INVESTIGATION OF CHANGES IN AMINO ACIDS DURING DIFFERENT FERMENTATION CONDITIONS

AMİNOASİTLERİN FARKLI FERMANTASYON KOŞULLARINDA ORTAYA ÇIKAN DEĞİŞİMLERİNİN İNCELENMESİ

CEMİLE YILMAZ

PROF. DR. VURAL GÖKMEN

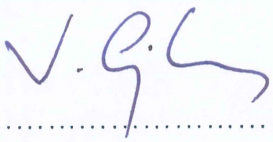
Supervisor

Submitted to Graduate School of Science and Engineering of Hacettepe University
as a Partial Fulfillment to the Requirements
for the Award of the Degree of Doctor of Philosophy
in Food Engineering

2017

This work named '**Investigation of Changes in Amino Acids during Different Fermentation Conditions**' by **CEMİLE YILMAZ** has been approved as a thesis for the Degree of **DOCTOR OF PHILOSOPHY IN FOOD ENGINEERING** by the below mentioned Examining Committee Members.

Prof. Dr. Zekiye Yeşim Özbaş
Head ..... 

Prof. Dr. Vural Gökmen
Supervisor ..... 

Prof. Dr. Hami Alpas
Member ..... 

Assoc. Prof. Dr. Yeşim Soyer
Member ..... 

Asst. Prof. Dr. Burçe Ataç Mogol
Member ..... 

This thesis has been approved as a thesis for the Degree of **DOCTOR OF PHILOSOPHY IN FOOD ENGINEERING** by Board of Directors of the Institute for Graduate School of Science and Engineering.

Prof. Dr. Menemşe GÜMÜŞDERELİOĞLU
Director of the Institute of
Graduate School of Science and Engineering

YAYINLAMA VE FİKRİ MÜLKİYET HAKLARI BEYANI

Enstitü tarafından onaylanan lisansüstü tezimin/raporumun tamamını veya herhangi bir kısmını, basılı (kağıt) ve elektronik formatta arşivleme ve aşağıda verilen koşullarla kullanıma açma iznini Hacettepe üniversitesine verdiğimi bildiririm. Bu izinle Üniversiteye verilen kullanım hakları dışındaki tüm fikri mülkiyet haklarım bende kalacak, tezimin tamamının ya da bir bölümünün gelecekteki çalışmalarda (makale, kitap, lisans ve patent vb.) kullanım hakları bana ait olacaktır.

Tezin kendi orijinal çalışmam olduğunu, başkalarının haklarını ihlal etmediğimi ve tezimin tek yetkili sahibi olduğumu beyan ve taahhüt ederim. Tezimde yer alan telif hakkı bulunan ve sahiplerinden yazılı izin alınarak kullanması zorunlu metinlerin yazılı izin alarak kullandığımı ve istenildiğinde suretlerini Üniversiteye teslim etmeyi taahhüt ederim.

☑ **Tezimin/Raporumun tamamı dünya çapında erişime açılabilir ve bir kısmı veya tamamının fotokopisi alınabilir.**

(Bu seçenekle teziniz arama motorlarında indekslenebilecek, daha sonra tezinizin erişim statüsünün değiştirilmesini talep etseniz ve kütüphane bu talebinizi yerine getirse bile, tezinin arama motorlarının önbelleklerinde kalmaya devam edebilecektir.)

☐ **Tezimin/Raporumun ..... tarihine kadar erişime açılmasını ve fotokopi alınmasını (İç Kapak, Özet, İçindekiler ve Kaynakça hariç) istemiyorum.**
(Bu sürenin sonunda uzatma için başvuruda bulunmadığım taktirde, tezimin/raporumun tamamı her yerden erişime açılabilir, kaynak gösterilmek şartıyla bir kısmı ve ya tamamının fotokopisi alınabilir)

☐ **Tezimin/Raporumun ..... tarihine kadar erişime açılmasını istemiyorum, ancak kaynak gösterilmek şartıyla bir kısmı veya tamamının fotokopisinin alınmasını onaylıyorum.**

☐ **Serbest Seçenek/Yazarın Seçimi**

02 / 01/ 2018

Cemile Yılmaz

ETHICS

In this thesis study, prepared in accordance with the spelling rules of Institute of Graduate Studies in Science of Hacettepe University,

I declare that

- all the information and documents have been obtained in the base of the academic rules
- all audio-visual and written information and results have been presented according to the rules of scientific ethics
- in case of using others Works, related studies have been cited in accordance with the scientific standards
- all cited studies have been fully referenced
- I did not do any distortion in the data set
- and any part of this thesis has not been presented as another thesis study at this or any other university.

23/11/2017

CEMİLE YILMAZ

ABSTRACT

INVESTIGATION OF CHANGES IN AMINO ACIDS DURİNG DIFFERENT FERMENTATION CONDITIONS

Cemile YILMAZ
Doctor of Philosophy, Department of Food Engineering
Supervisor: Prof. Dr. Vural GÖKMEN
December 2017, 110 pages

Fermentation is one of the food processing techniques, which can change the chemical composition of foods. Changes in chemical composition during fermentation stem from metabolism of microorganisms found in fermented foods, which leads to formation of microbial metabolites. Their formation in fermented foods is of critical importance due to the fact that microbial metabolites can affect human health positively or negatively. The main focus of this thesis was to understand changes of amino acids and thereby, formation of bioactive amines and tryptophan derivatives during fermentation.

At the beginning of this study, the effect of *S. cerevisiae* on the formation of gamma-aminobutyric acid (GABA) and the other bioactive amines during wort fermentation was investigated. For this purpose, spoiled and unspoiled worts were evaluated in terms of content of bioactive amines. Unspoiled wort was prepared by adding antibiotic to the wort. During fermentation, concentration of GABA increased in both unspoiled and spoiled worts. In spite of that, tyramine and histamine were found only in spoiled wort. Decreased concentrations of tyrosine and histidine were associated with increased concentrations of tyramine and histamine, respectively, in spoiled wort. The results indicated that occurrence of

GABA in beers should not be considered as one of the indicators of microbial contamination differently from tyramine and histamine.

In the second part, the formation of tyramine during yoghurt fermentation with the focus on interaction between *Streptococcus thermophilus* RSKK 04082, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 and *Lactobacillus plantarum* RSKK 02030 was investigated. These microorganisms were used in the yoghurt fermentation as single strains or mixed cultures containing double or triple strains. The interactions between microorganisms have been also revealed by determining total free amino acids and the pH of the medium together with the microbial count of the strains. It was observed that *L. delbrueckii* subsp. *bulgaricus* DSM 20081 did not produce tyramine while *S. thermophilus* RSKK 04082 and *L. plantarum* RSKK 02030 could produce tyramine depending on the fermentation conditions. Synergistic interactions between *S. thermophilus* RSKK 04082 and *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and, between *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and *L. plantarum* RSKK 02030 were found in terms of tyramine production. It was observed in this study that *L. delbrueckii* subsp. *bulgaricus* DSM 20081 had indirect impact for accumulation of tyramine in the yoghurts.

In the third part, a method for the detection of tryptophan derivatives in kynurenine pathway using tandem mass spectrometry in various fermented food products (bread, beer, red wine, white cheese, yoghurt, kefir and cocoa powder) was developed. The method entails an aqueous extraction and reversed phase chromatographic separation using pentafluorophenyl (PFP) column. It allowed quantitation of low ppb levels of tryptophan and its derivatives in different fermented food matrices. Dairy products (yoghurt, white cheese and kefir) were found to contain kynurenine. Although bread samples analyzed did not contain kynurenic acid, beer and red wine samples as yeast-fermented foods were found to contain kynurenic acid. Among foods analyzed, cacao powder had the highest amounts of kynurenic acid, which is a neuroprotective compound.

In the fourth part, the formation of tryptophan derivatives in kynurenine pathway by *S. cerevisiae* NCYC 88 and *S. pastorianus* NCYC 203 during wort fermentation by using Gompertz model was investigated. As a result of this study, more tryptophan was utilized by *S. cerevisiae* NCYC 88 during fermentation as compared to *S.*

pastorianus NCYC 203. The kynurenic acid concentration of wort fermented with *S. cerevisiae* NCYC 88 and *S. pastorianus* NCYC 203 increased during fermentation. When tryptophan was added into the wort fermented with *S. cerevisiae* NCYC 88 and *S. pastorianus* NCYC 203, the concentrations of the kynurenic acid increased. Moreover, the kynurenine content of worts fermented with *S. cerevisiae* NCYC 88 increased when tryptophan was added into the wort. It was also observed in this study that *S. pastorianus* NCYC 203 used more niacin than *S. cerevisiae* NCYC 88 during beer fermentation.

Keywords: fermentation, beer, yoghurt, bioactive amines, tryptophan derivatives, kynurenine pathway

ÖZET

AMİNOASİTLERİN FARKLI FERMANTASYON KOŞULLARINDA ORTAYA ÇIKAN DEĞİŞİMLERİNİN İNCELENMESİ

Cemile YILMAZ
Doktora, Gıda Mühendisliği Bölümü
Tez Danışmanı: Prof. Dr. Vural GÖKMEN
Aralık 2017, 110 sayfa

Fermantasyon, gıdaların kimyasal bileşimini değiştirebilen gıda işleme tekniklerinden biridir. Fermantasyon sırasında kimyasal bileşimdeki değişiklikler fermente gıdalardaki mikroorganizmaların metabolizmalarından kaynaklanır ve bu da mikrobiyel metabolitlerin oluşumuna neden olur. Fermente gıdalarda mikrobiyel metabolitlerin oluşumu, insan sağlığını olumlu veya olumsuz etkileyebilecekleri için kritik öneme sahiptir. Bu tezin esas amacı, fermantasyon sırasında amino asitlerdeki değişiklikleri ve dolayısıyla biyoaktif amin ve triptofan türevlerinin oluşumunu anlamaya yöneliktir.

Bu çalışmanın başında, *S. cerevisiae* mayasının şıra fermantasyonu sırasında gama-aminobütirik asit (GABA) ve diğer biyoaktif aminlerin oluşumu üzerindeki etkisi araştırılmıştır. Bu amaçla, bozulmuş ve bozulmamış şıralar biyoaktif amin içeriği açısından değerlendirilmiştir. Bozulmamış şıra, antibiyotik eklenerek hazırlanmıştır. Fermantasyon süresince, bozulmamış ve bozulmuş şıralarda GABA konsantrasyonu artmıştır. Buna rağmen, tiramin ve histamin sadece bozulmuş şırada bulunmuştur. Bozulmuş şıradaki azalan konsantrasyonlardaki tirozin ve histidin, artan tiramin ve histamin konsantrasyonları ile ilişkilendirilmiştir. Sonuçlar, tiramin ve histaminden farklı olarak, GABA'nın bir mikrobiyel

kontaminasyon göstergesi olarak düşünülmemesi gerektiğini göstermiştir.

İkinci bölümde, *Streptococcus thermophilus* RSKK 04082, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 ve *Lactobacillus plantarum* RSKK 02030 arasındaki etkileşimlere odaklanılarak yoğurt fermantasyonu sırasında tiramin oluşumu incelenmiştir. Bu mikroorganizmalar yoğurt fermantasyonunda tek suş veya ikili veya üçlü suşlar içeren karışık kültürler olarak kullanılmıştır. Mikroorganizmalar arasındaki etkileşimler, suşların mikrobiyel sayımı ile birlikte toplam serbest amino asitlerin ve pH'ın belirlenmesiyle de ortaya çıkarılmıştır. *S. thermophilus* RSKK 04082 ve *L. plantarum* RSKK 02030 fermantasyon koşullarına bağlı olarak tiramin üretebilirken, *L. delbrueckii* subsp. *bulgaricus* DSM 20081 tiramin üretmemiştir. Tiramin üretimi açısından *S. thermophilus* RSKK 04082 ve *L. delbrueckii* subsp. *bulgaricus* DSM 20081 arasında ve, *L. plantarum* RSKK 02030 ve *L. delbrueckii* subsp. *bulgaricus* DSM 20081 arasında sinerjik etkileşimler görülmüştür. Bu çalışmada, *L. delbrueckii* subsp. *bulgaricus* DSM 20081 bakterisinin yoğurtlarda tiramin birikimi üzerine dolaylı etkisi olduğu görülmüştür.

Üçüncü bölümde, çeşitli fermente gıda ürünlerinde (ekmek, bira, kırmızı şarap, beyaz peynir, yoğurt, kefir ve kakao tozu) tandem kütle spektrometrisini kullanarak kynurenin yolundaki triptofan ve türevlerinin tayini için analitik bir yöntem geliştirilmiştir. Metot, pentaflorofenil (PFP) kolonu kullanılarak sulu ekstraksiyon ve ters faz kromatografik ayrımını içermektedir. Metot, farklı fermente gıda matrislerinde düşük ppb düzeyindeki triptofan ve türevlerinin miktarlarının belirlenmesini sağlamıştır. Süt ürünlerinin (yoğurt, beyaz peynir ve kefir) kynurenin içerdiği tespit edilmiştir. Analiz edilen ekmek örnekleri kynurenik asit içermezken, maya fermantasyonu ile üretilen bira ve kırmızı şarap örneklerinin kynurenik asit içerdiği bulunmuştur. Analiz edilen gıdalardan kakao tozu, nöroprotektif bir bileşik olan kynurenik asidi yüksek konsantrasyonlarda içermektedir.

Dördüncü bölümde, şıra fermantasyonu sırasında kynurenin yolundaki triptofan türevlerinin *S. cerevisiae* NCYC 88 ve *S. pastorianus* NCYC 203 tarafından oluşumu Gompertz modeli kullanılarak araştırılmıştır. Bu çalışmanın sonucu olarak, *S. cerevisiae* NCYC 88 fermantasyon boyunca *S. pastorianus* NCYC 203'e kıyasla daha fazla triptofan kullanmıştır. *S. cerevisiae* NCYC 88 ve *S. pastorianus* NCYC 203 ile fermente edilen şıranın kynurenik asit konsantrasyonu fermantasyon sırasında artmıştır. *S. cerevisiae* NCYC 88 ve *S. pastorianus* NCYC

203 ile fermente edilmiş şıraya triptofan ilave edildiğinde kynurenik asit konsantrasyonu artmıştır. Ayrıca, şıraya triptofan ilave edildiğinde *S. cerevisiae* NCYC 88 ile fermente edilen şıradaki kynurenin içeriği artmıştır. Bu çalışmada bira fermantasyonu sırasında, *S. pastorianus* NCYC 203 mayasının, S. cerevisiae NCYC 88 mayasına göre daha fazla niasin kullandığı görülmüştür.

Anahtar kelimeler: fermantasyon, bira, yoğurt, biyoaktif aminler, triptofan türevleri, kynurenin yolu

ACKNOWLEDGEMENTS

Firstly, I thank my supervisor Prof. Dr. Vural Gökmen for his support and guidance during my studies at Hacettepe University.

I would like to thank to Dr. Tolgahan Kocadağlı for not only his help in the laboratory but also for his ideas about my studies.

I am very grateful to TUBITAK BIDEB for the scholarship provided during my PhD study.

I also would like to thank to my friends from laboratory especially Dr. Burçe Ataç Mogol, Dr. Kübra Özdemir, Dr. Halise Gül Akıllıoğlu, Dr. Neslihan Taş, Dr. Aytül Hamzalıoğlu, Ecem Evrim Çelik, Ezgi Doğan Cömert, Işıl Gürsul Aktağ and Özge Dönmez for their support, discussing ideas or help in lab work.

I owe a debt of gratitude to my mother Sevim Yılmaz for always believing in me, supporting me and sharing her love with me.

CONTENTS

page

LIST OF TABLES

page

LIST OF FIGURES

page

SYMBOLS AND ABBREVIATIONS

Symbols

A	Asymptotic concentration
μ_m	Maximum production rate
e	Euler number
λ	Lag period
t	Time

Abbreviations

CFU	Colony forming Unit
DAO	Diamine oxidase
DNA	Deoxyribonucleic acid
DOPA	Dihydroxyphenylalanine
EMP	Embden-Meyerhof-Parnas
EPS	Exopolysaccharides
ESI-MS	Electrospray Ionisation-Mass Spectrometry
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
IDO-1	Indoleamine-2,3-dioxygenase-1
IDO-2	Indoleamine-2,3-dioxygenase-2
LOD	Limit of detection
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MRS	De Man, Rogosa & Sharpe
MS	Mass spectrometry
NAD	Nicotinamide adenine dinucleotide
PCA	Plate Count Agar
PEP	Phosphoenolpyruvate
PFP	Pentafluorophenyl
PTS	Phosphotransferase
TDO	Tryptophan-2,3-dioxygenase

UHT	Ultra high temperature
UPLC	Ultra high performance liquid chromatography
YPD	Yeast Extract Peptone Dextrose

INTRODUCTION

Fermentation causes many changes in food properties. Bioactive amines and some neuroactive compounds can be formed during fermentation in consequence of metabolism of microorganisms found in the fermented foods. Bioactive amines are generally synthesized by decarboxylation of amino acids. However, some hydroxylation and condensation reactions are essential for synthesis of some bioactive amines such as dopamine and serotonin. Investigation of formation of bioactive amines during fermentation is of critical importance because of the fact that bioactive amines have some physiological roles on human health. Moreover, if they are taken up from foods in high amounts, humans can be affected negatively [1]. Beside bioactive amines, tryptophan derivatives in kynurenine pathway have significant roles on human health since they are physiological and physiopathological modulators in the central nervous system [2]. Beside humans, kynurenine pathway is found in yeasts and some bacteria [3-5]. Therefore, tryptophan derivatives in kynurenine pathway can be found in fermented foods. The principal focus of this thesis was to understand changes of amino acids and thereby, formation of bioactive amines and tryptophan derivatives during fermentation. Taking into account this aim, this thesis is presented as five chapters:

Chapter 1 gives the general information about the fermentation, bioactive amines and tryptophan derivatives in kynurenine pathway.

Chapter 2 discusses the effect of *S. cerevisiae* on the formation of GABA and the other bioactive amines during wort fermentation. The results reported in this chapter have been published in the following article;

Yılmaz, C., Gökmen, V., Comparative evaluation of the formations of gamma-aminobutyric acid and other bioactive amines during unhopped wort fermentation, *Journal of Food Processing and Preservation*, doi: 10.1111/jfpp.13405, **2017**.

Chapter 3 discusses the formation of tyramine during yoghurt fermentation with the focus on interaction between *Streptococcus thermophilus* RSKK 04082, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 and *Lactobacillus plantarum* RSKK 02030. The results reported in this chapter have been published in the following article;

Yılmaz, C., Gökmen, V., Formation of tyramine in yoghurt during fermentation-Interaction between yoghurt starter bacteria and *Lactobacillus plantarum*, *Food Research International*, 97, 288-295, **2017**.

Chapter 4 discusses the determination of tryptophan and its derivatives in kynurenine pathway using tandem mass spectrometry in various fermented food products. The results reported in this chapter have been published in the following article;

Yılmaz, C., Gökmen, V., Determination of Tryptophan Derivatives in Kynurenine Pathway in Fermented Foods Using Liquid Chromatography Tandem Mass Spectrometry, *Food Chemistry*, 243, 420-427, **2018**.

Chapter 5 discusses the formation of tryptophan derivatives in kynurenine pathway by *S. cerevisiae* NCYC 88 and *S. pastorianus* NCYC 203 during wort fermentation by using Gompertz kinetic model.

1 GENERAL INFORMATION

1.1 Fermentation

Fermentation is one of the oldest processing technologies as archaeological studies have demonstrated that bread and beer are mostly consumed foods in ancient Egypt [6]. The history of fermentation began with the detection of the microorganisms in 1665 by Van Leeuwenhoek and Hook [7]. In 1857, Pasteur investigated the effects of microorganisms on fermentation. Besides, it was revealed by him that there are several fermentation types [8]. Furthermore, in 1877, Sir John Lister investigated the role of *Lactococcus lactis* in fermented milk [9]. To date, many developments about the fermentation have been carried out since fermentation has several advantages on food quality. Fermentation is a preservation technique for foods, which requires low energy [10]. It increases the microbial safety by preventing the growth of the undesired microorganisms. For instance, lactic acid synthesized in yoghurt prevents the growth of spoilage bacteria. Fermentation also improves the digestibility since microbial enzymes break down carbohydrates, proteins and other food components. Furthermore, it adds more qualified properties to foods in terms of functionality, taste and texture [11].

