E., Piraino, P., Paraggio, M. and Romano, P., 2005. Phenotypic characterization of lactic acid bacteria from sourdoughs for Altamura bread produced in Apulia (Southern Italy). International Journal of Food Microbiology, 98 (1): 63-72.

Rizzello, C. G., Calasso, M., Campanella, D., De Angelis, M. and Gobbetti, M., 2014. Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread International Journal of Food Microbiology, 180: 78-87.

Rizzello, C. G., Coda, R. and Gobbetti, M., 2017. Use of Sourdough Fermentation and Nonwheat Flours for Enhancing Nutritional and Healthy Properties of Wheat-Based Foods (F. Juana. C. Martinez-Villaluenga and E. Peñas Editors). Fermented Foods in Health and Disease Prevention, Academic Press, Boston, p 433-452.

Rizzello, C. G., De Angelis, M., Di Cagno, R., Camarca, A., Silano, M., Losito, I., De Vincenzi, M., De Bari, M. D., Palmisano, F., Maurano, F., Gianfrani, C. and Gobbetti, M., 2007. Highly Efficient Gluten Degradation by Lactobacilli and Fungal Proteases during Food Processing: New Perspectives for Celiac Disease. Applied and Environmental Microbiology, 73 (14): 4499-4507.

Rizzello, C. G., Nionelli, L., Coda, R., Di Cagno, R. and Gobbetti, M., 2010. Use of sourdough fermented wheat germ for enhancing the nutritional, texture and sensory characteristics of the white bread. European Food Research and Technology, 230 (4): 645-654.

Rocha, J. M. and Malcata, F. X., 1999. On the microbiological profile of traditional Portuguese sourdough. Journal of Food Protection, 62 (12): 1416-1429.

Rocha, J. M. and Malcata, F. X., 2016. Microbial Ecology Dynamics in Portuguese Broa Sourdough. Journal of Food Quality, 39:634-648.

Rollán, G., Angelis, M. D., Gobbetti, M. and Valdez, G. F. D., 2005. Proteolytic activity and reduction of gliadin-like fractions by sourdough lactobacilli. Journal of Applied Microbiology, 99 (6): 1495-1502.

Romanelli, A. M., Sutton, D. A., Thompson, E. H., Rinaldi, M. G. and Wickes, B. L., 2010. Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: a cautionary note. Journal of Clinical Microbiology, 48 (3): 741-752.

Rosenquist, H. and Hansen, Å., 2000. The microbial stability of two bakery sourdoughs made from conventionally and organically grown rye. Food Microbiology, 17 (3): 241-250.

Rossi, F., Iacumin, L., Siano, F., Coppola, R. and Reale, A., 2012. *Lb. paracasei* in sourdough: growth, acidifying capability, and VOCs production in different acidity values and temperatures.

Ruiz Rodriguez, L., Vera Pingitore, E., Rollan, G., Cocconcelli, P. S., Fontana, C., Saavedra, L., Vignolo, G. and Hebert, E. M., 2016a. Biodiversity and technological-functional potential of lactic acid bacteria isolated from spontaneously fermented quinoa sourdoughs. Journal of Applied Microbiology, 120 (5): 1289-1301.

Ruiz Rodriguez, L., Vera Pingitore, E., Rollan, G., Martos, G., Saavedra, L., Fontana, C., Hebert, E. M. and Vignolo, G., 2016b. Biodiversity and technological potential of lactic acid bacteria isolated from spontaneously fermented amaranth sourdough. Letters in Applied Microbiology, 63 (2): 147-154.

Rzhetsky, A. and Nei, M., 1992. A Simple Method for Estimating and Testing Minimum-Evolution Trees. Molecular Biology and Evolution, 9 (5): 945-945.

Saeed, M., Anjum, F. M., Zahoor, T., Nawaz, H. and Rehman, S. U., 2009. Isolation and Characterization of Starter Culture from Spontaneous Fermentation of Sourdough. The International Journal of Agricultural and Biological Engineering, 11: 329–332.

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

Salim Ur, R., Paterson, A. and Piggott, J. R., 2006. Flavour in sourdough breads: a review. Trends in Food Science & Technology, 17 (10): 557-566.

Salovaara, H., 1998. Lactic acid bacteria in cereal-based products (S. Salminen and A. Von Wright Editors). Lactic Acid Bacteria-Microbiology and Functional Aspects, 2nd Ed. Marcel Dekker, New York, p 115-137.

Salovaara, H. and Gänzle, M., 2012. Lactic Acid Bacteria in Cereal-Based Products (S. Lahtinen, A. C. Ouwehand, S. Salminen and A. Von Wright Editors). Lactic acid bacteria Microbiological and Functional Aspects, Fourth Edition. Taylor & Francis Group, LLC, Boca Raton, p 227-245.

Salovaara, H. and Katunpaa, H., 1984. An approach to the classification of lactobacilli isolated from Finnish sour rye dough ferments. Acta Aliment Pol, 10: 231-239.

Sarilar, V., Sterck, L., Matsumoto, S., Jacques, N., Neuvéglise, C., Tinsley, C. R., Sicard, D. and Casaregola, S., 2017. Genome sequence of the type strain CLIB 1764T (=CBS 14374T) of the yeast species *Kazachstania saulgeensis* isolated from French organic sourdough. Genomics Data, 13: 41-43.

Scardovi, V., 1986. Genus Bifidobacterium Orla-Jensen 1924, 472AL (P. H. A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt Editors). Bergey's Manual of Systematic Bacteriology, vol. 2, Williams & Wilkins, Baltimore, p 1418-1434.

Scheirlinck, I., Van Der Meulen, R., De Vuyst, L., Vandamme, P. and Huys, G., 2009. Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. Journal of Applied Microbiology, 106 (4): 1081-1092.

Schillinger, U. and Lücke, F.-K., 1987. Identification of lactobacilli from meat and meat products. Food Microbiology, 4 (3): 199-208.

Schillinger, U. and Lücke, F. K., 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Applied and Environmental Microbiology, 55 (8): 1901-1906.

Schleifer, K. H. and Ludwig, W., 1995. Phylogenetic relationships of lactic acid bacteria (B. J. B. Wood and W. H. Holzapfel Editors). The Genera of Lactic Acid Bacteria, The Lactic Acid Bacteria Volume 2, Springer Science+Business Media, Germany, p 7-18.

Schober, T. J., Bean, S. R. and Boyle, D. L., 2007. Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological, and microstructural background. Journal of Agricultural and Food Chemistry, 55 (13): 5137-5146.

Schober, T. J., Dockery, P. and Arendt, E. K., 2003. Model studies for wheat sourdough systems using gluten, lactate buffer and sodium chloride. European Food Research and Technology, 217 (3): 235-243.

Settanni, L. and Corsetti, A., 2008. Application of bacteriocins in vegetable food biopreservation. International Journal of Food Microbiology, 121 (2): 123-138.

Settanni, L., Di Grigoli, A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G. and Bonanno, A., 2012. Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a traditional Caciocavallo type cheese. International Journal of Food Microbiology, 155 (1): 73-81.

Settanni, L., Massitti, O., Van Sinderen, D. and Corsetti, A., 2005. In situ activity of a bacteriocin-producing *Lactococcus lactis* strain. Influence on the interactions between lactic acid bacteria during sourdough fermentation. Journal of Applied Microbiology, 99 (3): 670-681.

Settanni, L., Tanguler, H., Moschetti, G., Reale, S., Gargano, V. and Erten, H., 2011. Evolution of fermenting microbiota in tarhana produced under controlled technological conditions. Food Microbiology, 28 (7): 1367-1373.

Settanni, L., Ventimiglia, G., Alfonzo, A., Corona, O., Miceli, A. and Moschetti, G., 2013. An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production. Food Research International, 54 (2): 1569-1578.

Sicard, D. and Legras, J.-L., 2011. Bread, beer and wine: Yeast domestication in the Saccharomyces sensu stricto complex. Comptes Rendus Biologies, 334 (3): 229-236.

Singh, J., Dartois, A. and Kaur, L., 2010. Starch digestibility in food matrix: a review. Trends in Food Science & Technology, 21 (4): 168-180.

Siragusa, S., Di Cagno, R., Ercolini, D., Minervini, F., Gobbetti, M. and De Angelis, M., 2009. Taxonomic structure and monitoring of the dominant population of lactic acid bacteria during wheat flour sourdough type I propagation using *Lactobacillus sanfranciscensis* starters. Applied and Environmental Microbiology, 75 (4): 1099-1109.

Smid, E. J. and Hugenholtz, J., 2010. Functional genomics for food fermentation processes. Annual Review of Food Science and Technology, 1: 497-519.

Sneath, P. H. A. and Sokal, R. R., 1973. Numerical Taxonomy. Freeman, San Francisco, 573 p.

Sobczak, I. and Lolkema, J. S., 2005. The 2 - hydroxycarboxylate transporter family: Physiology, structure, and mechanism. Microbiology and Molecular Biology Reviews, 69: 665-695.

Solomon, T. P. and Blannin, A. K., 2007. Effects of short-term cinnamon ingestion on in vivo glucose tolerance. Diabetes, Obesity and Metabolism, 9 (6): 895-901.

Spicher, G. and Lonner, C., 1985. The microflora of sourdough. The *Lactobacillus* species of sourdough from Swedish bakeries. Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung, 181: 9-13.

Spicher, G., 1983. Baked goods (G. Reed Editor). Biotechnology, Vol. 5: Food and Feed Productions with Microorganisms, Verlag Chemie, Weinheim, Germany, p 1-80.

Stanbury, P. F., 2000. Fermentation Technology (J. Walker and R. Rapley Editors). Molecular Biology and Biotechnology. Royal Society of Chemistry, Cambridge, Great Britain, p 1-24.

Stenlid, J., Karlsson, J.-O. and Högberg, N., 1994. Intraspecific genetic variation in Heterobasidion annosum revealed by amplification of minisatellite DNA. Mycological Research, 98 (1): 57-63.

Sterr, Y., Weiss, A. and Schmidt, H., 2009. Evaluation of lactic acid bacteria for sourdough fermentation of amaranth. International Journal of Food Microbiology, 136 (1): 75-82.

Stolz, P., 1999. Mikrobiologie des Sauerteiges. G. Spicher and H. Stephan. Handbuch Sauerteig: Biologie, Biochemie, Technologie 5th edition. Behr's Verlag, Hamburg, p 35-60.

Stolz, P. and Bocker, G., 1996. Technology, properties and applications of sourdough products. Advances in Food Science, 18: 234-236.

Struyf, N., Maelen, E., Hemdane, S., Verspreet, J., Verstrepen, K. J. and Courtin, C. M., 2017. Bread Dough and Baker's Yeast: An Uplifting Synergy. Comprehensive Reviews in Food Science and Food Safety, 16 (5): 850-867.

Succi, M., Reale, A., Andrighetto, C., Lombardi, A., Sorrentino, E. and Coppola, R., 2003. Presence of yeasts in southern Italian sourdoughs from Triticum aestivum flour. FEMS Microbiology Letters, 225 (1): 143-148.

Sugihara, T. F., Kline, L. and Mccready, L. B., 1970. Nature of the San Francisco sour dough French bread process: II.Microbiological aspects. Bakers Digest, 44: 51-57.

Sutherland, I. W., 1972. Bacterial exopolysaccharides. Advances in Microbial Physiology, 8: 143-213.

Swain, M. R. and Ananadharaj, M., 2017. Regional Fermented Vegetables and Fruits in Asia-Pacific (S. Paramithiotis Editor). Lactic Acid Fermentation of Fruits and Vegetables, CRC Press, Boca Raton, p 181-203.

Swanson, R. B., 2008. Bakery: Yeast-leavened Breads (J. S. Smith and Y. H. Hui Editors). Food Processing: Principles and Applications. Blackwell Publishing, Iowa, USA, p 183-201.

Şimşek, Ö., Çon, A. H. and Tulumoğlu, Ş., 2006. Isolating lactic starter cultures with antimicrobial activity for sourdough processes. Food Control, 17 (4): 263-270.

Taccari, M., Aquilanti, L., Polverigiani, S., Osimani, A., Garofalo, C., Milanovic, V. and Clementi, F., 2016. Microbial Diversity of Type I Sourdoughs Prepared and Back-Slopped with Wholemeal and Refined Soft (*Triticum aestivum*) Wheat Flours. Journal of Food Science, 81 (8): M1996-2005.