1.1.1 Fermented Foods

The principal groups of fermented foods are beverages, dairy products, cereal-based products and fermented foods produced from meat, fish, fruits and vegetables [12]. Fermented foods are generally produced using lactic acid bacteria, yeasts and moulds. Yeasts are key microorganisms for production of wine, beer and bread. Lactic acid bacteria are used for manufacturing of yoghurt, cheese, kefir, pickled cucumbers, sauerkraut, olives, sourdough breads, and fermented sausages. Moulds are used for production of tempeh and soy sauce [10]. Common fermented foods are demonstrated in Table 1.1 [13].

Table 1.1. Common fermented foods and starter cultures [13]

Fermented Foods	**Microorganisms**
Wine	*Saccharomyces cerevisiae*
	Oenococcus oeni
Beer	*Saccharomyces cerevisiae*
	Saccharomyces pastorianus
Bread	*Saccharomyces cerevisiae*
Yoghurt	*Streptococus thermophilus*
	Lactobacillus delbrueckii subsp. *bulgaricus*
Cheese	*Propionibacterium freudenreichii*
	Leuconostoc mesenteroides
	Leuconostoc lactis
	Streptococus thermophilus
	Lactobacillus lactis
	Lactobacillus helveticus
	Lactobacillus casei
Sausage	*Lactobacillus plantarum*
	Lactobacillus curvatus
	Lactobacillus sakei
	Pediococcus pentosaceus
	Pediococcus acidilactici
Fermented vegetables	*Lactobacillus plantarum*
	Pediococcus acidilactici
	Leuconostoc mesenteroides
Soy sauce	*Pediococcus halophilus*
Sourdough bread	*Lactobacillus sanfranciscensis*

1.1.1.1 Beer

Beer, a yeast-fermented beverage, is produced after several processing steps including malting, mashing, boiling with hop, fermentation and packaging. In malting step, germination of barley is carried out and some enzymes such as amylases and proteases are activated during germination. After milling of the barley malt, grist is suspended with water and heated to a certain temperature for degradation of starch to glucose by amylase. Afterwards, malt particles are removed from the medium and sweet wort is boiled with hop, which provides a specific aroma and bitter taste to the beer. Boiling also serves several functions such as sterilization of wort and inactivation of enzymes [10, 14]. *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* are starter cultures using for fermentation of wort. Lager fermentation is carried out using *Saccharomyces pastorianus*, which flocculates to the bottom of wort. On the other hand, *Saccharomyces cerevisiae*, which flocculates to the top of wort, is used for production of ale beers. Temperature of lager fermentation is 8-12 ^{0}C while temperature of ale fermentation 18-25 ^{0}C. Furthermore, the extent of the fermentation time is 5-7 days for ale beers and 7-12 days for lager beers. During the first hours of fermentation, dissolved oxygen is consumed by yeast. At approximately 8-16 hours of fermentation, carbon dioxide begins to form and the temperature of the medium increases. In the 24-48 hours of fermentation, maximum growth of yeast and maximum carbohydrate assimilation are observed. Meanwhile, lower pH is observed in the medium because of the fact that organic acids are synthesized by yeast. After consumption of carbohydrates, ethanol is formed. During fermentation, yeast synthesizes two α-acetohydroxy acids, which are then transformed into diacetyl and vicinal diketones. After fermentation, beer is stored at 0 ^{0}C and when concentrations of vicinal diketones are low enough, beer filtered to remove the yeasts. Moreover, beer can be pasteurized to inactivate the spoilage microorganisms [10, 15].

1.1.1.2 Yoghurt

Yoghurt, a dairy fermented product, is produced after milk standardization in terms of fat content and solid-non-fat (protein, lactose and mineral) content. Fat content of milk can be arranged by removing fat from milk, addition of cream into the milk or combinations of these methods. For standardization of solid-non-fat

concentration, there are some various methods including heating of milk, concentration by vacuum evaporation, concentration by membrane filtration, addition of milk powder, whey powder and casein powder. After standardization of milk, milk is heated to 95-97 ^{0}C for 7-10 minutes and then homogenization (55-80 ^{0}C and 10-20 MPa) is carried out. The homogenized milk is cooled to 41-42 ^{0}C and milk is inoculated with *Streptococcus thermophilus* and *L. bulgaricus*. Then, starter microorganisms began to ferment lactose, which leads to formation of lactic acid. Moreover, aroma compounds including acetaldehyde, acetoin and diacetyl and exopolysachharides are formed. In set style of yoghurt, it is packaged as soon as it is inoculated with starter cultures. Fermentation is carried out until the pH of yoghurts decrease to 4.5-4.6. The yoghurt is then cooled to about 5 ^{0}C to control the acidity of the final product [16-18].

1.1.2 Microorganisms in Fermented Foods and Their Interactions

Yeast and lactic acid bacteria are mostly utilized microorganisms in fermented foods. *Saccharomyces cerevisiae* is utilized for production of bread, wine and beer. *Saccharomyces pastorianus* is the other yeast used as starter culture in beer. *Debaryomyces hansenii* is utilized for manufacturing of cheeses. *Galactomyces geotrichum* is the starter culture of some mold ripened and surface ripened cheeses. Lactic acid bacteria utilized in milk are primarily *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis* [19]. *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are starter microorganisms in yoghurt production. *Streptococcus thermophilus* is also used for production of some cheeses, especially Parmesan. *Leuconostoc mesenteroides* subsp. *mesenteroides, Leuconostoc mesenteroides* subsp. *cremoris, Leuconostoc mesenteroides* subsp. *dextranicum* are microorganisms that are used in the fermentation of vegetables. Due to production of polysaccharides, they are used as thickeners in foods. They are also related with the carbon dioxide formation in Gouda cheeses. Pediococci has a role on the fermentation of vegetables and meats. *Pediococcus halophilus* takes part in the production of soy sauce [13].

Fermented foods are generally produced with mixed cultures instead of single strain cultures. Therefore, each strain/species interacts not only with food matrix

but also with other microorganisms composing the mixed culture. Interactions between microorganisms divide into two categories as direct and indirect interactions. Direct interaction occurs via signaling molecules excreted by some microorganisms to provide cell-to-cell communication. This communication is termed as “quorum sensing”, which has important roles to regulate some physiological activities such as biofilm formation, antibiotic resistance and sporulation [20]. Indirect interaction might occur if physicochemical properties of growth medium are changed by strains. The characteristics of medium generally change in consequence of competition for nutrition and synthesis of new beneficial or harmful compounds. Considering one strain’s effect on other strain, indirect interaction divides into five categories: competition, mutualism, commensalism, parasitism and amensalism [21].

Amensalism is an interaction type between microorganisms in which one microorganism affects the other microorganism adversely while it is not affected. Alcohols, carboxylic acids and antimicrobial compounds such as bacteriocins produced by one microorganism can affect negatively the other microorganisms [21]. In competition, microorganisms compete for nutrients. In this interaction type, both interacting microorganisms are affected in a negative way [22]. In dairy fermentations, free amino acids are generally competitive factors for microorganisms since nitrogenous compounds is limiting in dairy products [21]. Commensalism is an interaction type in which one microorganism affects the other microorganism positively whereas it is not affected. In some cheeses, propionic acid bacteria produce acetate and propionate using lactic acid [21, 22]. $PrtP^+$ strains produce peptides from milk protein owing to having extracellular proteases and $PrtP^-$ strains utilize these peptides as nitrogen sources in some cheeses. Parasitism is an interaction type in which one microorganism benefits at the expense of another. Bacteriophages can be a good instance of parasitism. Mutualism is described as each microorganism derives a benefit from the interaction [21]. Growth rates of *S. thermophilus* and *L. bulgaricus* are greater when mixture of them is used for yoghurt production. This interaction type in yogurt is termed as mutualism [23, 24].

In yoghurt, *S. thermophilus* incrases the count of *L. bulgaricus* by synthesizing folic

acid and carbon dioxide, which have important roles for synthesis of purines and amino acids [16, 25]. Under favour of having the cell-wall protease PrtB, *L. delbrueckii* subsp. *bulgaricus* provides nitrogen sources for *S. thermophilus* [26]. Furthermore, aromatic compounds and exopolysaccharides are produced much more in yogurts fermented with mixed culture than single strain culture [27, 28].

In wine, due to producing ethanol, *Saccharomyces cerevisiae* can affect the non-*Saccharomyces* species negatively [29]. The synthesizing of extracellular proteins and glycoproteins by killer yeasts can be a good example for amensalism in wine. Some compounds including ethanol, sulfur compounds, protein and peptides released by *Saccharomyces cerevisiae* affect negatively the malolactic fermentation in wine [30]. In addition to these negative interactions, most of the positive interactions are also observed in wine. Non-*Saccharomyces* species having extracellular proteolytic activity provides amino acids for *Saccharomyces cerevisiae* as a nitrogen source [31]. Moreover, bacterial growth can be stimulated due to the fact that nitrogen compounds release into the medium during yeast autolysis [30].

In cheeses, the studies demonstrated that utilization of lactate by yeasts causes decreasing of pH of the cheese surface, which leads to the growth of less acid tolerant bacteria during cheese ripening [32]. Moreover, production of bacteriocins by lactic acid bacteria affects negatively the spoilage bacteria [33].

1.1.3 Metabolism of Food Components during Beer and Yoghurt Fermentation

1.1.3.1 Metabolism of Nitrogen

Nitrogenous components, which are amino acids, peptides and proteins, comprise approximately 4-5% of the total dissolved solids in wort. The amount of nitrogenous components in wort depends on the chemical composition of malt, type of adjunct, proteolysis during mashing, and wort boiling. Free amino acid concentration of wort is approximately 150-230 mg/L. Most of the free amino acids in wort are generated during mashing and malting stages of brewing. In addition to amino acids, peptides and proteins, wort also contains nucleic acids and ammonia, whose concentrations are 280-330 mg/L and 25-30 mg/L, respectively. Moroever, amines including methylamine, ethylamine, butylamine, tyramine,

hordenine and choline can be found in wort depending on wort production conditions [14]. Amino acids are important for synthesis of amino acids, proteins, cell viability, fermentation rate and ethanol tolerance [34]. Amino acids are utilized by yeast in a certain order during beer fermentation. Amino acids are classified into four classes based on their assimilation during fermentation. In classes A and B, the amino acids (arginine, asparagine, aspartate, glutamate, glutamine, lysine, serine, threonine, histidine, isoleucine, leucine, methionine, valine) are generally used for protein synthesis. Amino acids in class C (alanine, phenylalanine, tryptophan and tyrosine) are only used when amino acids in class A are consumed in the medium. Amino acid in class D (proline) is not used by yeast during fermentation since mitochondrial oxidase, which is necessary for dissimilation of proline, is not found under the anaerobic conditions of fermentation. Dipeptides and tripeptides can be utilized by *S. cerevisiae* although oligopeptides are not transported into the cells [35, 36]. Amino acids transport into the cell by several transport enzymes [34].

Milk proteins consist of casein and whey proteins. The count of free amino acids in yoghurt depends on starter microorganisms since *S. thermophilus* and *L. bulgaricus* have different exopeptidase and endopeptidase activities. Although *S. thermophilus* has more exopeptidase activity than *L. delbrueckii* subsp. *bulgaricus,* endopeptidase activity is higher in the *L. delbrueckii* subsp. *bulgaricus.* Therefore, *L. delbrueckii* subsp. *bulgaricus* hydrolyses the casein to obtain polypeptides and then, amino acids are formed from polypeptides by the *S. thermophilus,* which has exopeptidase activity. Free amino acid concentration of the yoghurt depends on the rate of the assimilation and proteolysis by the bacteria. In yoghurt, glutamic acid, proline, serine and alanine are less preferable amino acids by bacteria [16, 37].

In amino acid synthesis, amination and transamination reactions are important for microorganisms. Amination reactions require ammonium ions and keto acids forming the carbon skeleton of the amino acids. The pyruvate and 2-oxoglutarate occurring in the catabolism provide amino groups from ammonium ions. If the concentration of ammonium ions in the medium is high, L-glutamate is formed from 2-oxoglutarate by L-glutamate dehydrogenase. Alanine and glutamate are

the primary amino acids. The synthesis of alanine and glutamate is sufficient for the formation of other amino acids by transamination [38].

1.1.3.2 Metabolism of Carbohydrate

Carbohydrates comprise 90-92% of wort solids. [39]. Wort contains sucrose, fructose, glucose, maltose, maltotriose and dextrin. Although *S. cerevisiae* and *S. pastorianus* use sucrose, glucose, fructose, maltose and maltotriose, maltotetraose and dextrins do not utilized by yeasts. The first utilized sugars in wort are sucrose, glucose and fructose, which are consumed in the 24 hours of the fermentation. Then, maltose is consumed and when concentration of maltose in the medium is low, maltotriose begins to assimilate. Maltose and maltotriose are transfered into the cell, while sucrose is firstly hydrolyzed to glucose and fructose by invertase outside the cell. Maltose and maltotriose are hydrolyzed into the cell by α-glucosidase enzymes. Two glucose uptake systems termed as low and high affinity have been detected to date. Moreover, the maltose uptake system is found to require cellular energy [34, 35]. During the first hours of beer fermentation, Embden-Meyerhof-Parnas (EMP) pathway and Krebs cycle are effective for oxidative degradation of carbohydrates. However, in the later period of the fermentation, alcoholic fermentation begins to predominate [39]. In alcoholic fermentation, after pyruvate is formed from hexose by glycolysis, it is decarboxylated to acetaldehyde. Then, ethanol is formed by NAD^+ linked alcohol dehydrogenase [36].

In yoghurt, lactose is metabolized via homofermentative or heterofermentative pathways depending on starter culture. *S. thermophilus*, *L. bulgaricus* and *Lactobacillus acidophilus* use homofermentative metabolic pathway. However, *Bifidobacterium* spp. ferments lactose heterofermentatively [16]. Lactococci has a lactose transport system, phosphoenolpyruvate dependent (PEP)-phosphotransferase system (PTS). Due to containing phospho-β-galactosidase (β-P-gal), this system provides conversion of lactose to galactose and glucose [40]. On the other hand, *S. thermophilus* has lactose permease (LacS), which transfers lactose into the cell [40]. *S. thermophilus*, *L. bulgaricus* and *Lactobacillus acidophilus* use EMP pathway after lactose converts to glucose and galactose. These microorganisms can also use galactose via Leloir pathway if all glucose in the medium is consumed. Pyruvate, metabolized by EMP, is then converted to

lactic acid [16, 41]. In heterofermentative pathway, lactose is transferred into the cell and hydrolyzed into galactose and glucose. Fructose-6-phosphate shunt is utilized for catabolism of hexoses. The end products of heterofermentative pathway are lactate and acetate. Moreover, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* produce exopolysaccharides (EPS) using carbohydrates. Acetaldehyde is also one of the flavor components produced by yoghurt bacteria via lactose metabolism in which pyruvate is converted to acetaldehyde by α-carboxylase. The other patway for synthesis of acetaldehyde involves production of acetyl-CoA from pyruvate by pyruvate dehydrogenase and formation of acetaldehyde from acetyl-CoA by aldehyde dehydrogenase [16, 40].

1.1.3.3 Metabolism of Lipid

Malt, adjuncts and hops are the source of lipid in wort. Predominant lipid group found in barley and malt is triacylglycerols. The amount of lipid in wort depends on processing conditions during wort production [14]. High concentration of fatty acids is taken up through simple diffusion by *Saccharomyces cerevisiae* whereas low concentration of fatty acids is taken up via facilitated diffusion system. Fatty acids are produced from Acetyl-CoA. Acetyl-CoA carboxylase and fatty acid synthase are two principal enzymes for the production of fatty acids [34, 36].

Most abundant lipid in milk is acylglycerols. Beside acylglycerols, phospholipids, sterols, fatty acids and fat-soluble vitamins are found in milk [16]. Rao and Reddy reported that during fermentation of milk by *S. thermophilus*, *L bulgaricus* and *Lactobacillus acidophilus,* concentrations of saturated fatty acids increased [42]. Furthermore, level of volatile fatty acids can increase based on starter bacteria, type of milk and fermentation conditions [16].

1.2 Bioactive Amines

1.2.1 Classification

Bioactive amines are synthesized as a result of decarboxylation of amino acids [43, 44]. Depending on the number of the amino groups, they are grouped as monoamines (tyramine, tryptamine and serotonin), diamines (putrescine and cadaverine) and polyamines (spermine and spermidine) [45]. They might also be classified as aromatic (tyramine), aliphatic (putrescine and cadaverine) and heterocyclic (histamine and tryptamine) amines [46]. Moreover, some bioactive amines can be classified as catecholamines (dopamine, norepinephrine and epinephrine) and indoleamines (serotonin) [47]. Figure 1.1 indicates the chemical structure of some bioactive amines.

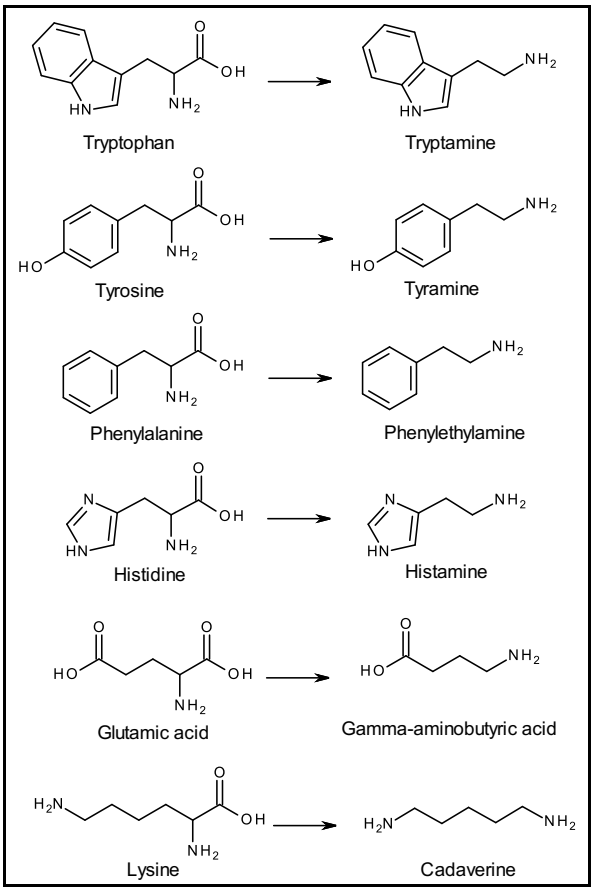


Figure 1.1. Chemical structures of some bioactive amines and their precursor amino acids

1.2.2 Physiological and Toxicological Effects

Histamine, which is formed by decarboxylation of histidine, is produced in mast cells, basophils, platelets, histaminergic neurons and enterochromaffine cells. In addition to being a neurotransmitter, histamine has several physiological effects on human health including, gastric acid secretion, circadian rhythm and cognition [48]. In healthy people, bioactive amines are metabolized by amine oxidases including monoamine oxidases [49] and diamine oxidases (DAO) [43, 50]. Beside amine oxidases, methyl or acetyltransferases might metabolize histamine [51]. If high level of dietary histamine is consumed, histamine intoxication, which is known as “scombroid poisoning” can be occurred [51]. Histamine causes several adverse symptoms such as headaches, sweating, blood pressure disorders [52]. Drugs containing monoamine oxidase inhibitors might also trigger the histamine intoxication [45]. Moreover, availability of other amines might affect the histamine intoxication [51]. Histamine is the only bioactive amine, which has legal limits for scombrid fish (200 mg/kg) and fish products (400 mg/kg) [53, 54]. The legal limit for histamine in wine detected by the European Union was 2-10 mg/L [1].