Tamang, J. P. and Fleet, G. H., 2009. Yeasts Diversity in Fermented Foods and Beverages (T. Satyanarayana and G. Kunze Editors). Yeast Biotechnology: Diversity and Applications. Springer, Netherlands, p 169-198.

Tamani, R. J., Goh, K. K. T. and Brennan, C. S., 2013. Physico-Chemical Properties of Sourdough Bread Production Using Selected Lactobacilli Starter Cultures. Journal of Food Quality, 36 (4): 245-252.

Tamura, K., Nei, M. and Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America, 101 (30): 11030-11035.

Tayuan, C., Tannock, G. W. and Rodtong, S., 2011. Growth and exopolysaccharide production by *Weissella* sp. from low-cost substitutes for sucrose. African Journal of Microbiology Research, 5(22): 3693-3701.

Thiele, C., Gänzle, M. G. and Vogel, R. F., 2002. Contribution of Sourdough Lactobacilli, Yeast, and Cereal Enzymes to the Generation of Amino Acids in Dough Relevant for Bread Flavor. Cereal Chemistry, 79 (1): 45-51.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 25 (24): 4876-4882.

Tieking, M., Korakli, M., Ehrmann, M. A., Gänzle, M. G. and Vogel, R. F., 2003. In Situ Production of Exopolysaccharides during Sourdough Fermentation

by Cereal and Intestinal Isolates of Lactic Acid Bacteria. Applied and Environmental Microbiology, 69 (2): 945-952.

Torrieri, E., Pepe, O., Ventorino, V., Masi, P. and Cavella, S., 2014. Effect of sourdough at different concentrations on quality and shelf life of bread. LWT - Food Science and Technology, 56 (2): 508-516.

Tortora, G. J., Funke, B. R. and Case, C. L., 2010. Microbiology: An Introduction, Pearson Education, San Francisco, 960 p.

Trofa, D., Gácser, A. and Nosanchuk, J. D., 2008. *Candida parapsilosis*, an Emerging Fungal Pathogen. Clinical Microbiology Reviews, 21 (4): 606-625.

Tucker, G. S., 2016. Food Preservation and Biodeterioration, 2nd Edition. John Wiley & Sons, Ltd., West Sussex, 257 p.

Tuukkanen, K., Loponen, J., Mikola, M., Sontag-Strohm, T. and Salovaara, H., 2005. Degradation of Secalins During Rye Sourdough Fermentation. Cereal Chemistry, 82 (6): 677-682.

Tye-Din, J. and Anderson, R., 2008. Immunopathogenesis of celiac disease. Current Gastroenterology Reports, 10 (5): 458-465.

Ulloa, M., Herrera, T. and Lappe, P., 1987. Fermentaciones tradicionales indígenas de México, 13, 20, Mexico, Instituto Nacional Indigenista.

Valcheva, R., Korakli, M., Onno, B., Prevost, H., Ivanova, I., Ehrmann, M. A., Dousset, X., Ganzle, M. G. and Vogel, R. F., 2005. *Lactobacillus hammesii* sp. nov., isolated from French sourdough. International Journal of Systematic and Evolutionary Microbiology, 55 (Pt 2): 763-767.

Valmorri, S., Settanni, L., Suzzi, G., Gardini, F., Vernocchi, P. and Corsetti, A., 2006. Application of a novel polyphasic approach to study the lactobacilli composition of sourdoughs from the Abruzzo region (central Italy). Letters in Applied Microbiology, 43 (3): 343-349.

Valmorri, S., Tofalo, R., Settanni, L., Corsetti, A. and Suzzi, G., 2010. Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). Antonie Van Leeuwenhoek, 97 (2): 119-129.

Van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie, S., Somoza, V., Knorr, D., Jasti, P. R. and Eisenbrand, G., 2010. A review on the beneficial aspects of food processing. Molecular Nutrition & Food Research, 54 (9): 1215-1247.

Van Der Meulen, R., Scheirlinck, I., Van Schoor, A., Huys, G., Vancanneyt, M., Vandamme, P. and De Vuyst, L., 2007. Population Dynamics and Metabolite Target Analysis of Lactic Acid Bacteria during Laboratory Fermentations of Wheat and Spelt Sourdoughs. Applied and Environmental Microbiology, 73 (15): 4741-4750.

Varshney, R. K., Thudi, M. and Muehlbauer, F. J., 2017. The chickpea genome: an introduction. R. K. Varshney, M. Thudi and F. Muehlbauer. The Chickpea Genome. Springer International Publishing AG, Switzerland, p1-4.

Ventimiglia, G., Alfonzo, A., Galluzzo, P., Corona, O., Francesca, N., Caracappa, S., Moschetti, G. and Settanni, L., 2015. Codominance of *Lactobacillus plantarum* and obligate heterofermentative lactic acid bacteria during sourdough fermentation. Food Microbiology, 51: 57-68.

Venturi, M., Guerrini, S., Granchi, L. and Vincenzini, M., 2012. Typing of Lactobacillus sanfranciscensis isolates from traditional sourdoughs by combining conventional and multiplex RAPD-PCR profiles. International Journal of Food Microbiology, 156 (2): 122-126.

Vermeulen, N., Pavlovic, M., Ehrmann, M. A., Gänzle, M. G. and Vogel, R. F., 2005. Functional Characterization of the Proteolytic System of

Lactobacillus sanfranciscensis DSM 20451(T) during Growth in Sourdough. Applied and Environmental Microbiology, 71 (10): 6260-6266.

Vernocchi, P., Valmorri, S., Gatto, V., Torriani, S., Gianotti, A., Suzzi, G., Guerzoni, M. E. and Gardini, F., 2004. A survey on yeast microbiota associated with an Italian traditional sweet-leavened baked good fermentation. Food Research International, 37 (5): 469-476.

Viiard, E., Mihhalevski, A., Ruhka, T., Paalme, T. and Sarand, I., 2012. Evaluation of the microbial community in industrial rye sourdough upon continuous back-slopping propagation revealed *Lactobacillus helveticus* as the dominant species. Journal of Applied Microbiology, 114 (2): 404-412.

Vishniac, H. and Kurtzman, C., 1992. *Cryptococcus antarcticus* sp. nov. and *Cryptococcus albidosimilis* sp. nov., Basidioblastomycetes from Antarctic Soils. International Journal of Systematic Bacteriology, 42: 547-553.

Vogel, R. F., Bocker, G., Stolz, P., Ehrmann, M., Fanta, D., Ludwig, W., Pot, B., Kersters, K., Schleifer, K. H. and Hammes, W. P., 1994. Identification of lactobacilli from sourdough and description of *Lactobacillus pontis* sp. nov. International Journal of Systematic Bacteriology, 44 (2): 223-229.

Vogel, R. F., Ehrmann, M. A. and Gänzle, M. G., 2002. Development and potential of starter lactobacilli resulting from exploration of the sourdough ecosystem. Antonie Van Leeuwenhoek, 81 (1): 631-638.

Vogel, R. F., Knorr, R., Muller, M. R., Steudel, U., Ganzle, M. G. and Ehrmann, M. A., 1999. Non-dairy lactic fermentations: the cereal world. Antonie Van Leeuwenhoek, 76 (1-4): 403-411.

Vogel, R. F., Pavlovic, M., Ehrmann, M. A., Wiezer, A., Liesegang, H., Offschanka, S., Voget, S., Angelov, A., Böcker, G. and Liebl, W., 2011. Genomic analysis reveals *Lactobacillus sanfranciscensis* as stable element in traditional sourdoughs. Microbial Cell Factories, 10 (1): S6.

Vogelmann, S. A., Seitter, M., Singer, U., Brandt, M. J. and Hertel, C., 2009. Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. International Journal of Food Microbiology, 130 (3): 205-212.

Von Wright, A. and Axelsson, L., 2012. Lactic Acid Bacteria: An Introduction (S. Lahtinen, A. C. Ouwehand, S. Salminen and A. Von Wright Editors). Lactic Acid Bacteria Microbiological and Functional Aspects, Fourth Edition. CRC Press, Boca Raton, p 1-16.

Vrancken, G., De Vuyst, L., Van Der Meulen, R., Huys, G., Vandamme, P. and Daniel, H.-M., 2010. Yeast species composition differs between artisan bakery and spontaneous laboratory sourdoughs. FEMS Yeast Research, 10: 471-481.

Vrancken, G., Rimaux, T., Weckx, S., Leroy, F. and De Vuyst, L., 2011. Influence of Temperature and Backslopping Time on the Microbiota of a Type I Propagated Laboratory Wheat Sourdough Fermentation. Applied and Environmental Microbiology, 77 (8): 2716-2726.

Waites, M. J., Morgan, N. L., Rockeyn, J. S. and Higton, G., 2001. Industrial Microbiology: An Introduction. Blackwell Science, Hong Kong, 288 p.

Wallace, T. C., Murray, R. and Zelman, K. M., 2016. The Nutritional Value and Health Benefits of Chickpeas and Hummus. Nutrients, 8 (12): 766.

Wang, P., Jin, Z. and Xu, X., 2015. Physicochemical alterations of wheat gluten proteins upon dough formation and frozen storage – A review from gluten, glutenin and gliadin perspectives. Trends in Food Science & Technology 46: 189-198.

Wang, Z. X., Zhuge, J., Fang, H. and Prior, B. A., 2001. Glycerol production by microbial fermentation: a review. Biotechnology Advances, 19 (3): 201-223.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J., 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173 (2): 697-703.

Wiander, B., 2017. Sauerkraut Fermentation (S. Paramithiotis Editor). Lactic Acid Fermentation of Fruits and Vegetables, CRC Press, Boca Raton, p 65-81.

Yagmur, G., Tangüler, H., Leventdurur, S., Bagder Elmacı, S., Unal Turhan, E., Francesca, N., Settanni, L., Moschetti, G. and 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.

Yang, S.-T., Zhu, Y., Yu, M. and Tang, C., 2011. Anaerobic reactions (D. R. Heldman and C. I. Moraru Editors). Encyclopedia of Agricultural, Food, and Biological Engineering, Second edition, CRC press, Boca Raton, p 46-54.

Yarza, P., Yilmaz, P., Pruesse, E., Glockner, F. O., Ludwig, W., Schleifer, K. H., Whitman, W. B., Euzeby, J., Amann, R. and Rossello-Mora, R., 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nature Reviews Microbiology, 12 (9): 635-645.

Yousef, A. E. and Carlstrom, C., 2003. Food Microbiology: A Laboratory Manual. John Wiley & Sons, Inc., New Jersey, 288 p.

Zhang, G., Ao, Z. and Hamaker, B. R., 2008. Nutritional property of endosperm starches from maize mutants: a parabolic relationship between slowly digestible starch and amylopectin fine structure. Journal of Agricultural and Food Chemistry, 56 (12): 4686-4694.

Zhang, G. and Hamaker, B. R., 2009. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Critical Reviews in Food Science and Nutrition, 49 (10): 852-867.

Zhang, G., Sadiq, F. A., Zhu, L., Liu, T., Yang, H., Wang, X. and He, G., 2015. Investigation of Microbial Communities of Chinese Sourdoughs Using Culture-Dependent and DGGE Approaches. Journal of Food Science, 80 (11): M2535-2542.

Zhang, J., Liu, W., Sun, Z., Bao, Q., Wang, F., Yu, J., Chen, W. and Zhang, H., 2011. Diversity of lactic acid bacteria and yeasts in traditional sourdoughs collected from western region in Inner Mongolia of China. Food Control, 22 (5): 767-774.