Tyramine, which is synthesized by decarboxylation of tyrosine, plays role as a hormonal mediator and neurotransmitter [52, 55]. Tyramine increases cardiac output and respiration, elevates blood glucose [52]. Furthermore, it has a role in the central nervous system as a releasing agent of catecholamines [56]. If tyramine rich foods are consumed, hypertensive crisis, called as cheese reaction, can occur [55].

GABA is one of the bioactive amines. It is synthesized by decarboxylation of glutamic acid, catalysed by glutamic acid decarboxylase enzyme [57]. Low level of GABA or alteration in GABAergic circuits in brain causes several psychiatric and neurological disorders [58-61].

Serotonin, known also as 5-hydroxytryptamine, is a neurotransmitter in humans [62]. Serotonin is found in the serotonergic neurons in the brain. On the other hand, it is mostly produced in the enterochromaffin cells [63]. Serotonin is synthesized from tryptophan in two enzymatic steps in humans. First step includes conversion of tryptophan to 5-hydroxytryptophan. Further, 5-hydroxytryptophan is transformed to serotonin by L-amino acid decarboxylase [64]. Serotonin has many important roles on human health, including regulation of appetite, mood and blood

pressure [65-67]. Decreasing or depletion of the synthesis of serotonin might cause several diseases, including depression, obesity and schizophrenia [68-70].

Phenylethylamine is produced by decarboxylation of phenylalanine and it may cause increasing of blood pressure, headaches, sweating and vomiting [71]. Tryptamine is synthesized by decarboxylation of tryptophan. Tryptamine is a serotonin-releasing agent [1]. High amounts of tryptamine and phenylethylamine can cause adverse effects on human health. Phenylethylamine and tryptamine might inhibit uptake of catecholamines [72].

Dopamine is a catecholamine and synthesized in the central nervous system [73]. Dopamine is synthesized by decarboxylation of L-DOPA [1]. It has many important roles on human health such as motor activity, coordination, muscle tension, emotional processes and appetite control [74].

Putrescine and cadaverine are formed by decarboxylation of ornithine and lysine [75]. Putrescine can be transformed into spermidine and spermine. These polyamines have significant role especially on cell growth [46]. Moreover, it was reported that they prevent oxidation of fatty acids [76]. On the other hand, since they inhibit the histamine and tyramine detoxifying enzymes, they can increase the concentration of histamine and tyramine in blood [55]. Due to reacting with nitrite, putrescine and cadaverine can form carcinogenic nitrosamines [48].

1.2.3 Bioactive Amines in Fermented Foods

Bioactive amines are synthesized in humans, plants and microorganisms [43]. Microorganisms having amino acid decarboxylase activity can synthesize bioactive amines in foods [77]. Amino acid decarboxylase enzymes can be found in spoilage microorganisms or cultures used for manufacturing of fermented foods [43]. Decarboxylation of amino acids is carried out as microorganism response to acid stress. In decarboxylation reactions, intracellular proton is consumed and carbon dioxide is formed therefore, pH of environment is regulated. Decarboxylation activity also provides energy for the microorganisms [78, 79]. Level of free amino acids and availability of amino acid decarboxylase enzymes in the medium are two main factors for the formation of bioactive amines [49]. In addition to these factors, environmental conditions are significant for the synthesize bioactive amines in fermented foods [55].

Beer, a fermented beverage, contains histamine, tyramine, putrescine, cadaverine, phenylethylamine, tryptamine, spermine and spermidine [80-84]. Slomkowska et al [84] reported that total concentration of biogenic amines in Polish beers was 16.15 mg/L. Moreover, Polish beers were reported to have high concentration of spermine (8.43 mg/L), spermidine (3.37 mg/L) and putrescine (1.75 mg/L). Czech beers were found to contain histamine (0.55 mg/L), tryptamine (1.21 mg/L), tyramine (6.85 mg/L), putrescine (8.84 mg/L) and cadaverine (12.9 mg/L) [83]. Izquierdo-Pulido (1996) reported that the concentrations of spermine, spermidine, tryptamine and phenylethylamine were lower than 2 mg/L and the concentration of histamine ranged from 0.5 mg/L to 1.1 mg/L in European beers [85]. Raw materials and brewing conditions are effective for accumulation of bioactive amines in beers [86]. Kalac et al [87] reported that malt, yeasts and hops can contain spermidine, phenylethylamine, agmatine and spermine. It was also reported in this study that formation of histamine, tyramine and cadaverine are related with low hygienic conditions during brewing. In beer, formation of bioactive amines depends on the microorganisms, especially lactic acid bacteria, which contaminate beer [88, 89]. According to a study reported by Izquierdo-Pulido (1995), *Saccharomyces cerevisiae* var. *uvarum* did not synthesize tyramine and histamine during fermentation [90]. Besides, it was reported that tyramine and histamine were formed by *Pediococcus* spp. which were isolated from beers [89]. Furthermore, *L. brevis* increased the concentration of putrescine and tyramine during fermentation in beer [88, 89].

Total bioactive amine concentration of wine reaches up to 50 mg/L [91]. Precursor bioactive amines in wines are histamine, tyramine, putrescine, cadaverine and phenylethylamine [92]. Nalazek-Rudnicka et al [93] found putrescine (3300 μg/L), spermidine (2600 μg/L) and agmatine (1160 μg/L) in wine samples. Moreover, methylamine, dimethylamine, spermine, tyramine, *β*-phenylethylamine, isopentylamine, histamine, and cadaverine were also determined in these wine samples. Raw material and processing conditions affect the formation of bioactive amines in wine [72]. Grape varieties, maturity degree, soil type, fertilization, irrigation, climatic conditions, maceration, degree of autolysis, alcohol content, microbial growth and fermentation conditions affect the synthesis of bioactive amines in wine [94]. It was reported that nitrogenous fertilization of soil was a

significant factor on bioactive amine formation in wine [95]. Landete et al [96] reported that concentrations of putrescine and histamine in wines produced with different grape varieties are significantly different. Ancin-Azpilicueta [97] reported that high amounts of bioactive amines are formed in wines produced with grapes which have high amount of amino acids. It was revealed that the extent of the skin maceration time was effective on the formation of histamine, tyramine and putrescine in wine [98]. It was also reported that the concentration of bioactive amines increased when sulfur dioxide level in wine decreased [99].

In fermented dairy products, cheeses contain generally high level of bioactive amines [72]. Tyramine and histamine are mostly found bioactive amines in cheeses [100, 101]. In addition, low concentrations of spermidine and spermine are found in cheeses [72]. It was reported in a study that cheeses produced with raw milk and ripened in a cave contained 1500 mg/kg total bioactive amine [102]. Pachlova et al [103] investigated the bioactive amine content of brine-ripened cheeses. The predominant bioactive amines in these cheeses were putrescine, tyramine and histamine and, total bioactive amine content were above 120 mg/kg. Poveda et al [104] reported that tyramine (4.2-50.7 mg/kg) and histamine (10.2-60.5 mg/kg) were the most abundant bioactive amines in pasteurized goat milk cheeses and total concentration of bioactive amine ranges from 26 mg/kg to 175.1 mg/kg in these cheeses. The amount of bioactive amines in cheeses depends on parameters including starter and non-starter microorganisms, ripening time, ripening conditions, pH, concentration of salt, moisture content, and storage temperature [79, 105, 106]. Moreover, proteolytic enzymes, which are added for ripening of cheeses can increase the formation of bioactive amines [1]. Among dairy products, yoghurt is one of the dairy products containing less bioactive amines. Bodmer et al [107] reported that histamine concentration of yoghurt was up to 13 mg/kg. It was also reported that the bioactive amine level of the yoghurt ranged from 2.5 mg/kg to 26.7 mg/kg [108].

In dry fermented sausages, phenylethylamine, putrescine, cadeverine, tyramine and tryptamine were observed [109]. Van Ba et al [110] reported that putrescine (88.64-455.39 mg/kg) and tyramine (223.85-444.67 mg/kg) were the most abundant bioactive amines in fermented sausages. Moreover, Sun et al [111] reported that histamine (196.06 mg/kg), tyramine (164.67 mg/kg) and cadaverine

(141.65 mg/kg) were observed in Sichuan-style spontaneously fermented sausages. Some other fermented foods such as cocoa and bread can contain bioactive amines. Oracz et al [112] reported that raw cocoa beans contain dopamine, serotonin, tyramine, tryptamine and phenylethylamine. Moroever, tryptamine, tyramine, spermidine and spermine were determined in cocoa beans during fermentation [113]. Diana et al [114] reported that sourdough breads were found to contain GABA and tyramine. It was also reported that vinegar was found to contain histamine, putrescine, spermidine, spermine, agmatine and tyramine [1]. Putrescine, histamine, tyramine and cadaverine were found in fermented vegetables such as sauerkraut and fermented soybean [1].

1.3 Tryptophan Derivatives

1.3.1 Kynurenine Pathway

Tryptophan is a precursor of many compounds having biological importance. Tryptophan is utilized for protein synthesis or metabolized through three pathways, which are serotonin, indole and kynurenine pathways [57]. Approximately 95% of tryptophan catabolism proceeds via kynurenine pathway and only 1% is transformed into serotonin [115]. In serotonin pathway, hydroxylation of tryptophan to 5-hydroxytryptophan and further, decarboxylation of 5-hydroxytryptophan to 5-hydroxytryptamine (serotonin) are carried out [57]. In kynurenine pathway, tryptophan is transformed to kynurenine via tryptophan-2,3-dioxygenase (TDO), indoleamine-2,3-dioxygenase-1 (IDO-1) and indoleamine-2,3-dioxygenase-2 (IDO-2) [116]. Figure 1.2 indicates the schematic illustration of kynurenine pathway in humans. TDO is generally localized in the liver while IDO is localized in gut, lung and brain [117]. Kynurenine may be converted to kynurenic acid, anthranilic acid, 3-hydroxykynurenine, and last two may be further converted to 3-hydroxyanthranilic acid. It is metabolized into 2-amino-3-carboxymuconate semialdehyde, which is further transformed into quinolinic acid or picolinic acid. In the later stages, quinolinic acid participates in synthesis of nicotinamide adenine dinucleotide (NAD^+) [116, 118].

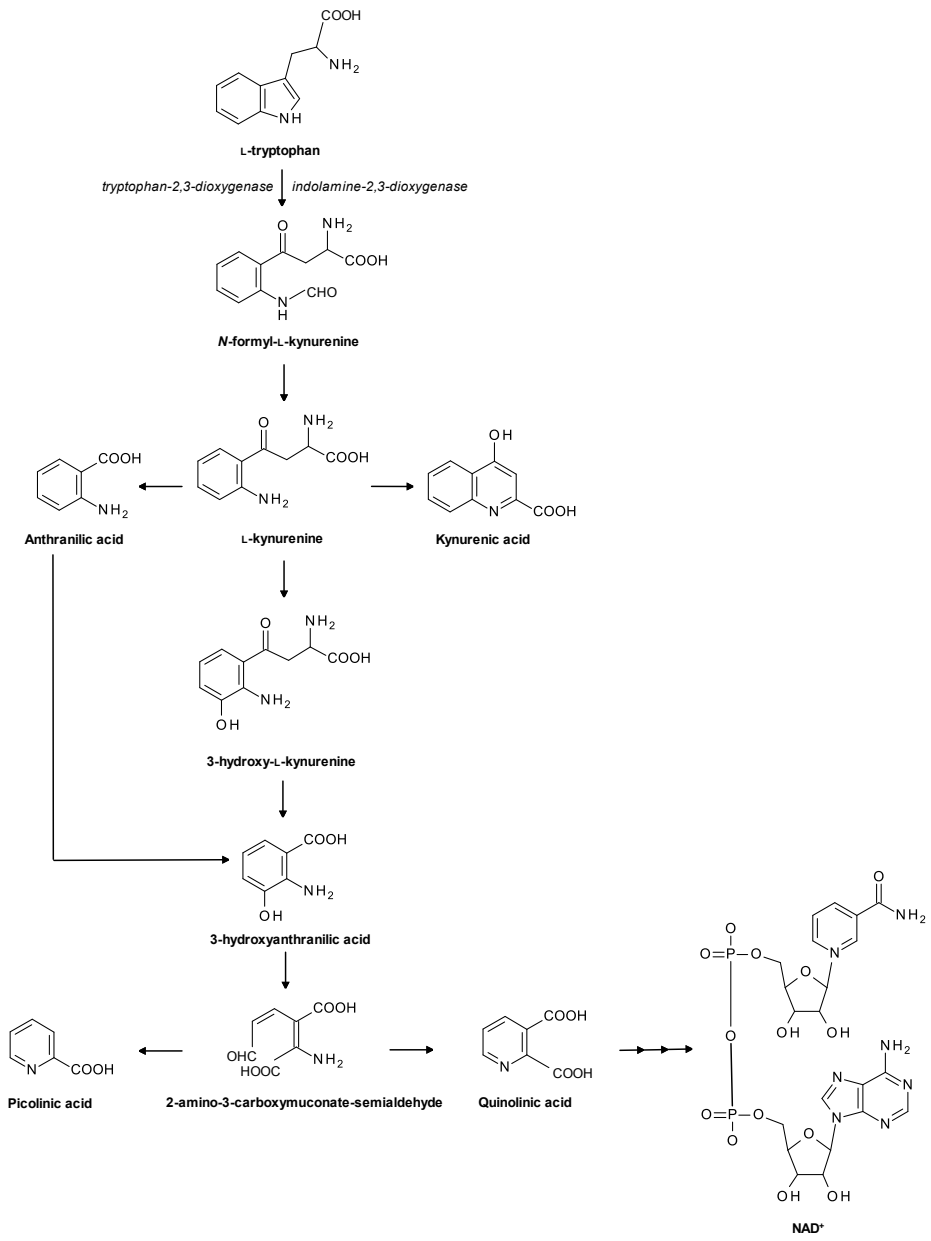


Figure 1.2. Schematic illustration of kynurenine pathway in humans

Kynurenine is a ligand at the aryl-hydrocarbon receptor and regulates gene expression and immune function [118, 119]. Kynurenic acid, α7 nicotinic acetylcholine receptor antagonist and *N*-methyl-D-aspartate receptor antagonist, is a neuroprotective compound [115, 120]. Kynurenic acid has also antioxidant properties [121]. Quinolinic acid, agonist of the glutamatergic *N*-methyl-D-aspartate receptor, is the precursor for synthesis of NAD^+, which is the cofactor in many cellular reactions such as ATP synthesis and DNA repair [118]. However, production of high levels of quinolinic acid results in neurotoxic effects on the cells [122]. Picolinic acid, a neuroprotectant and an iron/zinc chelator, has antitumoral, antifungal and antiviral properties [122]. In spite of being an immunoregulator, 3-hydroxyanthranilic acid is a neurotoxin [122].

Kynurenine pathway has an important role as a physiological and physiopathological modulator in the central nervous system [2]. Studies have showed that kynurenine pathway is involved in certain diseases including Alzheimer's disease, schizophrenia, Huntington's disease and cancer [123-127]. Besides, abnormal kynurenine pathway metabolism occurred in animals with hypoglycaemia, ischemia and perinatal hypoxia [2, 128]. According to a study, in patients having Parkinson's disease, kynurenine and kynurenic acid levels decreased and 3-hydroxykynurenine concentration increased by comparison with healthy people [2, 129]. It was reported that quinolinic acid level increased in Alzheimer's disease and Huntington's disease patients [130, 131]. It was also reported that seizure occurred when quinolinic acid was injected to the mice [132]. A study has showed that patients with schizophrenia have higher kynurenic acid level in cerebrospinal fluid than healthy people [133]. In another study, it was reported that kynurenic acid level was lower in the suicidal patients having schizophrenia compared to non-suicidal patients [134]. Moreover, quinolinic acid/kynurenic acid ratio in the cerebrospinal fluid of the suicide attempters was higher than healthy people [135]. It was reported that the level of kynurenic acid is higher in patients suffering from inflammatory bowel disease whereas it is lower in patients suffering from irritable bowel syndrome [136-138]. Besides, tryptophan derivatives in kynurenine pathway could take part in prooxidative and antioxidative processes in the brain [139]. 3-hydroxykynurenine and 3-hydroxyanthranilic acid

produce reactive hydroxyl radicals [140]. Besides, quinolinic acid triggers the lipid peroxidation [141].

Kynurenine pathway is found not only in humans but also in yeasts [3, 4]. Existence of the kynurenine pathway in yeast was demonstrated and 3-hydroxyanthranilic acid dioxygenase was characterized [4]. The genes encoding the different enzymes of the kynurenine pathway in *Saccharomyces cerevisiae* were identified [3]. It was reported that formylkynurenine formamidase and 3-hydroxy-anthranilic acid oxidase, which are enzymes in kynurenine pathway, were detected in yeast [142]. Moreover, it was reported that Bna3p is the yeast kynurenine aminotransferase [143].

Synthesis of NAD^+ in yeasts occurs via quinolinic acid in two major pathways. First pathway includes the *de novo* biosynthesis pathway. Second pathway is the salvage biosynthesis pathway [144]. In other words, in various NAD^+-consuming pathways, NAD^+ is transformed into nicotinamide, which might be recycled back to NAD^+ via nicotinic acid (NA) and some other compounds [145, 146]. Figure 1.3 indicates NAD^+ synthesis via *de novo* pathway (kynurenine pathway) and salvage pathway in yeasts [145]. It has been shown that biosynthesis of NAD^+ and niacin via kynurenine pathway is present in *Saccharomyces cerevisiae* and *Saccharomyces uvarum* [147, 148]. It was found that *Saccharomyces cerevisiae* excretes quinolinic acid and then used this compound for biosynthesis of NAD^+ [147]. Moreover, in a study, it was reported that when kynurenine and 3-hydroxyanthranilic acid were added into the medium, concentration of niacin in the medium increased [148].

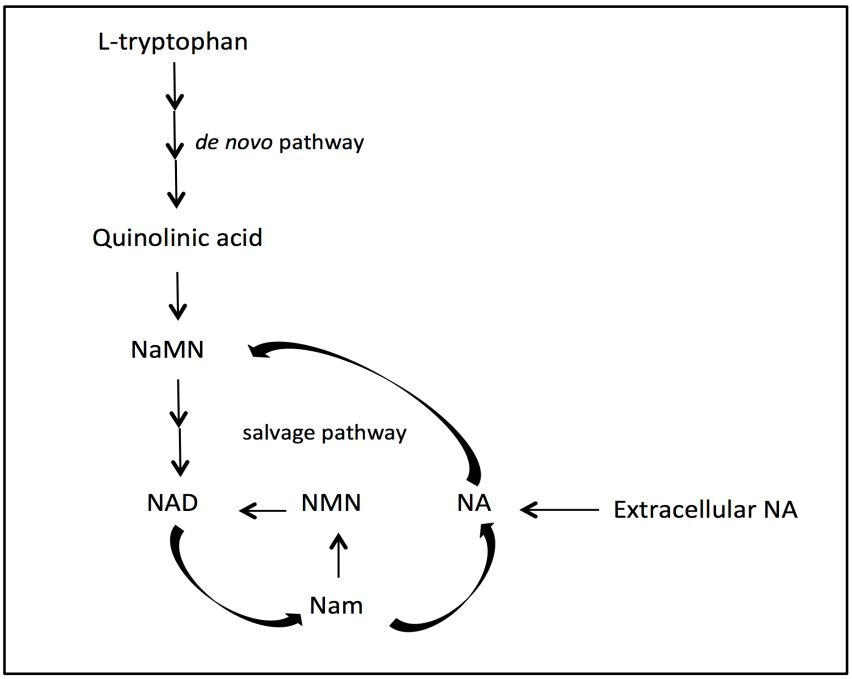


Figure 1.3. NAD^+ synthesis via *de novo* pathway (kynurenine pathway) and salvage pathway in yeasts, NaMN: nicotinic acid mononucleotide; NMN: nicotinamide mononucleotide; Nam: Nicotinamide; NA: Nicotinic acid (niacin)

1.3.2 Tryptophan Derivatives in Kynurenine Pathway in Foods

In the literature, there are limited studies including tryptophan derivatives in kynurenine pathway in foods. Additionally, most of these studies are mainly concentrated on kynurenic acid. A detailed study reported by Turski et al [149] determined concentration of kynurenic acid in several food products and they reported the highest concentrations in honey and honey products (947.7-8572.8 pmol/g), broccoli (2213.7 pmol/g), and potato (688.3 pmol/g) while lowest concentrations in Gerber-chicken (5.1 pmol/g) and red paprika (6.1 pmol/g). They also reported that although boiling did not change the kynurenic acid concentration of potato, it lowered the kynurenic acid content of carrot, broccoli and cauliflower. Kynurenic acid was also reported in sixteen varieties of potatoes and kynurenic acid concentration ranged from 0.239 to 3.240 µg/g dry weight [150]. In addition to that, other food products produced from potato including French fries, crisps and flour contained kynurenic acid. In this study, it was also showed that when storage time of potatoes increased, kynurenic acid content of potatoes decreased [150]. Chestnut honey was reported to have very high concentration of kynurenic acid (129-601 µg/g) although chestnut products contain significantly lower than in chestnut honey [138]. Furthermore, it was found that multiflorous honeys (0.09-

0.12 µg/g), lucerne honey (0.10 µg/g), honeydew honey (0.12 µg/g), thyme honey (0.14 µg/g), lavender honey (0.15 µg/g), eucalyptus honey (11.3 µg/g) and pine honey (14.2 µg/g) contained kynurenic acid [138]. Spices and some herbs were also found to contain kynurenic acid with highest concentration in basil (14.08 µg/g) and lowest concentration in black pepper (0.10 µg/g) [151]. Kynurenine was reported in honey samples (highest in multifloral honey, 4.68-5.47 mg/kg) as in the case of kynurenic acid [152]. In this study, the highest concentration of kynurenic acid (103.5-141.15 mg/kg) was observed in chestnut honey samples. Bertazzo et al [153] reported kynurenine in milk and some dairy products. Kynurenine concentration in milk was found approximately 0.07-0.08 µg/mL and did not change significantly between milk and their fermented products.

1.3.3 Analysis of Tryptophan Derivatives in Kynurenine Pathway

Determination of tryptophan derivatives in kynurenine pathway has been generally carried out using liquid chromatography with various detection methods such as ultraviolet and fluorimetric detection [118, 154-156]. To date, analyses of tryptophan derivatives in kynurenine pathway in foods have involved dilution of samples with water, precipitation of proteins and then centrifugation or isolation in solid extraction cartridges [138, 149, 150, 152]. In food samples, tryptophan derivatives were usually analyzed by using liquid chromatography with fluorescence detector [149, 151-153]. Turski et al [149] determined kynurenic acid content in various food samples using fluorescence detector. It was reported that kynurenic acid concentration in spices were also detected using fluorescence detector [151]. Furthermore, tryptophan, kynurenine, 5-hydroxytrptophan and serotonin were analyzed in milk and some milk products with fluorescence detector [153]. On the other hand, Soto et al [152] used atmospheric pressure chemical ionization mass spectrometry to quantify levels of tryptophan derivatives in honey products.

2 COMPARATIVE EVALUATION OF THE FORMATIONS OF GAMMA-AMINOBUTYRIC ACID AND OTHER BIOACTIVE AMINES DURING UNHOPPED WORT FERMENTATION

2.1 Introduction

Bioactive amines are mainly formed by decarboxylation of related amino acids or by amination/transamination of aldehydes and ketones [157]. They have significant physiological roles in the body [158] as mentioned in Chapter 1. However, foods including high amounts of bioactive amines might cause negative symptoms on human health [157].

GABA is found naturally in some plants. Besides, some microorganisms have ability to produce GABA because of their GAD activity [159]. Fermented foods might be a good source of GABA depending on the presence of GABA-producing strains. It was reported that lactic acid bacteria in several fermented foods including cheeses and wheat sourdough synthesized GABA [160-162]. Hudec et al [163] found that baker's yeast and wine yeast (*S. cerevisiae*) produced low amount of GABA. It was also reported that the level of GABA in bread fermented with baker's yeast increased during fermentation [164].

Biogenic amine formation in beer depends on raw materials, brewing techniques and microbial contamination [87]. Beer is fairly rare infected as pasteurization and sterile filtration prior to packaging are applied. However, occasional microbiological failures might occur even in the best-managed breweries before pasteurization step [36]. Biogenic amine in beer is generally synthesized by lactic acid bacteria, especially *Lactobacillus* and *Pediococcus*, that are primary beer-spoilage microorganisms [165]. Studies have recently shown that histamine and tyramine are the amines that are generally found in bottled beers [84, 86, 165-168]. However, GABA formation during beer fermentation has not been investigated in detail. It is also not known whether *S. cerevisiae* or beer-spoilage microorganisms are responsible for the formation of GABA during beer fermentation.

This study aimed to investigate the effect of *S. cerevisiae* on the production of GABA and other bioactive amines during unhopped wort fermentation together with changes in concentration of individual amino acids. Furthermore, correlation between bioactive amines and related amino acids in spoiled and unspoiled wort samples during fermentation was investigated.

2.2 Materials and Methods

2.2.1 Chemicals and Consumables

Acetonitrile (HPLC grade), methanol (HPLC grade), serotonin hydrochloride (≥98%), tryptamine (>98%), tyramine (99%), dopamine hydrochloride, histamine (≥97%) were obtained from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%) and high-purity (>98%) alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), tryptophan (Trp), tyrosine (Tyr), valine (Val), and GABA, Plate Count Agar (PCA) were supplied from Merck Co. (Darmstadt, Germany). Yeast Extract Peptone Dextrose (YPD) and Agar were obtained from Lab M (Lancashire, UK). Ampicillin-sulbactam (Ampisid) was purchased from MN Pharmaceuticals (Istanbul, Turkey).

Ultrapure water was used throughout the experiments (Milli Q-System, Millipore, Milford, MA, USA). Syringe filters (nylon, 0.45 µm) and Atlantis HILIC Silica column (2.1 x 150 mm i.d., 3 µm) were supplied by Waters Corp. (Milford, MA, USA).

2.2.2 Preparation of Wort

Malt was obtained from the Anadolu Efes Beverage Company (Ankara, Turkey). Its quality properties are given in Table 2.1 Malt was ground with a grinder to obtain fine particles. Ground malt was mixed with pure water (1:6 (w/v)) to prepare fresh wort. It was incubated at 45 °C for 30 min and 70 °C for 60 min afterwards. During incubation, wort was agitated every 10 minutes. After incubation, the wort was immediately cooled to 25 °C and filtered through layers of cloth [226].

Table 2.1. Quality characteristics of malt

Kolbach index (g/kg)	405
Moisture (g/kg)	47
Saccharification time (min)	12
Soluble nitrogen (g/kg)	8
Total protein (g/kg dry malt)	120

2.2.3 Fermentation

Fermentation was performed in two groups of worts as spoiled and unspoiled worts. Unspoiled wort was prepared by adding ampicillin-sulbactam antibiotic (20 mg/L) to the wort. Spoiled wort was prepared without adding antibiotic. Fermentation started just after the addition of 1% of instant yeast (*S. cerevisiae;* Dr. Oetker, Turkey) into the wort in glass bottle. Glass bottle was closed tightly with a tap having an airlock. Three separate glass bottles for each group were placed in an incubator at 28 °C for 8 days. Sampling was carried out every 24 hours of fermentation from each bottle.

2.2.4 Analysis of Bioactive Amines and Free Amino Acids

One milliliter of sample collected every 24 hours of fermentation was mixed with one mililiter of methanol and stored - 80 °C to prevent yeast activation. The samples were mixed by vortexing for 5 min just before analysis. After vortexing, the samples were centrifuged at 3000x*g* for 5 min and the supernatant was collected for further dilution. The samples were diluted 200 times with acetonitrile:water (60:40, v/v) mixture and filtered through a 0.45 µm syringe filter into an autosampler vial prior to analyses of bioactive amines and free amino acids.

The analysis of bioactive amines was performed according to the method carried out by our laboratory. Bioactive amines were determined by ultra high performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) (Waters Corp., Milford, MA, USA). Chromatographic separations were performed on Atlantis HILIC column (2.1 × 150 mm, 3 µm) by using a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (40:60, v/v) at a flow rate of 0.3 mL/min at 40 °C. The injection volume was 3 µL. The electrospray source operated in positive ionization mode had the following settings: capillary voltage of

0.80 kV; cone voltage of 20 V; extractor voltage of 4 V; source temperature of 120 °C; desolvation temperature of 370 °C; desolvation gas (nitrogen) flow of 800 L/h and cone gas flow of 100 L/h. Bioactive amines were detected by MRM (Table 2.2) Concentration of amines in samples was calculated according to the calibration curve prepared by using the standard solutions of each amine in the concentration range of 5 and 100 µg/L.

The analysis of free amino acids was performed according to the method reported previously [169].

Table 2.2. The MRM parameters used to detect individual bioactive amine compounds by UPLC-MS/MS

Compound	Parent ions $[M+H]^+$	Fragment ions $[M+H]^+$	Cone voltage (V)	Collision energy (V)
Serotonin	177	160	15	10
Tryptamine	161	144.1	15	12
Dopamine	154	137	20	10
		91	20	23
Histamine	112	95.1*	18	17
		68	18	20
GABA	104	87*	10	13
		69	10	13
		45	10	16
Tyramine	138	121.1	15	12
		77	15	20
		91	15	20
		103	15	20

*Quantitation of histamine and GABA was performed according to the area of their fragment ion chromatograms and all other bioactive amines were quantified by using the area of their total ion chromatograms.

2.2.5 Yeast Count and Total Microbial Count

Samples collected every 24 hours of the fermentation were inoculated by spread plate technique to both YPD Agar and PCA. Yeast count was performed by using YPD Agar after incubation for 24 h at 30 °C. PCA was utilized for determination of total microbial count after incubation for 48 h at 37 °C.

2.2.6 Analysis of pH

The pH of the samples was measured directly by using a pH meter (PHM210 MeterLab, France) at room temperature during fermentation.

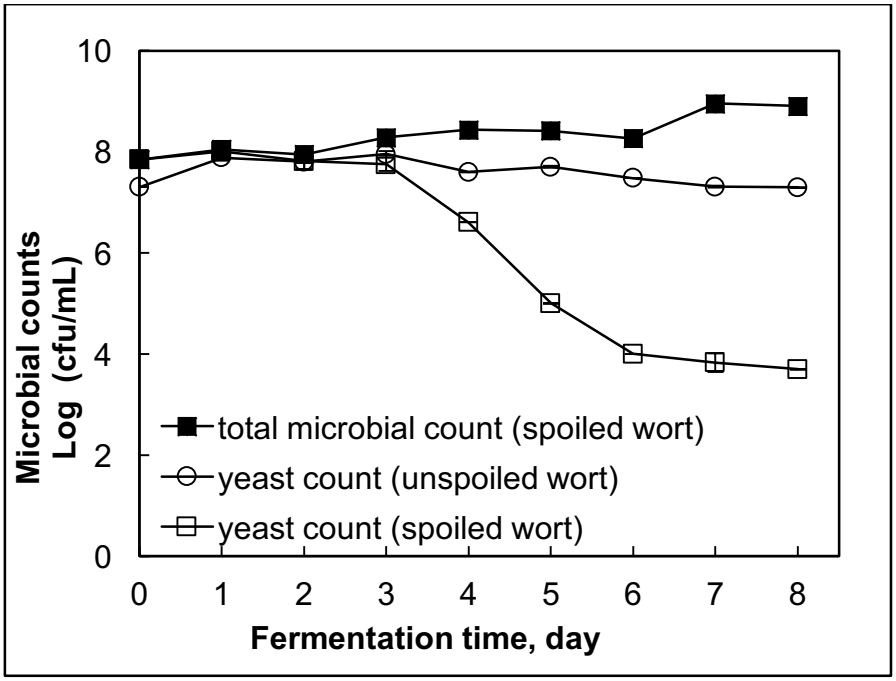
2.2.7 Statistical Analysis

The results were reported as mean ± standard deviations. Significant differences ($p < 0.05$) were evaluated by Tukey HSD test after the analysis of variance by using SPSS 17.0 (Chicago, IL, USA). The degree of linear dependence between two variables was examined by using the Pearson's correlation coefficient, *r*, giving a value between +1 and −1. The value +1 represents total positive correlation, while −1 represents total negative correlation, and zero means no correlation.

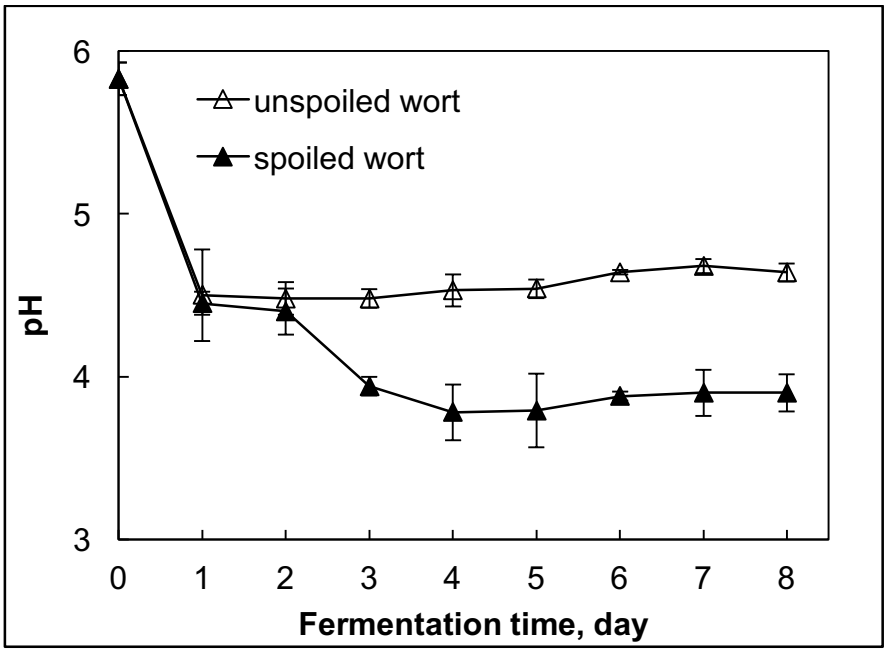
2.3 Results and Discussion

2.3.1 Changes of Yeast Count, Total Microbial Count and pH

In this study, spoiled and unspoiled wort samples were produced to reveal the effect of *S. cerevisiae* on bioactive amine formation during fermentation. Differently from spoiled wort, unspoiled wort was produced by adding antibiotic to the wort to prevent the growth of bacteria. Figure 2.1a indicates changes of yeast and total microbial count during wort fermentation. The yeast count in unspoiled wort was 7.30 log cfu (colony forming unit)/mL at the beginning of fermentation. It was found to contain 7.95 log cfu/mL of yeast within 3 days and the yeast count decreased to 7.30 log cfu/mL after 8 days of fermentation.



(a)



(b)

Figure 2.1. Changes of (a) yeast and total microbial counts and (b) pH in unspoiled and spoiled worts during fermentation (n=3).

Spoiled wort had 7.85 log cfu/mL of yeast. The yeast count decreased sharply from 7.75 to 3.70 log cfu/mL within the 3rd and 8th days of fermentation. On the other hand, total microbial load reached to 8.90 log cfu/mL after 8 days of fermentation. In other words, there was approximately 5.0 log cfu/mL of spoilage microorganisms in spoiled wort after 8 days of fermentation. Decreasing in the number of yeast in spoiled wort might be due to repression of yeast growth by spoilage microorganisms.

Figure 2.1b indicates pH changes of unspoiled and spoiled worts during fermentation. The initial pH value of wort was 5.8. After 24 hours of fermentation, the pH value decreased to 4.5 in both worts. Although the pH value did not change in unspoiled wort, it decreased from 4.4 to 3.9 in spoiled wort between 2nd and 8th days of fermentation.

2.3.2 Formation of Bioactive Amines

Figure 2.2a indicates concentrations of GABA in spoiled and unspoiled worts during fermentation. GABA concentration of unspoiled wort decreased from 111.85±9.33 to 87.32±9.52 mg/L after 24 hours of fermentation. That might be the result of utilization of GABA as a nitrogen source. It was revealed that *S. cerevisiae* could utilize GABA, which is transported across the plasma membrane by three uptake systems [170]. It was also reported that *S. cerevisiae* used a substantial proportion of GABA found in the growth medium [170]. Moreover, de Barber et al [171] found that yeast consumed GABA at the beginning of the bread dough fermentation due to the high demand of nitrogen. Between 1st and 8th days of fermentation, concentration of GABA in unspoiled wort increased up to 182.80±3.54 mg/L. Owing to no bacterial growth in unspoiled wort, it could be stated that *S. cerevisiae* has ability to produce GABA in beer.

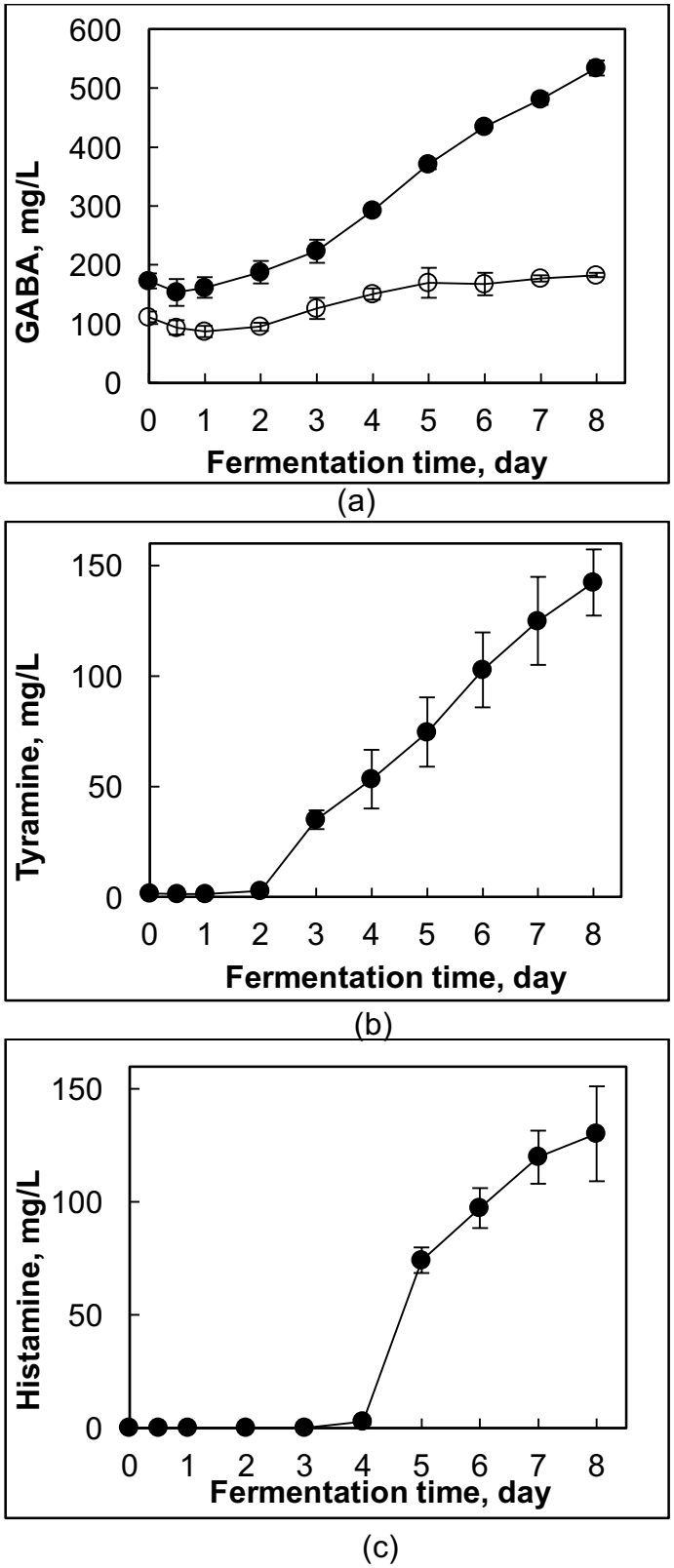


Figure 2.2. Concentrations of (a) GABA (b) tyramine and (c) histamine in unspoiled (open circles) and spoiled (closed circles) worts during fermentation (n=3)

There were no significant changes of tyramine and tryptamine concentration in unspoiled wort during fermentation ($p>0.05$) (Table 2.3). Besides, histamine, serotonin and dopamine were not formed as fermentation time increased. It was previously stated that *S. cerevisiae* var *uvarum* did not play a role to synthesize tyramine and histamine [90].