CURRICULUM VITAE

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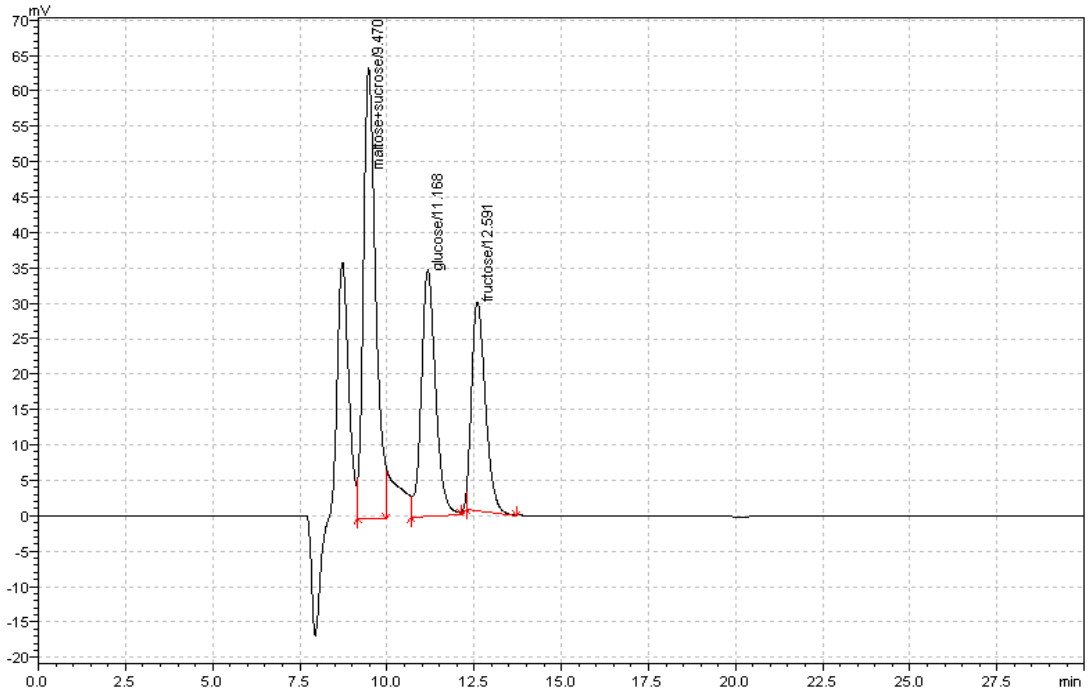
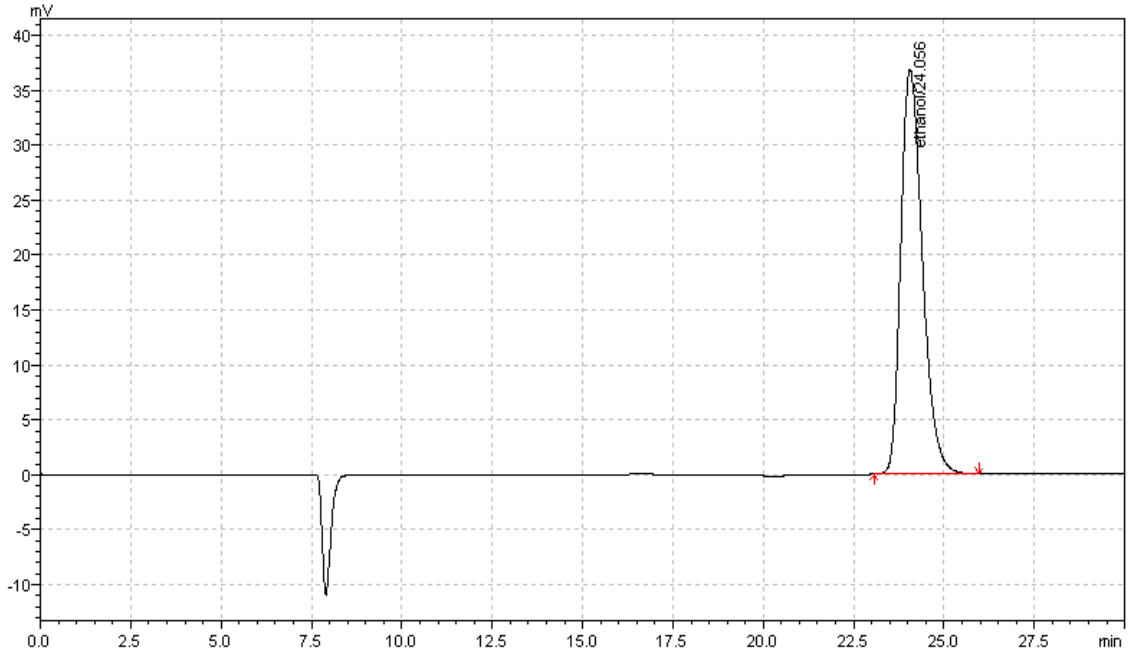
APPENDICES

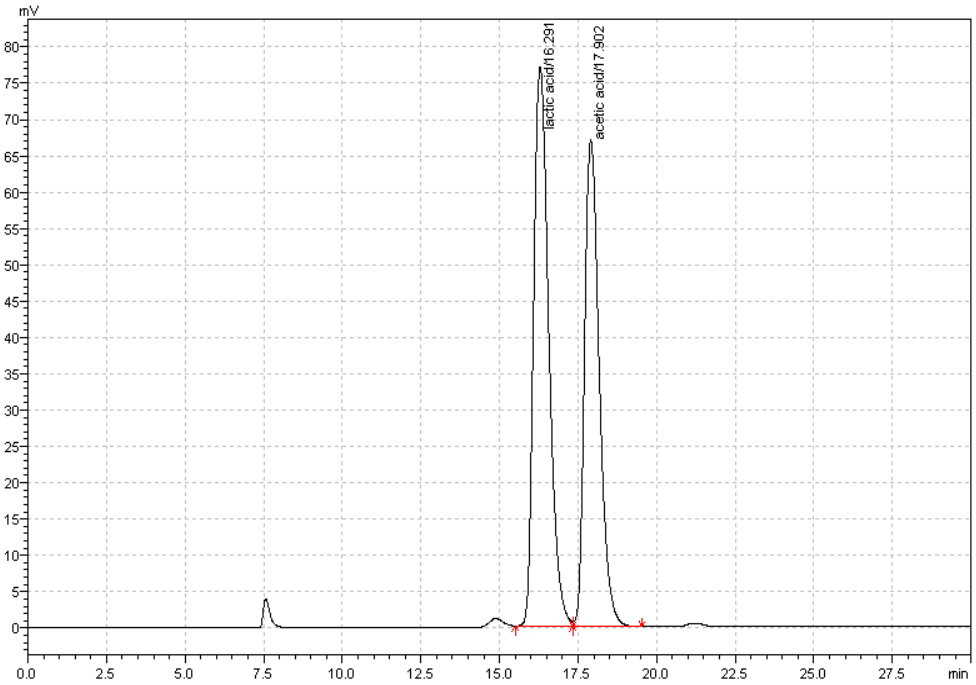
Appendix 1. Chromatogram image of the maltose+sucrose, glucose and fructose standards