Table 2.3. Concentrations of tyramine and tryptamine in unspoiled wort during fermentation (n=3)*

Time (day)	Tyramine (mg/L)	Tryptamine (mg/L)
0	1.26±0.07	0.45±0.10
0.5	1.16±0.13	0.46±0.12
1	1.31±0.37	0.45±0.16
2	1.14±0.08	0.43±0.15
3	1.11±0.12	0.43±0.17
4	1.27±0.21	0.42±0.17
5	1.36±0.15	0.42±0.17
6	1.11±0.09	0.40±0.09
7	1.21±0.38	0.43±0.09
8	1.15±0.07	0.45±0.14

* There were no significant changes in the concentrations of tyramine and tryptamine ($p>0.05$).

As shown in Figure 2.2a, GABA was also produced in spoiled wort. The initial concentration of GABA in wort was found 172.92±12.34 mg/L. In the 24 hours of fermentation, concentration of GABA decreased to 161.48±17.59 mg/L. At the end of fermentation, GABA concentration raised continuously reaching to 534.10±12.78 mg/L. The rate of GABA formation in spoiled wort was higher than that in unspoiled wort. It could be stated that beer-spoilage microorganisms are also responsible for the formation of GABA in wort during fermentation. Additionally, high positive correlation was detected between GABA and spoilage microorganisms ($r=0.865$; $p<0.01$).

Tryptamine concentration (0.58 mg/L) did not change in spoiled wort during fermentation. Differently from unspoiled wort, concentrations of tyramine and histamine increased after a certain fermentation time. Tyramine concentration did not change until the end of 2 days of fermentation (Figure 2.2b). However, its concentration increased from 2.63±1.15 to 142.22±15.00 mg/L within the 3rd and

8^{th} days of fermentation. Although the wort did not contain histamine, 2.78±1.44 mg/L of histamine was observed in 4^{th} day of fermentation and its concentration increased to 130.16±21.07 mg/L at the end of fermentation. Tyramine and histamine formation significantly correlated with the spoilage microorganisms in wort ($r=0.821$; $p<0.01$ and $r=0.827$; $p<0.01$, respectively). It was previously reported that tyramine and histamine was detected in worts inoculated with pediococci or lactobacilli [165, 172].

2.3.3 Changes of Amino Acid Concentration

Concentrations of individual amino acids of spoiled and unspoiled worts during fermentation were given in Table 2.4 and Table 2.5, respectively. Total free amino acid content decreased from 2582 to 1652 mg/L and from 2842 to 2051 mg/L at the end of 4 days of fermentation in unspoiled and spoiled worts, respectively. The reduction of amino acid concentrations was the reason of amino acid consumption of yeasts. It is known that amino acids in wort are utilized by yeast for synthesis of cellular proteins [173]. Within the 4^{th} and 8^{th} days of fermentation, total free amino acid concentration increased from 1652 to 2614 mg/L and from 2051 to 3344 mg/L in unspoiled and spoiled worts, respectively. Increasing of amino acid concentration after 4 days might be of excretion of protease enzymes from living and damaged yeasts in unspoiled wort. Similarly, the reason might be autolysis of some cells and excretion of protease enzymes from yeasts and beer spoilage microorganisms in spoiled wort. It was revealed in many studies that protease enzymes release from both living and damaged yeasts to provide more available nitrogen sources [174, 175].

When spoiled beer was evaluated in terms of precursor amino acid content of bioactive amines, concentration of Tyr reduced from 93.2 to 6.6 mg/L in 4 days of fermentation ($p<0.05$) and did not change significantly until the end of fermentation ($p>0.05$). Concentration of His also decreased from 74.2 mg/L to 45.2 mg/L in 4 days of fermentation and continued to decrease to 3.0 mg/L ($p<0.05$). In unspoiled wort, concentration of Tyr decreased from 114.6 mg/L to 42.7 mg/L in 4 days of fermentation and increased up to 97.6 mg/L ($p<0.05$). However, His concentration did not change significantly throughout fermentation ($p>0.05$).

Table 2.4. Concentrations of individual amino acids (mg/L) in unspoiled wort during fermentation (n=3)*

	Fermentation time, days			
	0	**4**	**6**	**8**
Ala	139.7±34.7^{a}	200.2±19.5^{ab}	230.1±6.9^{b}	246.9±2.2^{b}
Arg	172.6±31.6^{a}	119.6±15.5^{a}	176.1±26.4^{a}	166.1±9.0^{a}
Asn	110.9±0.4^{a}	51.6±21.0^{b}	98.1±16.9^{ab}	127.1±0.6^{a}
Asp	136.7±22.0^{a}	55.9±10.8^{b}	107.5±17.7^{a}	120.3±10.5^{a}
Glu	196.5±4.9^{a}	82.9±13.5^{b}	139.1±29.6^{ab}	168.5±5.8^{a}
Gln	201.9±12.8^{a}	49.0±19.4^{b}	57.5±10.7^{b}	76.5±20.1^{b}
Gly	32.6±10.7^{a}	85.9±1.9^{b}	82.8±11.0^{b}	81.8±16.7^{b}
His	60.4±0.3^{a}	39.4±7.7^{a}	43.4±1.8^{a}	48.6±9.4^{a}
Ile	92.1±10.9^{a}	24.3±8.1^{b}	67.7±14.4^{ab}	89.2±9.3^{a}
Leu	138.2±5.5^{ab}	71.2±15.8^{a}	152.1±31.3^{b}	150.2±1.7^{b}
Lys	141.7±1.3^{a}	111.7±11.8^{a}	188.6±24.9^{ab}	252.0±30.5^{b}
Met	35.9±4.8^{ab}	28.3±4.7^{a}	41.2±5.3^{ab}	51.0±5.5^{b}
Phe	165.8±0.4^{a}	53.9±22.1^{b}	105.3±31.0^{ab}	130.0±21.3^{ab}
Pro	466.3±19.6^{a}	495.9±18.3^{a}	479.3±27.4^{a}	504.2±19.5^{a}
Ser	88.2±12.0^{a}	40.5±7.7^{b}	73.0±16.3^{ab}	80.9±3.2^{ab}
Thr	83.0±8.3^{a}	30.5±3.9^{b}	67.4±16.7^{ab}	77.8±1.0^{a}
Trp	63.7±1.7^{a}	13.1±6.2^{b}	27.2±10.3^{b}	28.8±3.0^{b}
Tyr	114.6±15.1^{a}	42.7±16.2^{c}	65.6±6.2^{bc}	97.6±2.8^{ab}
Val	141.9±17.8^{a}	55.8±8.1^{b}	103.6±30.5^{ab}	116.6±0.1^{ab}
Total	2582.7±203.2^{a}	1652.3±195.7^{b}	2305.5±212.1^{ab}	2614.2±85.8^{a}

* Different letters within the same row indicates statistical significance ($p < 0.05$).

Table 2.5. Concentrations of individual amino acids (mg/L) in spoiled wort during fermentation (n=3)*

	Fermentation time, days			
	0	**4**	**6**	**8**
Ala	137.4±36.1^{a}	199.4±38.9^{ab}	272.6±16.2^{b}	292.2±6.9^{b}
Arg	199.5±12.2^{a}	66.5±22.4^{b}	14.8±3.9^{bc}	10.6±0.9^{c}
Asn	150.0±1.6^{a}	45.3±6.7^{b}	69.2±1.1^{c}	93.5±7.8^{d}
Asp	197.7±5.7^{ab}	125.2±22.6^{a}	214.9±21.1^{b}	261.7±16.5^{b}
Glu	68.1±9.8^{a}	68.3±17.5^{a}	47.5±6.1^{a}	48.7±6.4^{a}
Gln	203.2±16.6^{a}	30.9±5.9^{b}	49.7±11.1^{b}	70.5±15.3^{b}
Gly	60.7±18.6^{a}	86.4±14.4^{ab}	148.0±32.7^{bc}	169.7±40.3^{c}
His	74.2±2.4^{a}	45.2±8.1^{b}	3.4±1.6^{c}	3.0±1.4^{c}
Ile	132.9±7.7^{a}	143.4±42.5^{a}	297.9±2.9^{b}	380.7±51.3^{b}
Leu	148.6±10.9^{a}	152.1±37.2^{a}	270.1±41.0^{ab}	383.3±43.5^{b}
Lys	251.1±15.3^{a}	17.8±3.2^{b}	21.1±5.3^{b}	14.4±0.8^{b}
Met	43.5±3.2^{ab}	34.5±2.1^{a}	50.1±12.2^{ab}	69.0±3.0^{b}
Phe	163.1±5.6^{a}	92.4±19.1^{b}	170.3±14.6^{a}	206.1±15.2^{a}
Pro	506.6±19.9^{a}	687.7±30.9^{b}	646.1±51.3^{b}	742.0±4.0^{b}
Ser	105.3±20.5^{a}	59.0±5.9^{ab}	27.6±15.7^{b}	53.7±0.2^{ab}
Thr	82.1±1.5^{ab}	54.7±15.3^{a}	65.2±9.4^{ab}	132.2±28.3^{b}
Trp	56.5±3.8^{ab}	28.0±3.0^{a}	44.4±9.1^{ab}	62.8±8.5^{b}
Tyr	93.2±4.5^{a}	6.6±0.9^{b}	13.0±1.4^{b}	12.1±1.4^{b}
Val	169.0±23.4^{a}	106.2±1.7^{a}	174.0±31.8^{a}	338.2±52.3^{b}
Total	2842.8±68.7^{a}	2050.7±105.0^{b}	2599.8±54.5^{a}	3344.4±60.9^{c}

* Different letters within the same row indicates statistical significance ($p < 0.05$).

When compared to the unspoiled wort, decreasing of Tyr and His concentration in spoiled wort might be due to forming tyramine and histamine. It has been previously reported that *Lactobacillus* and *Pediococcus*, which adapt to the medium during beer fermentation, are the main beer spoilage microorganisms. These lactic acid bacteria can catabolize amino acids for energy production [172]. Hence, approximately 5.0 log cfu/mL of spoilage microorganisms in spoiled wort produce tyramine and histamine and, might consume their precursor amino acids. High negative correlations were observed between tyrosine and tyramine ($r=-0.77$; $p<0.05$) and, histidine and histamine ($r=-0.91$; $p<0.05$) in spoiled wort.

In unspoiled wort, concentration of Glu decreased from 196.5 mg/L to 82.9 mg/L in 4 days of fermentation and increased up to 168 mg/L after 8 days of fermentation ($p<0.05$). However, the level of Glu did not change significantly in throughout spoiled wort fermentation ($p>0.05$). In unspoiled and spoiled wort samples during fermentation, significant correlation was not observed between GABA and Glu ($r=0.31$; $p>0.05$, $r=-0.59$; $p>0.05$, respectively). It was reported in some studies that correlation was not observed between levels of bioactive amines and amino acids [176, 177]. It was also revealed that low correlation between bioactive amines and amino acids might be due to the degradation rate of wine proteins into amino acids quicker than the conversion rate of amino acids into bioactive amines [176, 177]. However, isotope-labeling method should be utilized to find correlation between amino acids and bioactive amines during fermentation.

Wort boiling with hop is one of the stages of the beer production. During wort boiling, some principal changes occur. Amino acids and reducing sugars can be incorporated into melanoidins. Polyphenols from hop can react with some proteins in wort [178]. Moreover, small amounts of nitrogen can be lost as volatile compounds [36]. Therefore, the amino acid composition of wort prepared in this study could be slightly different from the boiled worts produced in typical brewing procedure. However, it was reported that the amino acid composition of sweet wort ,a liquid rich in materials dissolved from the malt, and boiled wort is very similar [36].

Hops are used in beer production for their preservative value and pleasant flavour [36]. It was reported that hops inhibit Gram-positive bacteria [179]. On the other hand, it was reported that some Gram-positive bacteria are less sensitive to hops

[36, 180]. Therefore, the spectrum of microorganisms that contaminate unhopped and hopped wort could be different. It should be taken into consideration that unhopped wort prepared in this study could cause the growth of different spoilage microorganisms.

3 FORMATION OF TYRAMINE IN YOGHURT DURING FERMENTATION – INTERACTION BETWEEN YOGHURT STARTER BACTERIA AND *LACTOBACILLUS PLANTARUM*

3.1 Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [181]. Due to health benefits of probiotic microorganisms, there is an increasing interest in developing novel foods containing probiotics [182]. Probiotics reduce symptoms of lactose intolerance, prevent inflammatory bowel disease and improve balance of intestinal microflora [183-185].

Lactobacillus and *Bifidobacterium* are mostly used microorganisms in food products [186]. Dairy products are one of the most effective carriers for probiotics due to the fact that milk includes growth components for probiotics [187, 188]. Probiotics are generally added to milk products including cheese, yoghurt and ice cream [189-191].

Yoghurt is defined as milk fermented with symbiotic starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which shall be in a viable state, active and still present in the product through the end of shelf life [192]. Yoghurt is a famous matrix including probiotics [193]. Several probiotic bacteria such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis and Bifidobacterium bifidum* were added into yoghurts [188, 194-196]. Apart from these bacteria, *Lactobacillus plantarum* was also used as a probiotic culture in yoghurts in recent studies [182, 189, 197].

The significant bioactive amines in milk products are tyramine, putrescine, ß-phenylethylamine and tryptamine [51]. Tyramine, which is formed by decarboxylation of tyrosine, has a role as a releasing agent of catecholamines including adrenaline, noradrenaline and dopamine [56]. However, high amount of exogenous tyramine causes negative impacts on human health such as migraine [48]. The formation of these amines, including tyramine, is mainly related with microorganisms participating in fermentation process [198]. Therefore, interactions between microorganisms could have a role on the production of tyramine in fermented dairy products.

The aim of this study is to reveal the interactions between *S. thermophilus*, *L. bulgaricus* and *L. plantarum* in the formation of tyramine during yoghurt fermentation. For this reason, these microorganisms were used in the yoghurt fermentation as single strains or mixed cultures containing double or triple strains. Since their presence as mixed cultures might also affect some physicochemical properties of the yoghurt, the interactions between microorganisms have been revealed by also determining total free amino acids and the pH of the medium together with the microbial count of the strains.

3.2 Materials and Methods

3.2.1 Chemicals and Consumables

Acetonitrile (HPLC grade), serotonin hydrochloride (≥98%), tryptamine (>98%), tyramine (99%), dopamine hydrochloride were obtained from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%), lactic acid (90%) and high-purity (>98%) alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), glutamic acid [127], glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), tryptophan (Trp), tyrosine (Tyr), valine (Val), and GABA were purchased from Merck Co. (Darmstadt, Germany). De Man, Rogosa & Sharpe (MRS) broth and bacteriological agar were obtained from Lab M (Lancashire, UK). M17 agar was obtained from Liofilchem (Roseto Degli Abruzzi, Italy). Vancomycin was purchased from Koçak Pharmaceutical (Turkey).

Deionized water (5.6 µS/m) was used throughout the experiments (Milli Q-System, Millipore, Milford, MA, USA). Syringe filters (nylon, 0.45 µm) and Atlantis HILIC Silica column (2.1 x 150 mm i.d., 3 µm) were obtained by Waters Corp. (Milford, MA, USA).

Ultra high temperature (UHT) milk (3.0% protein, 3.3% fat and 4.7% carbohydrate) was supplied from a local market in Turkey. *Streptococcus thermophilus* RSKK 04082 and *Lactobacillus plantarum* RSKK 02030 were obtained from Refik Saydam National Type Culture Collection (Ankara, Turkey), a member of World Federation for Culture Collections. *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 was obtained from DSMZ-German Collection of Microorganisms and Cell Culture (Braunschweig, Germany).

3.2.2 Preparation of Yoghurts

3.2.2.1 Culture Preparation

Freeze-dried pellets of *S. thermophilus, L. bulgaricus* and *L. plantarum* were propagated in MRS broth and stored in a glycerol solution (40%) at -80 °C. Before fermentation, frozen bacteria were re-propagated in MRS broth at 37 °C for 24 h.

3.2.2.2 Fermentation

Sterile containers were filled with UHT milk (100 mL) aseptically and milk was heated to 42 °C. 100 µL of broth containing related culture (9 log cfu/mL broth) was added into the milk to perform fermentation. The milk was rapidly inoculated with *S. thermophilus* RSKK 04082 (S)*, L. delbrueckii* subsp. *bulgaricus* DSM 20081 (B) *and L. plantarum* RSKK 02030 (P), and combinations of these bacteria as S and B (SB), S and P (SP), B and P (BP), S, B and P (SBP). The initial counts of S were 6.5, 6.2, 6.5 and 6.2 log cfu/mL in milks fermented with S, SB, SP and SBP, respectively. The initial counts of P were 6.2, 6.2, 6.5 and 6.2 log cfu/mL in milks fermented with P, BP, SP and SBP, respectively. The initial counts of B were 5.6, 5.4, 5.4 and 5.4 log cfu/mL in milks fermented with B, BP, BS and SBP, respectively. Fermentation was completed in 8 hours and sampling was performed every 2 hours of the fermentation. Fermentation was carried out in duplicates.

The pH of yoghurts was measured directly by using a pH meter (PHM210 MeterLab, France) at room temperature during fermentation. Enumeration of bacteria was carried out after fermentation periods. For analyses of amino acids and bioactive amines, yoghurt samples were lyophilized. Samples were kept at -80 °C and freeze-drying was performed for 48 h (Christ Alpha 1-2 LD+, Osterode, Germany).

3.2.3 Analysis of Bioactive Amines and Free Amino Acids

3.2.3.1 Extraction

Extraction of free amino acids was performed according to the method reported by Kocadağlı et al [169]. Lyophilized samples (500 mg) were extracted with water in three stages (5, 2.5, 2.5 mL). After vortexing (10 min) and centrifugation (7500xg for 5 min) in each stage, supernatants were collected in a test tube. Combined supernatants were precipitated with acetonitrile and filtered through a 0.45 µm syringe filter into an autosampler vial prior to the analyses of tyramine, other bioactive amines and free amino acids. All samples were extracted in duplicates.

3.2.3.2 UPLC-MS/MS Analysis of Tyramine and Other Bioactive Amines

Analysis of tyramine and other bioactive amines was carried out as described in Chapter 2. Linearity was evaluated by plotting peak area against the concentrations of bioactive amine standards. Limit of detection (LOD) and limit of quantification (LOQ) were determined at a signal to noise ratio of 3 and 10, respectively. Reproducibility of the method was determined by analyzing three replicates in three consecutive days.

3.2.3.3 UPLC-MS/MS Analysis of Free Amino Acids

Free amino acids were also performed using an UPLC-MS/MS method described elsewhere [169].

3.2.4 Enumeration of Viable Bacteria

Yoghurt sample (1 mL) was diluted in dilution liquid (9 mL) (0.85% NaCl) and diluted samples were vortexed (Marienfeld Superior, Germany). 100 µL of diluted sample was seeded on the surface of plates. Counts of S, B and P were detected using methods reported previously [197, 200, 201].

3.2.5 Statistical Analysis

The results were reported as mean ± standard deviation. Significant differences ($p < 0.05$) were evaluated by Duncan test via analysis of variance by using SPSS 17.0 (Chicago, IL, USA).

3.3 Results and Discussion

3.3.1 pH Changes

pH values of yoghurts might change depending on using single strain or mixed culture for yoghurt production. Figure 3.1 indicates the changes in pH of yoghurts fermented with single strain and mixed cultures. pH values of milk samples fermented with S, B and P decreased from 6.6 to 5.4, 6.1 and 5.6 at the end of 8 hours of fermentation, respectively. When yoghurts were fermented with SB, SP, BP and SBP, pH values decreased to 4.8, 5.3, 5.0 and 4.6, respectively. It was obvious that pH values of yoghurts fermented with double and triple strains were lower than those of fermented with single strains. Considering the changes in pH values of yoghurts, it was found that acid development in B containing yoghurts (SB, BP and SBP) was significantly higher ($p<0.05$) than the others after 8 hours of fermentation. Similar to the findings of this study, stimulation of acid production

in mixed cultures of *S. thermophilus* and *L. bulgaricus* compared to their single strain cultures was also reported by other researchers [23, 24].

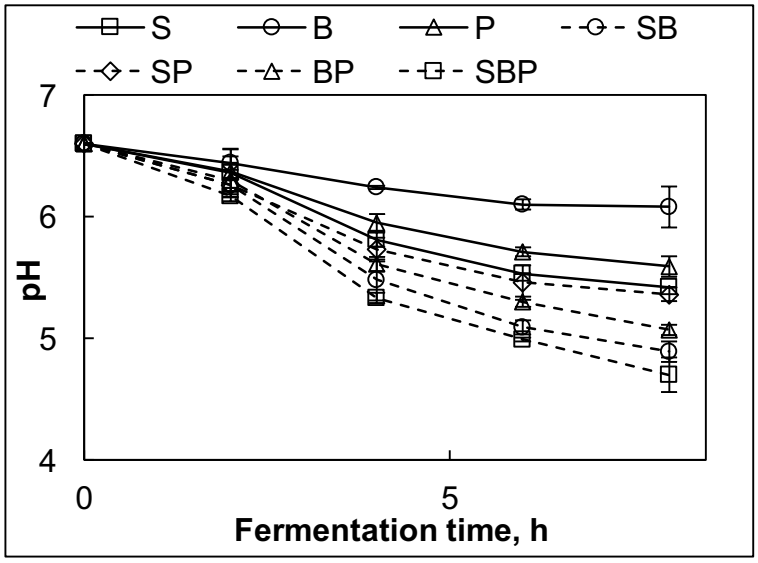


Figure 3.1. Changes in pH of yoghurts fermented with single strain cultures and mixed cultures during fermentation. S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*

3.3.2 Changes in Microbial Counts

Changes in viable counts of S, B and P of yoghurt samples fermented with single strain and mixed cultures are given in Table 3.1. After 8 hours of fermentation, count of S increased 1.6 log units. When yoghurt samples were fermented with SB and SBP, the increases in S count were 2.2 log and 2 log units, respectively. Nevertheless, 1.5 log units increase in S count was observed in yoghurt fermented with SP. Therefore, it could be said that B had positive impact on the growth of S. However, P did not have positive effect on the growth of S in SP fermented yoghurts. It was previously reported that association between *L. bulgaricus* and *S. thermophilus* caused higher level for *S. thermophilus* in milk [23, 24].

When milk was fermented with P, increase in P count was found to be 2 log units at the end of 8 hours of fermentation. Increases in P count were 2.2 log, 1.5 log, and 1.9 log units in yoghurts fermented with BP, SP and SBP, respectively. It was obvious that growth of P was affected positively by the incorporation of B.

In milk fermented with B, increase in B count was 1.1 log units at the end of 8 hours of fermentation. When yoghurts were produced with BP, BS and SBP,

increases in B count were 2.3 log, 1.8 log, and 2.4 log units, respectively. As a consequence, S and P promoted the growth of B in yoghurt samples. It was also reported by Herve-Jimenez et al [23] reported that count of *L. bulgaricus* was higher in milk fermented with mixed culture than single-strain culture.

Table 3.1. Changes in the count of bacteria (log cfu/mL) of yoghurt samples fermented with single strain cultures and mixed cultures during fermentation *

		Fermentation time (h)		
		0	4	8
S.thermophilus				
	S	6.5±0.1^{a}	7.5±0.0^{b}	8.1±0.0^{c}
	SB	6.2±0.3^{a}	8.0±0.0^{b}	8.4±0.1^{b}
	SP	6.5±0.2^{a}	7.6±0.0^{b}	7.9±0.0^{c}
	SBP	6.2±0.1^{a}	7.7±0.0^{b}	8.2±0.1^{c}
L. plantarum				
	P	6.2±0.2^{a}	7.8±0.0^{b}	8.2±0.1^{b}
	BP	6.2±0.2^{a}	8.0±0.1^{b}	8.4±0.0^{b}
	SP	6.5±0.2^{a}	7.6±0.0^{b}	8.0±0.1^{c}
	SBP	6.2±0.1^{a}	7.8±0.0^{b}	8.1±0.0^{c}
L. bulgaricus				
	B	5.6±0.1^{a}	5.8±0.2^{a}	6.7±0.2^{b}
	BP	5.4±0.2^{a}	6.8±0.1^{b}	7.7±0.1^{c}
	SB	5.4±0.4^{a}	6.2±0.2^{b}	7.2±0.1^{c}
	SBP	5.4±0.3^{a}	6.6±0.0^{b}	7.8±0.0^{c}

* S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*

Different letters within the same row indicate statistical significance ($p < 0.05$).

3.3.3 Formation of Bioactive Amines

There was a good linearity between the peak areas and concentrations (Table 3.2). LOD and LOQ values were demonstrated in Table 3.3. The method allowed quantitation of low ppb levels in yoghurt samples. Day-to-day reproducibility of the ESI-MS signal intensities of the ions was found high with a coefficient of variation of less than 5.0%

Table 3.2. Linearity equations and determination coefficients of bioactive amines

Bioactive	Linearity equation	Determination coefficient
GABA	y=325.6x-101.08	R^2=0.99829
Tyramine	y=1359.4x-47.75	R^2=0.99845
Tryptamine	y=1485.3x-422.03	R^2=0.99884
Dopamine	y=688.4x-1019.9	R^2=0.99323
Serotonin	y=339.4x-156.7	R^2=0.99918

The concentration of bioactive amine in the milk was determined before yoghurt was inoculated with selected cultures. GABA was the only bioactive amine with a concentration of 0.90 mg/kg d.w (dry weight) while tryptamine, tyramine, dopamine and serotonin were below the detection limit used. Concentrations of the GABA in yoghurts did not change significantly after 8 hours of fermentation ($p>0.05$). No other bioactive amines except tyramine were formed during 8 hours of fermentation.

The changes in tyramine concentration of yoghurts fermented with single strain and mixed cultures are given in Table 3.4. The cultures of S, B and P prepared in MRS broth and transferred to the milk resulted in the initial tyramine concentration of milk samples changing from 0.06±0.01 to 6.27±0.30 mg/kg d.w. After inoculation of strains to the milk, tyramine concentrations of milk samples fermented with single strain cultures increased significantly during fermentation ($p<0.05$) except for milk sample fermented with B ($p>0.05$). Concentrations of tyramine in milk samples fermented with S and P increased significantly from 2.60±0.02 to 6.75±0.35 mg/kg d.w and from 2.41±0.06 to 4.91±0.08 mg/kg d.w after 8 hours of fermentation, respectively ($p<0.05$). In a previous study, it was reported that only one strain produced tyramine within the eight strains of *L. bulgaricus* and within the twelve strains of *S. thermophilus* [202]. Moreover, it was found that some strains of *L. plantarum* in fermented foods have capability to produce tyramine [203].

Table 3.3. LOD and LOQ values (µg/kg) of bioactive amines in yoghurts fermented with single strain cultures and mixed cultures*

Yoghurts	GABA		Tyramine		Tryptamine		Dopamine		Serotonin	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOD
S	0.5	1.7	0.1	0.3	0.3	1.0	1.6	5.2	0.5	1.7
B	0.4	1.4	0.1	0.3	0.3	1.1	1.6	5.3	0.5	1.7
P	0.5	1.6	0.1	0.3	0.3	1.0	1.6	5.2	0.6	2.1
SB	0.5	1.7	0.2	0.7	0.4	1.4	1.6	5.4	0.5	1.8
SP	0.5	1.6	0.2	0.5	0.4	1.2	1.6	5.3	0.6	1.9
BP	0.5	1.6	0.1	0.3	0.3	1.1	1.6	5.2	0.7	2.2
SBP	0.4	1.4	0.1	0.3	0.3	1.0	1.6	5.2	0.5	1.6

* S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*

Table 3.4. Changes in the concentrations of tyramine (mg/kg d.w) of yoghurt samples fermented with single strain cultures and mixed cultures during fermentation*

Time (h)	S	B	P	SB	BP	SP	SBP
0	2.60±0.02^{a}	0.06±0.01^{a}	2.41±0.06^{a}	3.41±0.05^{a}	2.50±0.16^{a}	5.22±0.15^{a}	6.27±0.30^{a}
2	4.33±0.11^{b}	0.05±0.00^{b}	3.60±0.14^{b}	4.93±0.30^{ab}	2.92±0.02^{a}	6.84±0.20^{b}	7.89±0.38^{a}
4	4.13±0.24^{b}	0.05±0.00^{b}	3.94±0.03^{c}	9.34±1.17^{b}	6.53±0.07^{b}	6.54±0.18^{b}	15.31±0.27^{b}
6	5.27±0.29^{c}	0.05±0.00^{b}	4.62±0.22^{d}	21.76±3.64^{c}	11.01±0.77^{c}	9.29±0.35^{c}	23.70±2.11^{c}
8	6.75±0.35^{d}	0.06±0.01^{a}	4.91±0.08^{d}	20.46±2.62^{d}	8.75±0.17^{d}	8.54±0.10^{d}	29.23±2.19^{d}

* S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*

Different letters within the same column indicate statistical significance ($p < 0.05$).

When yoghurt samples were fermented with SB, BP, SP and SBP, concentrations of tyramine raised continuously reaching to 20.46±2.62, 8.75±0.17, 8.54±0.10 and 29.23±2.19 mg/kg d.w after 8 hours of fermentation, respectively ($p<0.05$) (Table 3). Considering the threshold toxic level of tyramine which was 600 mg and acceptable level of tyramine in cheese which was given as 100-800 mg/kg [56], concentrations of tyramine in yoghurt samples were not in the range of toxic levels.

Concentration of tyramine increased about 6 fold in yoghurt fermented with SB whereas it increased only 2.5 fold in milk fermented with S. On the other hand, tyramine concentration did not increase in milk fermented with B. It was obvious that there was a synergistic relationship between S and B in the conditions of yoghurt fermentation resulted in more tyramine production. A similar synergistic effect was also observed in yoghurt fermented with BP although it was not as strong as in case of SB. Tyramine concentration in yoghurt fermented with BP increased about 3.5 fold. As opposed to the SB and BP fermented yoghurts, tyramine concentration did not reach to the total tyramine concentration of their single strain yoghurts in SP fermented yoghurts. Therefore, relationship between S and P in terms of tyramine production could be explained as antagonism. The highest relative increase in tyramine concentration was observed in yoghurt fermented with SBP. In case of triple strain culture, SBP, the tyramine concentration increased compared to SP.

Considering the tyramine concentrations of mixed culture yoghurts, it was remarkable that whenever B involved in the mixed culture, the tyramine concentration increased much more than their additive effects during fermentation. Interestingly, B itself had no effect on tyramine concentration. The hidden role of B on the tyramine concentration of mixed cultures could be explained based on the two hypotheses that will be further explained and discussed in detail below. One of the hypotheses was when S or P was in the mixed culture with B, they reduce the pH of the medium to the optimum pH for B (Figure 3.1). Then, B might begin to produce tyramine. The other hypothesis was if B had proteolytic activity, it would provide sources for the production of tyramine for S and P. Under favor of these hypotheses, two modifications in fermentation medium were carried out to explain

the reason of the positive interaction between B and S and, B and P in mixed cultures.

3.3.4 Modifications in the Growth Medium

3.3.4.1 Decrease in pH

To reveal the first hypothesis, the pH of the milks were adjusted to 5.5 and 6, which were the optimum pH values for the activity of *L. bulgaricus*. It was detected that growth of B in milk samples fermented at pH 5.5 and pH 6.0 was higher than that of fermented at pH 6.6. However, concentration of tyramine did not change significantly during fermentation ($p>0.05$). These results proved that B could not synthesize tyramine even if pH of milk was optimum for B (Table 3.5).

3.3.4.2 Increase in the Concentrations of Free Amino Acids

The content of total free amino acids in yoghurts fermented with mixed cultures and single strain cultures are given in Table 3.6. In milk samples fermented with S and P, concentrations of total free amino acids decreased significantly from 949.9±29.2 to 673.7±48.6 mg/kg d.w and from 1005.4±22.8 to 918.6±12.5 mg/kg d.w at the end of 8 hours of fermentation, respectively. However, when milk was fermented with B, concentration of total free amino acid increased from 860.2±26.9 to 1280.2±11.2 mg/kg d.w. Due to having proteolytic activity [16, 204], *L. bulgaricus* increased total free amino acid content in yoghurt. It was previously demonstrated that the predominant proteolytic activity in yoghurt is due to PrtB, a cell-wall protease from *L. bulgaricus*. First step of proteolysis in yoghurt is carried out by cell-wall proteases, which hydrolyze the milk casein into peptides, and thereby *L. delbrueckii* subsp. *bulgaricus* has a significant role for enriching the medium with the nitrogen sources. The second step of proteolysis is the degradation of peptides into amino acids via endopeptidase and exopeptidase enzymes from S. *thermophilus* and *L. bulgaricus* [16]. As considered that only a few strains of *S. thermophilus* comprise cell-wall proteases [16], a reduction in the total amino acid concentration of milk fermented with S was observed. Approximately 9% reduction in total free amino acid content of milk fermented with P might be explained with low proteolytic activity of most of the *L. plantarum* strains [205].

When milk was fermented with SB, BP and SBP, approximately 28%, 13% and 10% increases in total free amino acid content were observed after fermentation,

respectively. Nevertheless, total free amino acid content of milk fermented with SP did not change significantly ($p>0.05$) (Table 3.6).

Table 3.5. Changes in pH, viable counts of related bacteria and tyramine concentrations of modified yoghurt samples during fermentation*

	Time (h)	pH	Count of bacteria (log cfu/mL)	Tyramine (mg/kg d.w)
S_{aa}	0	6.5±0.0^{a}	6.2±0.3^{a}	2.79±0.09^{a}
	4	5.6±0.1^{b}	8.7±0.0^{b}	11.42±040^{b}
	8	5.1±0.1^{c}	9.0±0.0^{b}	21.39±0.35^{c}
P_{aa}	0	6.5±0.0^{a}	6.1±0.2^{a}	3.13±0.10^{a}
	4	5.8±0.1^{b}	8.8±0.0^{b}	13.00±0.48^{b}
	8	5.2±0.1^{c}	8.7±0.2^{b}	23.05±0.04^{c}
B_{pH6}	0	5.9±0.1^{a}	5.0±0.0^{a}	0.03±0.02^{a}
	4	5.7±0.0^{a}	5.4±0.2^{b}	0.03±0.00^{a}
	8	5.5±0.1^{b}	6.4±0.0^{c}	0.06±0.01^{a}
$B_{pH5.5}$	0	5.5±0.1^{a}	5.1±0.3^{a}	0.03±0.02^{a}
	4	5.4±0.0^{ab}	5.7±0.0^{a}	0.04±0.00^{a}
	8	5.2±0.1^{b}	6.6±0.3^{b}	0.05±0.01^{a}

*S_{aa}: Yoghurt supplemented with amino acids and fermented with *S. thermophilus*,

P_{aa}: Yoghurt supplemented with amino acids and fermented with *L. plantarum,*

B_{pH6}: Yoghurt fermented with *L. delbrueckii* subsp. *bulgaricus* at pH 6,

$B_{pH5.5}$: Yoghurt fermented with *L. delbrueckii* subsp. *bulgaricus* at pH 5.5

Different letters within the same column in every panel indicate statistical significance ($p < 0.05$).

Table 3.6. Changes in the total free amino acid concentrations (mg/kg d.w) of yoghurt samples fermented with single strain cultures and mixed cultures during fermentation*

Time (h)	S	B	P	SB	BP	SP	SBP
0	949.9±29.2^{c}	860.2±26.9^{b}	1005.4±22.8^{d}	1050.3±15.9^{a}	959.2±15.4^{a}	1107.9±1.1^{bc}	1390.5±33.5^{b}
2	985.5±73.8^{c}	694.5±38.3^{a}	715.2±18.8^{a}	1104.5±49.7^{a}	904.4±29.4^{a}	1102.1±59.1^{bc}	1255.1±46.3^{a}
4	776.0±22.5^{ab}	826.9±38.6^{b}	813.0±20.7^{b}	1167.2±54.8^{a}	1153.4±46.5^{bc}	936.2±63.0^{a}	1215.3±3.7^{a}
6	821.5±10.0^{b}	1051.4±50.1^{c}	826.4±27.5^{b}	1360.1±31.3^{b}	1214.9±7.0^{c}	1082.0±31.1^{b}	1448.4±39.9^{bc}
8	673.7±48.6^{a}	1280.2±11.2^{d}	918.6±12.5^{c}	1344.7±66.4^{b}	1085.8±24.7^{b}	1222.6±53.7^{c}	1526.2±78.7^{c}

* S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*

Different letters within the same column indicate statistical significance ($p < 0.05$).

Concentrations of individual free amino acids of yoghurts fermented with single, double and triple strains are also given in Table 3.7. In milk fermented with S, concentration of all amino acids decreased except Ala, Asp, Cys, His and Pro. The concentrations of Ala, Asp, Cys and His did not change significantly ($p>0.05$) and concentration of Pro increased during fermentation ($p<0.05$). Due to the proteolytic activity, the content of most amino acids increased in yoghurt fermented with B. However, concentration of Asp decreased ($p<0.05$) and concentration of Glu and Cys did not change at the end of the 8 hours of fermentation ($p>0.05$). In yoghurt fermented with P, the concentrations of most amino acids decreased whereas the concentrations of Asp, Cys and Pro increased ($p<0.05$) and the concentration of Glu, Thr and Trp did not change during fermentation ($p>0.05$). It was previously reported that total free amino acid content of milk fermented with *S. thermophilus* decreased after fermentation [206]. However, total free amino acid content of milk fermented with *L. delbrueckii* subsp. *bulgaricus* increased from 8.67 mg/100g to 90.54 mg/100g after fermentation [206].

When milk was fermented with complex cultures, concentrations of amino acids decreased except Asp, Cys, Glu, His, Pro and Trp even though total free amino acid content increased after fermentation. Therefore, increase in total free amino acid content of yoghurts was due to accumulation of Asp, Cys, Glu, His, Pro and Trp. It was previously reported that Glu and Pro accumulated in large quantities in yoghurt samples, because they were not preferred as nitrogen source by S. *thermophilus* and *L. bulgaricus* [16].