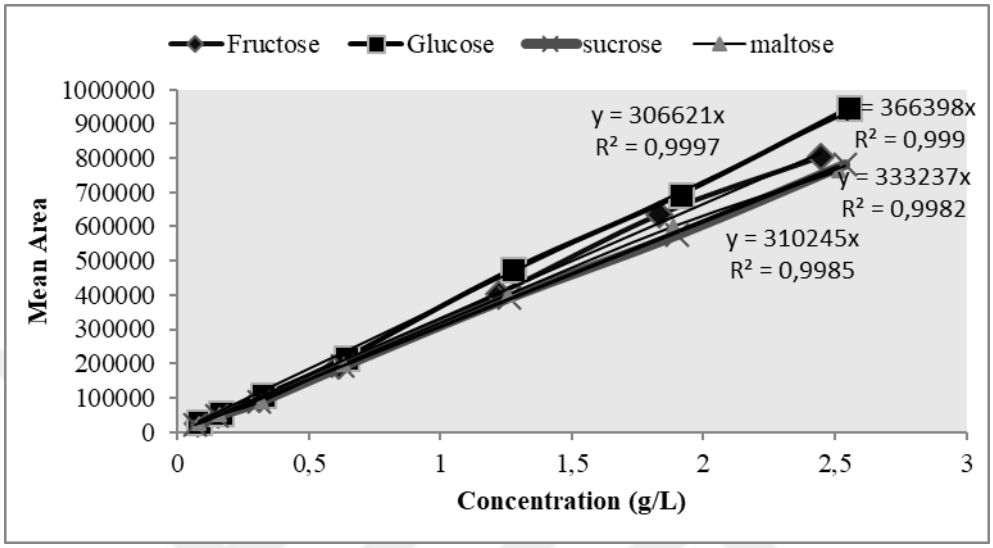
Appendix 2. Chromatogram image of the ethanol standard