Table 3.7. Changes in the individual free amino acid concentrations (mg/kg d.w) of yoghurt samples fermented with single strain cultures and mixed cultures during fermentation*

	Time, h	Ala	Arg	Asn	Asp	Cys	Gln	Glu
S	0	31.2±5.9^{a}	26.9±3.1^{a}	21.2±2.3^{a}	44.3±7.2^{a}	243.1±77.6^{a}	6.0±0.1^{a}	308.3±50.6^{a}
	2	45.3±1.7^{b}	6.7±0.1^{b}	29.2±1.6^{b}	93.8±16.7^{bc}	220.5±10.6^{a}	n.d	265.4±18.8^{ab}
	4	30.7±4.7^{a}	n.d	5.4±1.2^{c}	64.2±5.9^{ab}	221.7±11.4^{a}	0.6±0.2^{b}	270.7±4.6^{ab}
	6	45.2±6.2^{b}	n.d	2.9±1.0^{c}	99.0±21.2^{c}	239.2±70.9^{a}	1.6±0.1^{c}	220.5±0.5^{b}
	8	39.0±1.4^{ab}	n.d	1.8±0.4^{c}	57.5±3.5^{a}	210.0±35.4^{a}	0.9±0.1^{b}	207.5.5±3.5^{b}
B	0	50.2±4.2^{ab}	48.6±2.9^{a}	25.9±0.2^{a}	56.2±0.6^{a}	20.5±0.7^{a}	5.7±0.1^{a}	325.6±10.3^{b}
	2	43.7±4.2^{a}	34.0±2.6^{a}	25.4±2.0^{a}	40.0±5.0^{b}	28.5±4.2^{a}	7.0±0.6^{ab}	249.7±17.0^{a}
	4	48.3±9.8^{a}	43.8±14.1^{a}	37.5±8.6^{ab}	30.2±2.8^{a}	28.1±3.3^{a}	7.6±1.1^{b}	245±14.1^{a}
	6	63.4±4.6^{ab}	72.3±11.1^{b}	45.5±5.6^{bc}	38.2±5.5^{ab}	30.2±7.7^{a}	9.6±0.9^{c}	291.6±40.2^{ab}
	8	83.1±0.8^{c}	90.2±2.5^{b}	53.8±1.7^{c}	42.1±0.9^{b}	26.8±5.7^{a}	9.5±0.4^{c}	331.5±5.0^{c}
P	0	53.4±3.3^{a}	36.0±0.3^{a}	25.6±0.1^{a}	65.3±9.3^{ab}	148.4±0.9^{a}	4.3±0.3^{a}	322.9±1.1^{bc}
	2	31.8±0.4^{b}	5.8±0.8^{b}	15.8±0.2^{b}	56.2±8.5^{a}	157.4±3.4^{ab}	0.4±0.0^{b}	245.0±14.1^{a}
	4	32.8±1.2^{b}	n.d	10.5±0.5^{c}	80.5±0.8^{b}	161.9±5.1^{b}	0.4±0.2^{b}	321.8±2.6^{bc}
	6	28.8±1.1^{b}	n.d	2.6±0.6^{d}	104.0±6.2^{c}	186.8±4.9^{c}	0.2±0.0^{b}	316.7±14.6^{b}
	8	31.9±1.4^{b}	n.d	2.8±0.3^{d}	123.0±0.2^{c}	213.1±5.2^{d}	0.2±0.1^{b}	343.6±2.5^{c}
SB	0	54.1±1.8^{a}	37.1±3.3^{a}	36.7±1.2^{a}	59.4±1.3^{a}	194.8±6.3^{a}	3.9±0.1^{a}	295.1±5.4^{ab}
	2	54.0±4.3^{a}	6.2±0.5^{a}	35.4±4.5^{a}	86.1±10.9^{ab}	191.8±0.4^{a}	n.d	323.1±45.3^{ab}
	4	46.8±4.4^{a}	n.d	16.3±4.0^{b}	88.8±20.2^{ab}	276.6±6.0^{a}	0.1±0.0^{b}	364.8±53.7^{b}

	6	53.6±4.2^{a}	n.d	7.9±2.2^{b}	78.7±14.1^{ab}	700.6±73^{b}	n.d	240.6±50.4^{a}
	8	53.3±4.8^{a}	n.d	9.0±3.2^{b}	121.9±15.4^{c}	566.0±6.5^{c}	0.9±0.0^{b}	290.8±26.7^{ab}
BP	0	49.1±2.3^{a}	41.5±3.3^{a}	34.1±0.6^{a}	54.0±2.6^{a}	152.0±1.6^{a}	2.9±0.6^{a}	284.4±0.0^{a}
	2	48.1±7.5^{a}	12.7±1.7^{b}	38.3±1.4^{b}	59.0±1.6^{a}	131.9±10.5^{a}	n.d	245.5±10.1^{a}
	4	45.8±2.4^{a}	4.9±0.2^{c}	27.5±2.0^{c}	74.5±4.0^{b}	158.0±1.0^{a}	n.d	391.5±28.9^{b}
	6	59.1±2.1^{b}	n.d	19.0±1.6^{d}	83.1±2.6^{c}	310.9±16.9^{b}	n.d	373.9±23.2^{b}
	8	24.3±0.4^{c}	n.d	0.4±0.0^{e}	96.7±1.3^{d}	513.9±12.3^{c}	n.d	255.7±10.8^{a}
SP	0	51.5±2.0^{a}	29.5±1.9^{a}	34.6±0.3^{a}	54.4±1.8^{a}	317.8±1.6^{a}	1.8±0.3^{a}	286.4±3.4^{a}
	2	40.5±7.3^{b}	6.3±1.4^{b}	33.7±6.4^{a}	71.7±12.4^{a}	347.5±7.7^{a}	n.d	264.1±40.7^{a}
	4	63.5±1.9^{c}	4.8±0.1^{b}	16.6±1.9^{b}	65.9±3.8^{a}	165.5±0.1^{b}	n.d	294.7±32.2^{a}
	6	24.9±0.8^{d}	n.d	1.8±0.2^{c}	97.3±11.9^{b}	474.4±25.0^{c}	n.d	285.3±37.7^{a}
	8	27.1±1.7^{d}	n.d	1.8±0.8^{c}	112.9±0.4^{b}	509.4±16.2^{c}	n.d	341.2±8.1^{a}
SBP	0	66.5±0.7^{a}	39.4±3.8^{a}	52.7±2.1^{a}	74.8±1.8^{a}	336.7±19.2^{a}	2.9±0.8^{a}	301.3±34.8^{a}
	2	53.8±8.4^{ab}	7.2±0.7^{b}	39.7±8.2^{b}	81.5±10.7^{ab}	344.3±25.8^{a}	n.d	295.6±32.7^{a}
	4	46.8±1.8^{b}	n.d	11.3±0.0^{c}	85.5±2.0^{ab}	416.5±18.6^{b}	n.d	307.3±5.5^{a}
	6	52.9±3.7^{b}	n.d	9.7±0.3^{c}	103.0±6.7^{b}	654.6±29.2^{c}	n.d	278.9±21.8^{a}
	8	47.7±6.1^{b}	n.d	4.9±1.2^{c}	105.3±18.0^{b}	867.0±29.0^{d}	n.d	228.3±50.5^{a}
Milk		41.3±5.3	33.5±5.2	7.0±0.8	52.3±10.9	n.d	1.0±0.4	336.8±78.7
$Milk_{aa}$		108.0±13.9	73.0±7.9	67.3±4.5	133.9±18.6	4.1±0.4	4.5±0.8	428.4±20

	Time, h	Gly	His	Ile	Leu	Lys	Met	Phe
S	0	60.7±3.2^{a}	13.7±0.0^{a}	0.9±0.2^{a}	16.6±2.6^{a}	65.2±7.4^{ab}	7.6±0.8^{a}	8.1±1.4^{a}
	2	51.4±4.5^{ab}	15.5±0.8^{a}	1.2±0.0^{b}	22.4±1.6^{b}	75.7±6.1^{a}	9.2±1.3^{a}	19.1±4.0^{b}
	4	40.5±6.0^{bc}	14.1±1.3^{a}	0.1±0.0^{c}	4.9±0.6^{c}	33.9±5.6^{c}	0.7±0.3^{b}	1.6±0.0^{c}
	6	45.1±7.1^{bc}	15.2±2.8^{a}	0.1±0.0^{c}	3.1±0.6^{c}	44.7±18.0^{bc}	0.6±0.1^{b}	1.1±0.0^{c}
	8	33.5±2.1^{c}	13.6±0.3^{a}	n.d	1.9±0.2^{c}	27.5±3.5^{c}	0.1±0.0^{b}	0.4±0.1^{c}
B	0	64.2±2.2^{ab}	15.1±0.4^{a}	1.2±0.0^{a}	21.4±0.5^{a}	83.8±0.9^{ab}	4.5±0.1^{a}	18.2±2.4^{ab}
	2	53.0±1.8^{a}	11.9±0.6^{a}	1.2±0.0^{a}	20.4±1.2^{a}	60.3±0.7^{a}	4.2±0.8^{a}	15.1±2.3^{a}
	4	64.7±10.7^{ab}	13.8±2.0^{a}	1.8±0.4^{b}	29.7±5.7^{b}	72.6±24.2^{ab}	12.1±4.0^{b}	26.6±6.6^{bc}
	6	78.6±7.1^{bc}	18.7±1.1^{b}	2.1±0.2^{bc}	35.5±3.7^{bc}	96.4±16.1^{b}	17.8±4.9^{bc}	29.7±4.9^{c}
	8	89.0±2.2^{c}	19.0±1.4^{b}	2.5±0.0^{c}	41.9±0.5^{c}	141.9±0.2^{c}	24.8±0.3^{c}	42.8±1.3^{d}
P	0	66.3±6.5^{a}	26.7±0.2^{c}	1.2±0.0^{a}	20.8±0.6^{a}	80.0±0.8^{a}	9.4±0.2^{a}	15.7±0.9^{a}
	2	36.6±3.5^{b}	15.8±2.3^{b}	0.6±0.0^{b}	11.1±2.9^{b}	41.0±1.9^{b}	4.8±1.1^{b}	7.5±1.8^{b}
	4	42.2±1.7^{b}	11.3±0.9^{a}	0.3±0.0^{c}	8.8±1.5^{b}	41.2±3.2^{b}	0.7±0.1^{c}	6.6±0.8^{b}
	6	42.0±2.3^{b}	12.7±0.2^{ab}	0.1±0.0^{d}	3.8±0.4^{c}	32.3±3.7^{c}	0.7±0.0^{c}	7.2±0.3^{b}
	8	45.9±1.4^{b}	15.3±0.8^{b}	0.1±0.0^{d}	3.7±0.2^{c}	39.7±2.2^{b}	0.8±0.1^{c}	7.5±0.4^{b}
SB	0	58.0±0.9^{a}	13.0±0.4^{a}	1.6±0.1^{a}	29.0±0.6^{a}	92.5±4.4^{a}	13.8±0.4^{a}	26.8±1.9^{a}
	2	51.7±3.9^{a}	17.3±1.4^{ab}	1.4±0.1^{b}	28.3±3.7^{a}	90.5±6.4^{a}	12.3±1.8^{a}	27.1±5.7^{a}
	4	35.7±1.9^{b}	29.7±5.5^{c}	0.2±0.0^{c}	14.8±2.9^{b}	59.5±14.1^{b}	5.3±1.7^{b}	19.0±5.6^{ab}
	6	34.1±0.9^{b}	25.7±6.3^{bc}	0.2±0.0^{c}	8.5±2.1^{b}	49.9±6.3^{bc}	3.5±1.4^{b}	10.2±4.5^{bc}

	8	24.3±6.3^{b}	18.3±4.4^{abc}	0.2±0.0^{c}	9.3±2.0^{b}	31.3±0.9^{c}	2.1±0.9^{b}	5.1±3.7^{c}
BP	0	49.2±1.9^{a}	13.5±0.5^{a}	1.5±0.0^{a}	27.6±0.5^{a}	85.4±2.1^{a}	12.2±0.1^{a}	26.1±1.1^{a}
	2	49.5±8.1^{a}	14.5±0.2^{a}	1.6±0.0^{b}	30.4±1.3^{a}	80.9±1.9^{a}	14.0±1.7^{a}	31.0±3.7^{a}
	4	34.0±7.0^{ab}	34.3±4.0^{b}	0.5±0.0^{c}	24.0±2.0^{b}	84.6±5.3^{a}	13.1±1.3^{a}	26.5±1.7^{a}
	6	25.2±6.9^{b}	25.8±3.4^{c}	0.3±0.0^{d}	16.7±0.5^{c}	56.2±2.4^{b}	6.4±0.1^{b}	19.8±0.3^{b}
	8	32.0±5.9^{b}	18.6±0.8^{a}	0.1±0.0^{e}	2.4±0.2^{d}	30.8±0.3^{c}	0.6±0.1^{c}	1.3±0.5^{c}
SP	0	44.6±4.5^{ab}	13.2±0.0^{a}	1.5±0.0^{a}	28.1±0.1^{a}	82.1±8.1^{a}	12.4±0.3^{a}	24.9±0.2^{a}
	2	31.3±0.6^{a}	14.7±2.3^{a}	1.3±0.2^{a}	28.2±3.5^{a}	80.1±17.0^{a}	11.8±0.7^{a}	31.0±4.6^{b}
	4	56.7±6.6^{b}	20.3±2.9^{ab}	0.5±0.1^{b}	15.6±1.2^{b}	67.0±5.5^{a}	8.6±0.9^{b}	17.7±1.6^{c}
	6	35.6±4.8^{a}	15.3±0.5^{a}	0.1±0.0^{c}	3.3±0.1^{c}	24.5±1.8^{b}	0.5±0.0^{c}	1.6±0.3^{d}
	8	41.5±7.1^{a}	24.4±5.9^{b}	0.1±0.1^{c}	3.8±0.9^{c}	31.2±10.1^{b}	1.3±0.5^{c}	2.0±1.0^{d}
SBP	0	59.0±3.9^{a}	19.8±1.0^{a}	2.2±0.1^{a}	40.1±1.5^{a}	127.5±14.6^{a}	20.1±0.9^{a}	49.0±2.8^{a}
	2	51.0±4.8^{b}	18.7±4.3^{a}	1.6±0.3^{b}	32.9±6.8^{a}	94.5±28.4^{ab}	14.4±2.7^{b}	35.1±6.3^{b}
	4	38.7±1.7^{c}	28.0±0.4^{b}	0.2±0.0^{c}	11.5±0.2^{b}	71.7±1.3^{bc}	2.5±0.1^{c}	17.8±0.6^{c}
	6	30.8±1.7^{c}	28.4±1.4^{b}	0.2±0.0^{c}	10.2±0.6^{b}	62.1±0.5^{bc}	2.0±0.3^{c}	15.2±0.3^{cd}
	8	32.2±0.3^{c}	21.1±1.9^{a}	0.1±0.0^{c}	6.3±0.8^{b}	47.7±5.1^{c}	1.3±0.3^{c}	8.8±2.4^{d}
Milk		66.5±6.4	14.9±1.1	0.6±0.0	14.0±1.9	53.5±0.6	6.4±1.3	4.8±1.3
$Milk_{aa}$		126.6±15.6	61.7±5.7	3.6±0.5	54.7±8.9	116.7±1.1	46.4±2.9	36.7±11.3

	Time, h	Pro	Ser	Thr	Trp	Tyr	Val
S	0	27.9±5.1^{a}	17.7±4.0^{a}	13.6±2.3^{a}	7.1±0.1^{a}	5.8±0.5^{a}	22.2±4.3^{a}
	2	38.0±2.2^{a}	23.8±0.7^{b}	26.0±0.9^{c}	3.7±1.0^{b}	4.6±0.5^{b}	33.5±2.3^{b}
	4	57.9±10.1^{ab}	5.2±1.6^{c}	15.2±4.4^{a}	4.2±0.7^{b}	0.6±0.2^{c}	3.3±1.9^{c}
	6	83.3±21.4^{b}	1.9±0.5^{c}	10.5±2.0^{ab}	1.4±0.2^{c}	1.0±0.2^{c}	4.7±0.5^{c}
	8	75.0±7.1^{b}	1.2±0.3^{c}	6.0±1.4^{b}	n.d	0.4±0.1^{c}	2.7±0.5^{c}
B	0	37.4±1.4^{a}	21.5±2.0^{a}	16.2±0.8^{ab}	3.5±0.1^{ab}	8.5±0.0^{a}	31.5±2.7^{ab}
	2	27.9±1.8^{b}	21.0±0.6^{a}	13.3±1.6^{a}	2.7±0.2^{a}	7.3±1.4^{a}	27.2±1.5^{a}
	4	37.0±4.2^{a}	32.6±7.1^{a}	19.3±5.8^{ab}	5.5±1.2^{b}	15.5±4.2^{a}	54.7±13.3^{bc}
	6	49.4±4.2^{c}	45.8±2.0^{b}	23.1±1.4^{bc}	8.0±1.2^{c}	27.6±5.5^{b}	67.2±8.1^{cd}
	8	62.4±1.1^{d}	54.2±0.5^{b}	28.6±1.3^{c}	12.0±0.3^{d}	40.5±0.1^{c}	83.0±0.1^{d}
P	0	35.2±1.4^{a}	31.7±5.7^{a}	19.0±2.3^{a}	3.3±0.6^{ab}	9.2±1.5^{a}	30.9±0.8^{a}
	2	34.3±5.7^{a}	10.2±5.6^{b}	18.0±4.2^{a}	2.4±0.6^{a}	2.9±1.7^{b}	17.6±3.1^{b}
	4	42.3±3.2^{a}	4.9±1.5^{b}	20.4±4.9^{a}	3.1±0.1^{ab}	1.2±0.3^{b}	21.7±0.4^{b}
	6	59.1±2.9^{b}	6.7±3.1^{b}	15.8±1.8^{a}	3.9±0.4^{b}	1.1±0.2^{b}	1.5±0.4^{c}
	8	63.0±1.6^{b}	2.6±0.2^{b}	18.4±1.0^{a}	4.3±0.7^{b}	0.8±0.1^{b}	1.1±0.1^{c}
SB	0	35.1±0.8^{a}	22.0±0.0^{a}	20.8±0.8^{a}	6.3±0.7^{a}	6.2±0.1^{a}	43.3±0.4^{ab}
	2	51.8±4.2^{a}	30.4±1.1^{b}	29.8±3.1^{b}	8.1±2.0^{a}	4.0±0.9^{ab}	54.5±5.8^{b}
	4	102.5±12.5^{b}	5.3±1.2^{c}	22.1±4.7^{ab}	10.1±2.4^{a}	4.0±1.2^{ab}	64.9±15.8^{b}
	6	90.8±4.2^{b}	10.0±3.7^{c}	7.1±2.1^{c}	9.4±3.7^{a}	3.5±0.7^{b}	25.3±6.6^{a}
	8	129.8±9.1^{c}	8.7±1.6^{c}	12.1±3.2^{c}	10.5±3.2^{a}	2.6±0.9^{b}	47.8±7.9^{ab}

BP	0	29.8±0.0^{a}	22.9±3.7^{a}	20.7±2.4^{a}	5.5±0.5^{a}	5.2±0.1^{ab}	40.9±1.4^{a}
	2	34.3±0.7^{a}	31.9±5.7^{b}	22.5±0.8^{a}	7.2±0.4^{b}	4.6±0.6^{a}	45.6±1.7^{a}
	4	89.6±1.7^{b}	9.1±2.3^{c}	27.5±1.8^{b}	10.2±0.9^{c}	13.3±0.7^{c}	83.8±7.7^{b}
	6	95.7±11.2^{b}	5.9±0.3^{c}	19.7±0.9^{a}	11.4±0.4^{c}	6.9±1.6^{b}	78.1±4.6^{b}
	8	85.8±1.4^{b}	2.9±0.5^{c}	6.9±0.5^{c}	8.3±0.7^{b}	0.5±0.2^{d}	4.4±0.5^{c}
SP	0	29.7±0.1^{a}	19.5±0.8^{a}	21.1±0.2^{b}	6.1±0.0^{a}	6.8±0.3^{a}	41.0±0.7^{a}
	2	35.0±5.5^{a}	21.0±5.1^{a}	26.7±4.4^{c}	7.2±1.6^{ab}	4.6±0.1^{b}	44.6±8.7^{a}
	4	62.3±3.0^{b}	9.5±3.0^{b}	12.8±0.1^{a}	9.1±0.1^{b}	5.9±0.2^{ab}	38.5±4.5^{a}
	6	86.1±6.5^{c}	4.6±0.1^{bc}	11.2±1.2^{a}	7.1±0.5^{ab}	1.0±0.3^{c}	6.9±0.8^{b}
	8	92.6±2.8^{c}	0.41±0.1^{c}	13.6±1.3^{a}	8.7±0.5^{b}	1.3±0.4^{c}	8.7±2.4^{b}
SBP	0	35.8±0.4^{a}	47.2±5.2^{a}	33.1±0.0^{a}	11.5±0.2^{ab}	8.4±0.4^{a}	61.5±4.5^{a}
	2	54.8±9.8^{a}	26.1±0.5^{b}	29.8±6.6^{a}	10.2±3.8^{a}	3.4±0.8^{b}	58.8±9.4^{a}
	4	100.6±1.2^{b}	5.6±0.4^{c}	13.6±0.7^{b}	12.5±0.6^{ab}	6.6±0.0^{a}	38.0±0.6^{b}
	6	120.0±6.8^{b}	7.8±2.3^{c}	10.9±1.8^{b}	16.5±0.2^{b}	6.3±1.6^{a}	38.1±4.4^{b}
	8	100.9±11.5^{b}	6.6±0.3^{c}	6.3±1.0^{b}	13.0±1.7^{ab}	1.8±0.8^{b}	26.1±5.7^{b}
Milk		25.7±56.6	5.6±1.7	12.0±0.9	2.0±3.8	2.6±1.8	2.7±3.6
$Milk_{aa}$		75.0±6.7	62.9±9.9	78.4±4.7	43.0±9.6	29.4±4.2	46.1±9.1

* S: *S. thermophilus,* B: *L. delbrueckii* subsp. *bulgaricus,* P: *L. plantarum*, $Milk_{aa}$: Milk supplemented with amino acids
Different letters within the same column belonged to related yoghurt sample indicate statistical significance ($p < 0.05$).
n.d: not detected

To reveal the second hypothesis, milk was enriched with amino acids and fermented with either S or P. For this purpose, the total amino acid content of milk (683.2 mg/kg d.w) was increased about 2 fold to the value of 1600 mg/kg d.w prior to fermentation (Table 3.7). The count of S and P increased when the milk was enriched with amino acids. Concentrations of tyramine in milk samples supplemented with amino acids and fermented with S and P increased from 2.79±0.09 to 21.39±0.35 mg/kg d.w and from 3.13±0.10 to 23.0±0.04 mg/kg d.w at the end of 8 hours of fermentation, respectively (Table 3.5). The second hypothesis was proved as the S and P had capability of synthesizing tyramine depending on the amount of nitrogen source found in the food matrix. Although B did not produce tyramine, it had indirect effect on accumulation of tyramine in the yoghurts providing nitrogen sources for S and P. It was reported in several studies that increase in amino acid concentration during cheese ripening is critical for biogenic amine accumulation in cheeses [207, 208].