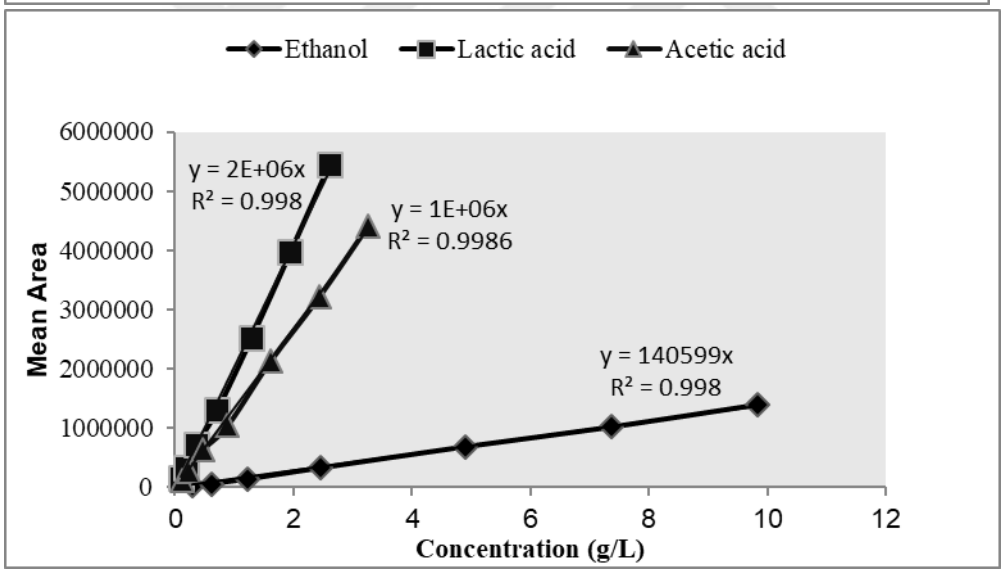


Appendix 3. Chromatogram image of the lactic acid and acetic acid standards

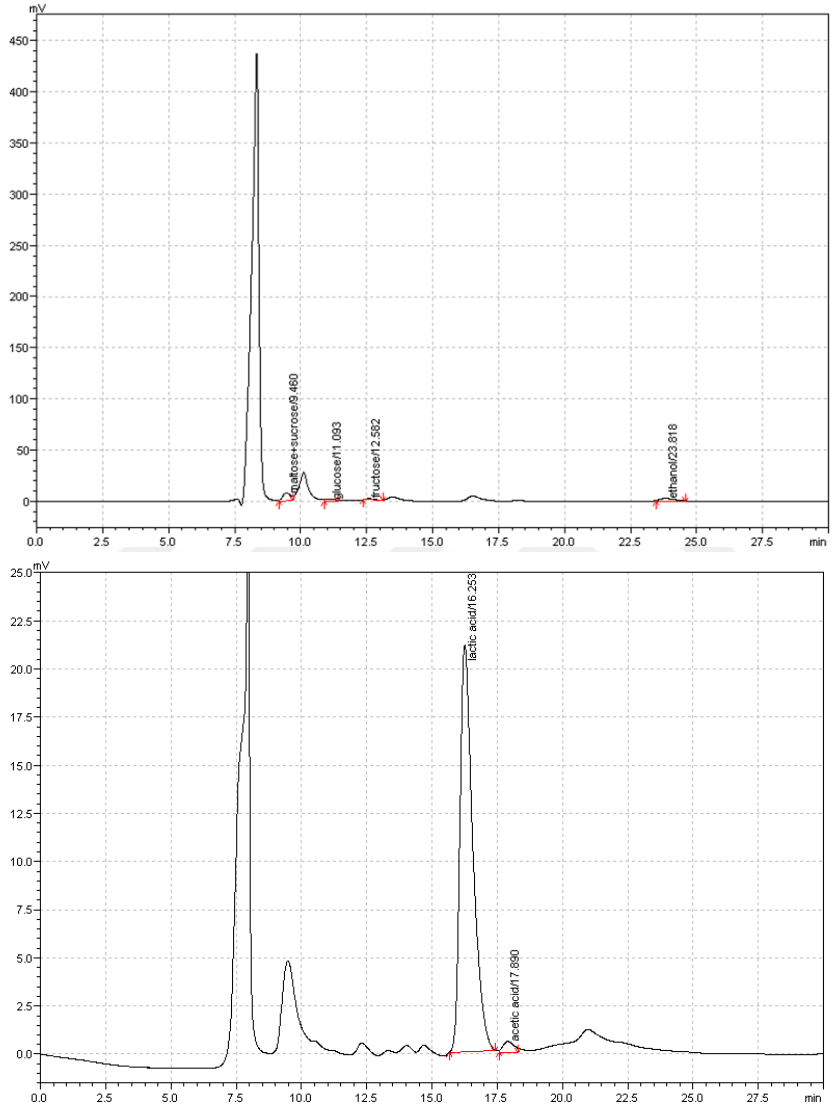


Appendix 4. Calibration curves for standards

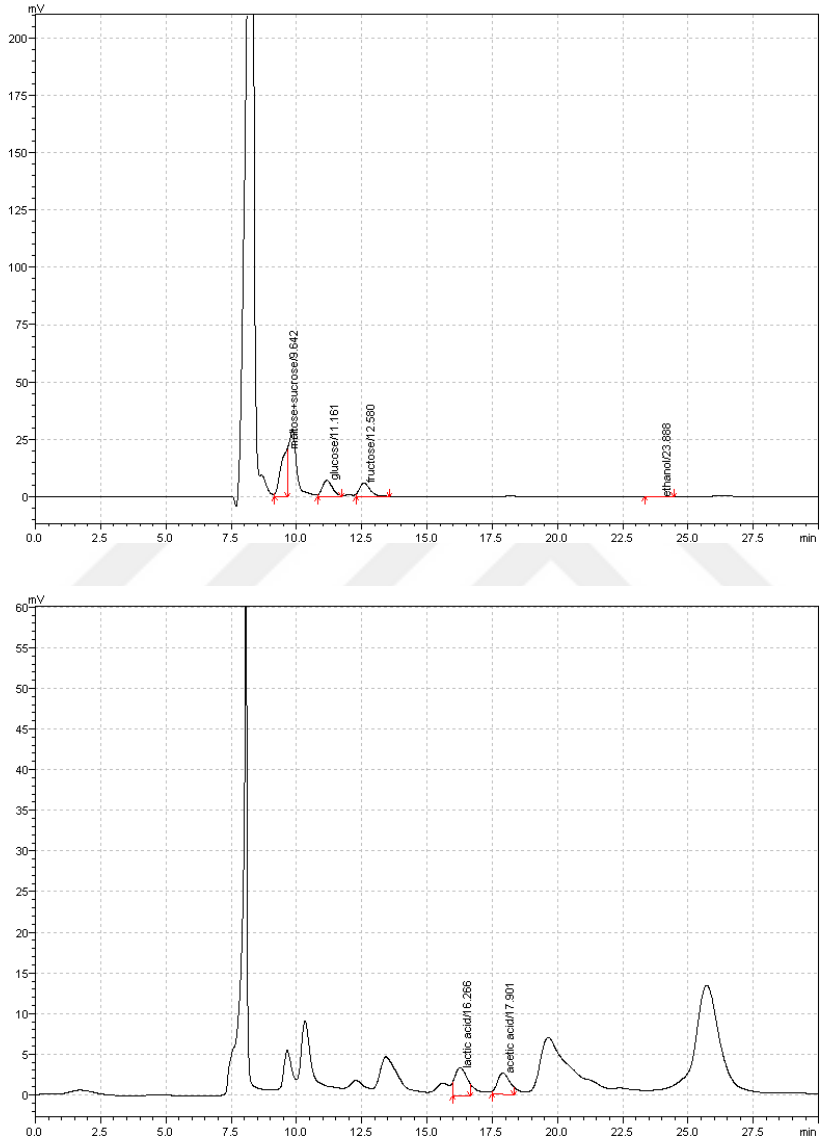




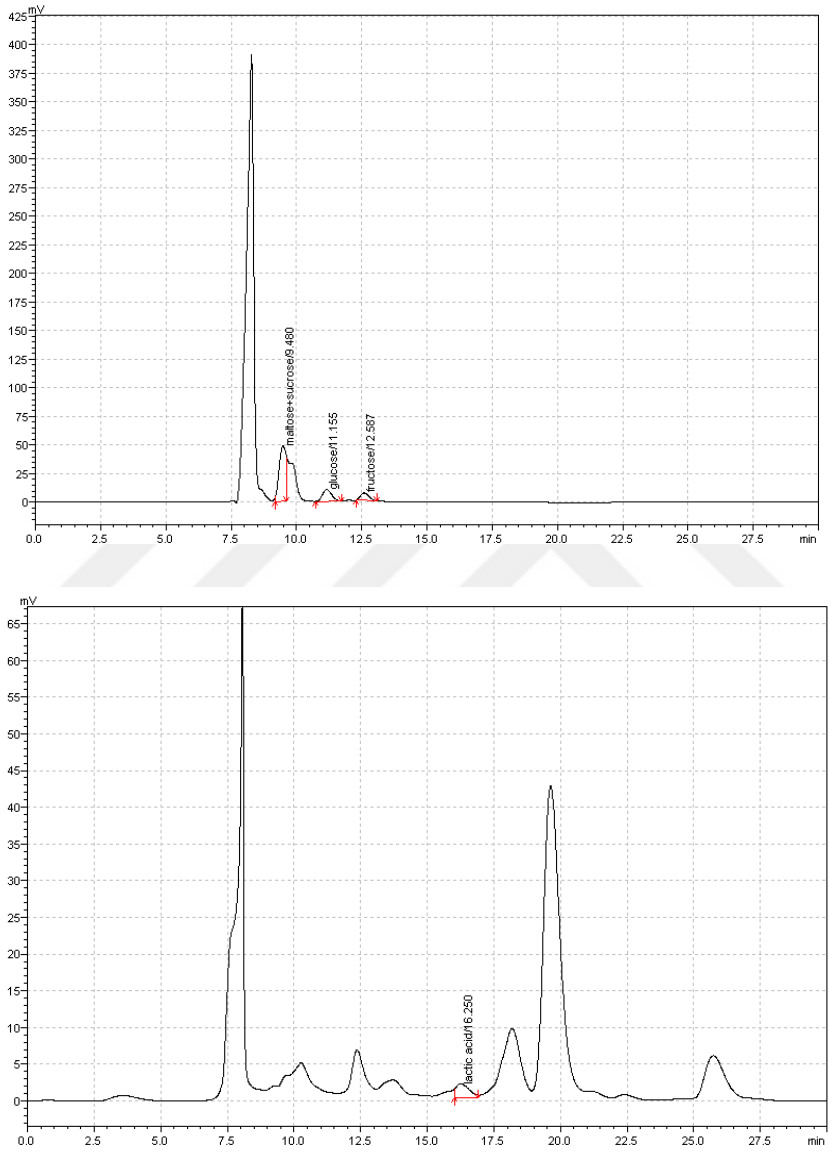
Appendix 5. Chromatogram images of a sourdough sample



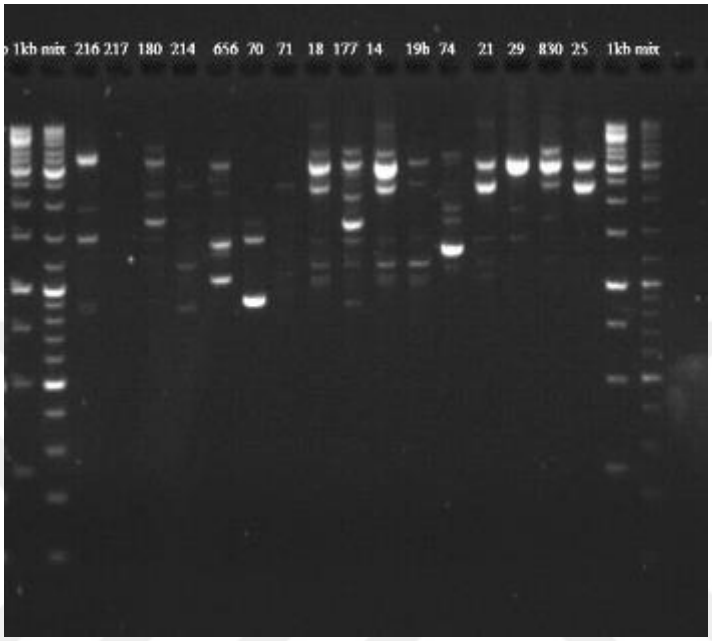
Appendix 6. Chromatogram images of a chickpea liquid starter



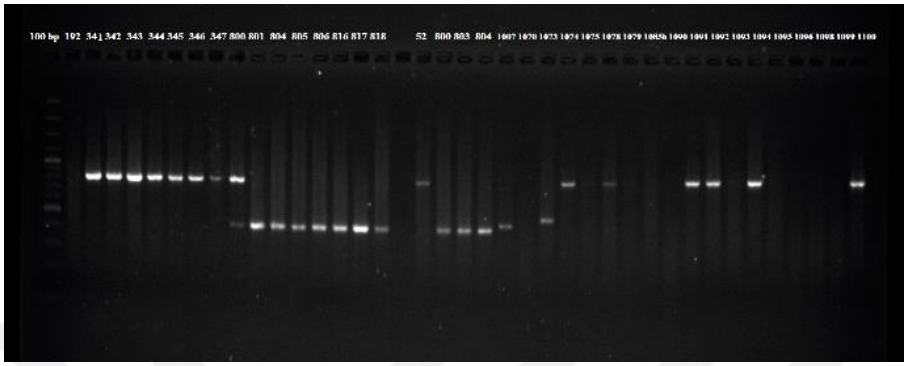
Appendix 7. Chromatogram images of a chickpea dough



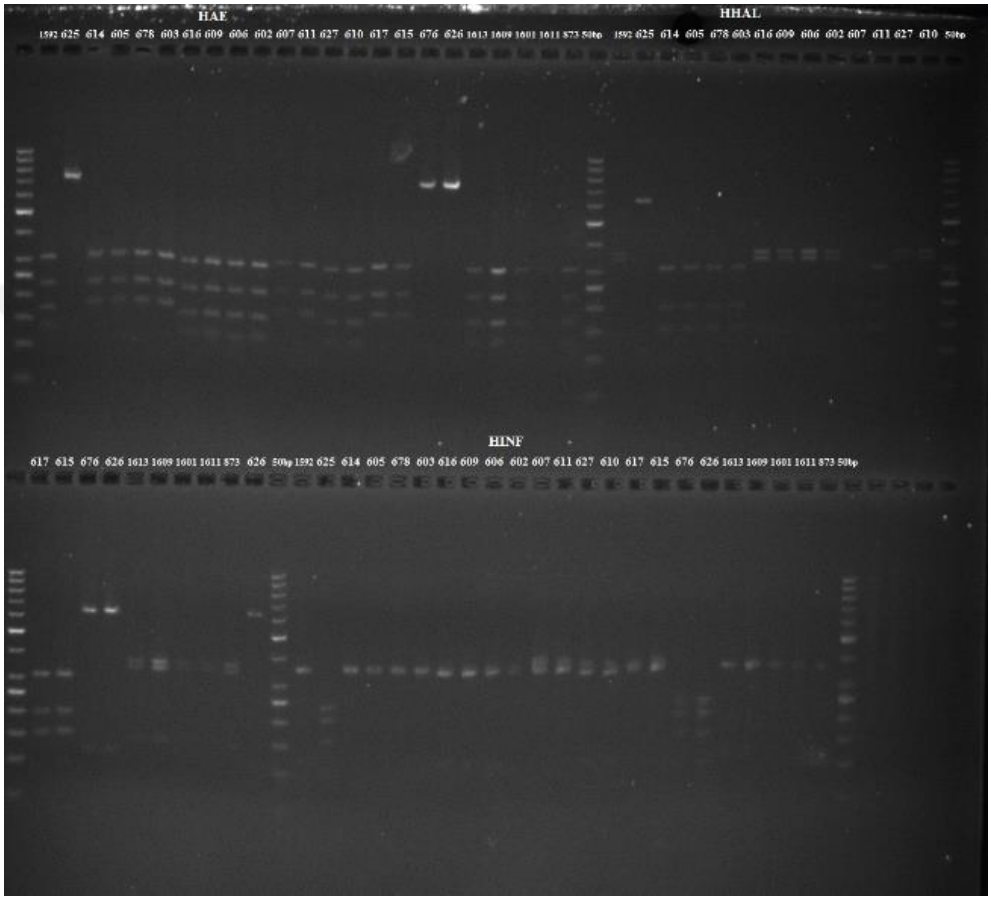
Appendix 8. A gel image of the RAPD-PCR analysis with M13 primer



Appendix 9. A gel image of the 5.8S ITS rRNA region amplification



Appendix 10. A gel image of the RFLP with restriction endonucleases *Hae* III, *Hha* I and *Hinf* I



Appendix 11. GC-MS chromatogram image of VOCs