Biogenic amine formation is an important topic for dairy industry because of its negative health concerns. Therefore, some strategies (e.g., pasteurization of milk, irradiation) could be carried out to limit biogenic amine levels in dairy products [56]. One of the strategies to avoid bioactive amines in foods is the use of decarboxylase-negative starter cultures. Moreover, antagonistic interaction between microorganisms in terms of tyramine production could be another way to limit the production of tyramine in dairy products.

The presence of precursor amino acids is a restricting parameter for accumulation of bioactive amines in food products. The precursor amino acids could be released from milk proteins in a consequence of proteolytic activity of microorganisms in dairy foods [56]. Therefore, reducing proteolytic activity could be suggested to control biogenic amine accumulation in dairy foods.

It was previously reported that some strains of *L. bulgaricus, S. thermophilus* and *L. plantarum* have capability to produce tyramine [202, 203]. In this study, *Streptococcus thermophilus* RSKK 04082 and *Lactobacillus plantarum* RSKK 02030 produced tyramine in yoghurts. It was found that tyramine formation is related with strains used for fermentation.

This study enlightened the possible mechanisms of tyramine formation when a new strain like *L. plantarum* RSKK 02030 was incorporated into a dairy product, yoghurt. Since *L. delbrueckii* subsp. *bulgaricus* increased the concentration of amino acids, formation of tyramine production of *L. plantarum* RSKK 02030 was triggered in the yoghurts. Therefore, microbial interactions were found to play an important role in the synthesizing of tyramine. It is suggested that strains that do not synthesize tyramine under the conditions of fermentation could be potentially used to produce tyramine free dairy products.

4 DETERMINATION OF TRYPTOPHAN DERIVATIVES IN KYNURENINE PATHWAY IN FERMENTED FOODS USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

4.1 Introduction

Tryptophan is the precursor of many compounds including kynurenine. Approximately 95% of tryptophan catabolism proceeds via kynurenine pathway in humans [115]. As mentioned in Chapter 1, tryptophan derivatives in kynurenine pathway have many significant roles on human health [123, 125, 126]. In addition to humans, tryptophan derivatives in kynurenine pathway were found in yeasts [3, 4]. Moreover, NAD^+ synthesis via kynurenine pathway was also observed in *Streptomyces antibioticus*, *Cyanidium caldarium*, *Karlingia rosea*, and *Xanthomonas pruni* [5]**.** Therefore, it was hypothesized in this study that the fermented foods could include tryptophan derivatives in kynurenine pathway due to metabolism of yeasts and bacteria.

There is no detailed investigation of the tryptophan derivatives involved in kynurenine pathway in fermented foods. Therefore, it was aimed to develop an analytical method to quantitate tryptophan derivatives in kynurenine pathway in various fermented food matrices. The method is based on aqueous extraction of tryptophan derivatives from foods, chromatographic separation with reversed phase PFP column, and detection by tandem mass spectrometry.

4.2 Materials and Methods

4.2.1 Chemicals and Consumables

Methanol (HPLC grade), L-kynurenine (≥98%), kynurenic acid (≥98%), quinolinic acid (99%), nicotinic acid (niacin) (≥98%) and nicotinamide (≥98%) were obtained from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%) and L-tryptophan were purchased from Merck Co. (Darmstadt, Germany). Syringe filters (nylon, 0.45 µm) were supplied by Waters Corp. (Milford, MA, USA). Hypersil GOLD PFP column (100 x 2.1 mm i.d., 1.9 µm) and Acquity UPLC HSS C18 column (150 x 2.1 mm i.d., 1.8 µm) were obtained from Thermo Scientific (Waltham, MA, USA) and Waters Corp. (Milford, MA, USA), respectively. Deionized water (5.6 µS/m) was used throughout the experiments.

4.2.2 Sample Preparation

Cacao powder, yoghurt, white cheese, kefir, bread, red wine, and beer were purchased in Ankara. Except liquid samples (red wine and beer), samples were freeze-dried. After samples were kept at -80 °C, freeze-drying was performed for 48 h (Christ Alpha 1-2 LD+, Osterode, Germany). Beer was degassed in an ultrasonic bath prior to analysis. Liquid samples were diluted up to five folds with the mixture of methanol-water (60:40, v/v). One milliliter of diluted sample was filtered through a 0.45 µm syringe filter into an autosampler vial prior to analysis.

Lyophilized samples (500 mg) were extracted with water in three stages (5, 2.5, 2.5 mL). After vortexing (3 min) and centrifugation (3000×g for 5 min) in each stage, supernatants were collected in a test tube. Four hundred µL of the combined extract was precipitated with six hundred µL of the methanol, and stored at -80 ^{o}C for 30 min. Methanol was used to precipitate the proteins in the extract. After storage at -80 ^{o}C for 30 min, the extract was centrifuged at 3000 xg for 5 min and the sediment was discarded. The supernatant was filtered through a 0.45 µm syringe filter into an autosampler vial prior to analysis. All samples were extracted in duplicates.

To choose most effective solvent, freeze-dried samples were extracted with water, the mixture of water-methanol at the ratio of 60:40 (v/v) and 30:70 (v/v), and methanol, respectively. Lyophilized samples (500 mg) were extracted with the solvent in three stages (5, 2.5, 2.5 mL). After vortexing (3 min) and centrifugation (3000×g for 5 min) in each stage, supernatants were collected in a test tube. Combined extracts were directly stored at -80 ^{o}C for 30 min. After that, the extract was centrifuged at 3000xg for 5 min and the sediment was discarded. The supernatant was filtered through a 0.45 µm syringe filter into an autosampler vial prior to analysis. All samples were extracted in duplicates.

4.2.3 LC-MS/MS Analysis

Tryptophan derivatives were determined by UPLC-MS/MS (Waters Corp., Milford, MA, USA). Chromatographic separation was performed on Hypersil GOLD PFP column (100 x 2.1 mm i.d., 1.9 µm) by using a gradient mixture of 0.1 % formic acid in water (A) and 0.1 % formic acid in methanol (B) at a flow rate of 0.2 mL/min at 40 °C. The gradient mixture was started from 10% B and remained for 2 min. Then, it was increased to 90% B in 1 min and 90% B remained 2 min. It was

decreased to 10%B in 1 min and %10 B remained for 2 min. The chromatographic run was completed in 8 min. The injection volume was 1 µL. The electrospray source operated in positive ionization mode, and had the following settings: capillary voltage of 2.5 kV; cone voltage of 20 V; extractor voltage of 3 V; source temperature of 130 °C; desolvation temperature of 320 °C; desolvation gas (Nitrogen) flow of 600 L/h and cone gas flow of 50 L/h. Tryptophan derivatives were identified by multiple reaction monitoring (MRM), using the conditions given in Table 4.1. Optimum cone voltage of each main ions, namely niacin, tryptophan, nicotinamide, kynurenine and kynurenic acid, was adjusted by changing the cone voltage to obtain the maximum intensity of the main ion during infusion of its standard solution. Optimum fragmentation voltages of fragment ions were found by changing collision energies to obtain the maximum intensity for the relevant fragment ion.

4.2.4 Validation of the Method

Concentrations of tryptophan derivatives in the samples were calculated according to the matrix-matched calibration curves prepared by spiking samples with standard solutions (0-50-100-500 and 1000 µg/L). LOD, LOQ and reproducibility of the method were determined according to description in Chapter 3. Solid and liquid food samples spiked with different levels of tryptophan derivatives (100-1000 µg/L and 50-100 µg/L, respectively) were analyzed to determine percentage recovery. On the other hand, for percentage recovery of tryptophan, 100 and 1000 µg/L of tryptophan levels were used for both solid and liquid samples.

4.2.5 Statistical Analysis

The results were reported as mean ± standard deviation. Significant differences ($p < 0.05$) were evaluated by Duncan test via analysis of variance (ANOVA) by using SPSS 17.0.

Table 4.1. The MRM transitions used to detect tryptophan and its derivatives in kynurenine pathway by UPLC-MS/MS

	Molecular ion $[M+H]^+$	Fragment ion $[M+H]^+$	Cone voltage (V)	Collision energy (V)
Tryptophan	205	159	20	12
		188*	20	12
Kynurenine	209	94	20	12
		146	20	15
		192*	20	10
Kynurenic acid	189.9	144*	25	20
		162	25	16
		172	25	12
Nicotinamide	123.1	80*	36	17
Niacin	124	80*	38	20

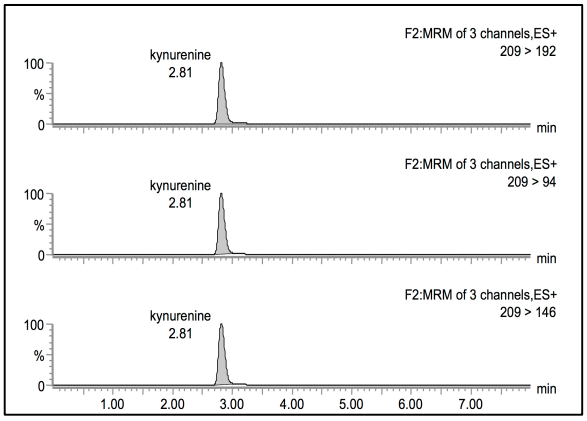
*Quantitation was performed according to the marked fragment ion.

4.3 Results and Discussion

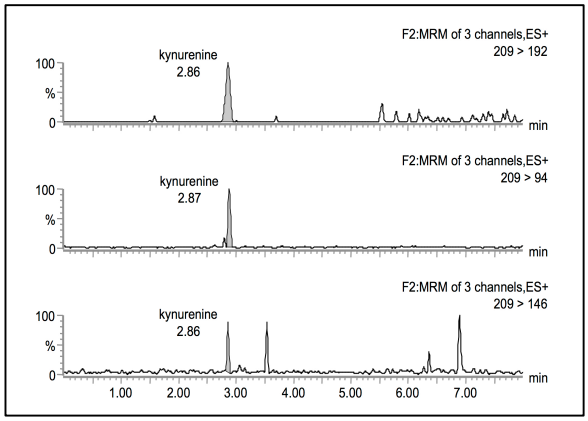
4.3.1 Evaluation of the Analytical Method

Two different columns (C18 and Phenyl columns) were tried to resolve tryptophan, kynurenine, kynurenic acid, quinolinic acid, nicotinamide and niacin. C18 column (Acquity HSS C18 column) was not found suitable in terms of separation and peak shapes (not shown). On the other hand, phenyl column (Hypersil GOLD PFP column) resulted in good peak shapes for all compounds analyzed, except quinolinic acid. Quinolinic acid was not well retained in both columns. These results were similar with the results reported by Fuertig et al [118].

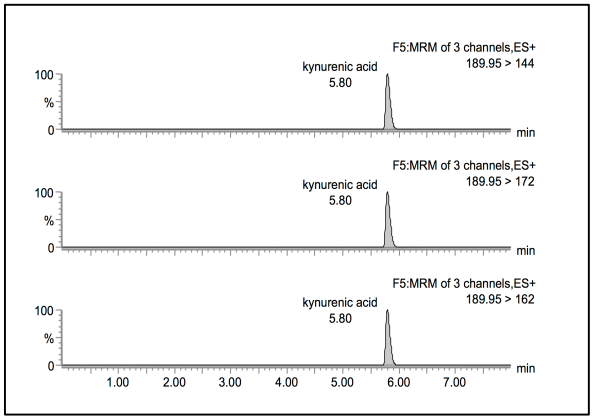
Tryptophan and its derivatives in kynurenine pathway were detected with their specific MRM transitions. Figure 4.1 illustrated the chromatographic separation and ion transitions of standard compounds (left panel). The compounds resolved approximately between 1.5 and 6 min in the chromatographic run. The chromatographic separation of tryptophan derivatives in bread or cacao samples was also illustrated in Figure 4.1 (right panel).


F2:MRM of 3 channels,ES+
209 > 192
kynurenine
2.81
F2:MRM of 3 channels,ES+
209 > 94
kynurenine
2.81
F2:MRM of 3 channels,ES+
209 > 146
kynurenine
2.81
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

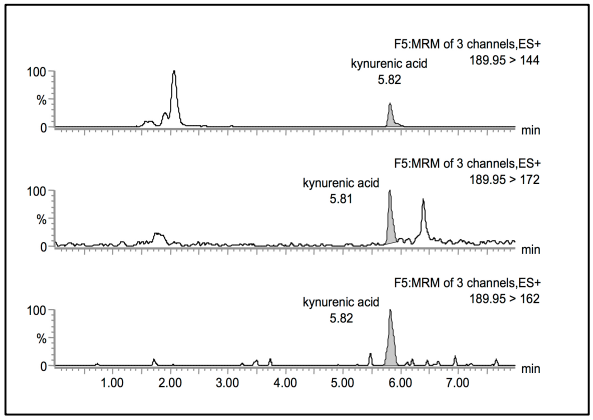
(a)


F2:MRM of 3 channels,ES+
209 > 192
kynurenine
2.86
F2:MRM of 3 channels,ES+
209 > 94
kynurenine
2.87
F2:MRM of 3 channels,ES+
209 > 146
kynurenine
2.86
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

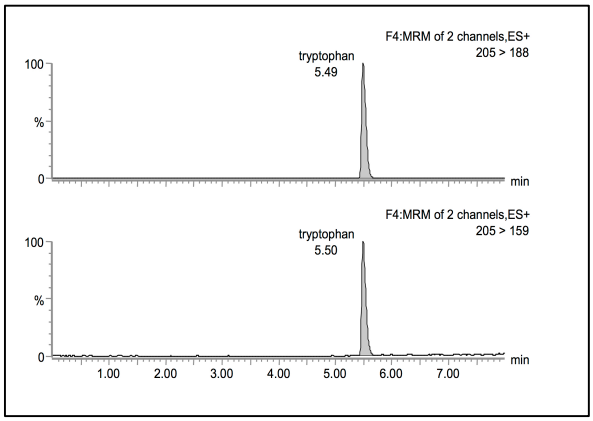
(b)


F5:MRM of 3 channels,ES+
189.95 > 144
kynurenic acid
5.80
F5:MRM of 3 channels,ES+
189.95 > 172
kynurenic acid
5.80
F5:MRM of 3 channels,ES+
189.95 > 162
kynurenic acid
5.80
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

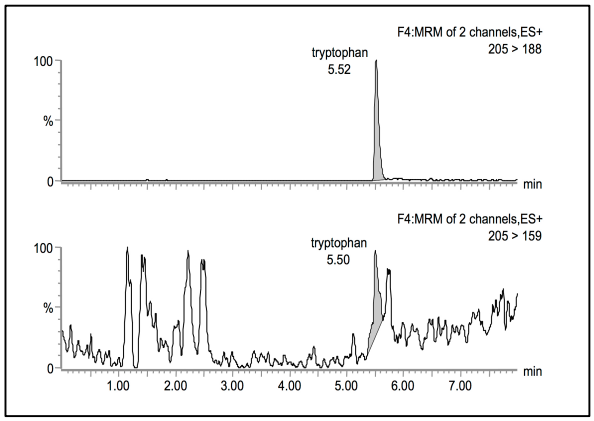
(c)


F5:MRM of 3 channels,ES+
189.95 > 144
kynurenic acid
5.82
F5:MRM of 3 channels,ES+
189.95 > 172
kynurenic acid
5.81
F5:MRM of 3 channels,ES+
189.95 > 162
kynurenic acid
5.82
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

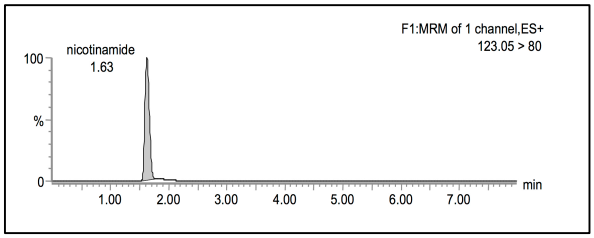
(d)


F4:MRM of 2 channels,ES+
205 > 188
tryptophan
5.49
F4:MRM of 2 channels,ES+
205 > 159
tryptophan
5.50
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

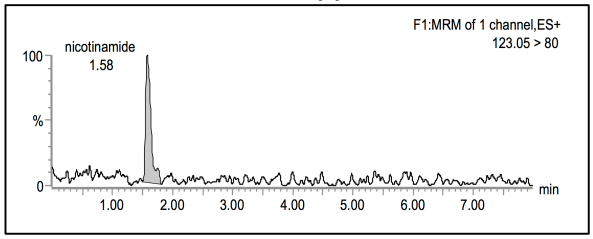
(e)


F4:MRM of 2 channels,ES+
205 > 188
tryptophan
5.52
F4:MRM of 2 channels,ES+
205 > 159
tryptophan
5.50
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

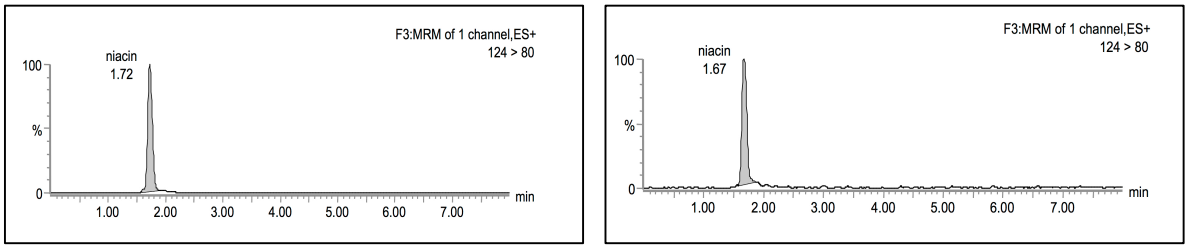
(f)


F1:MRM of 1 channel,ES+
123.05 > 80
nicotinamide
1.63
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

(g)


F1:MRM of 1 channel,ES+
123.05 > 80
nicotinamide
1.58
100
%
0
min
1.00
2.00
3.00
4.00
5.00
6.00
7.00

(h)



(i) **(j)**

Figure 4.1. UPLC-MS/MS chromatograms of (a) kynurenine standard solution (10 mg/L) (b) kynurenine in bread (c) kynurenic acid standard solution (10 mg/L) (d) kynurenic acid in cocoa powder (e) tryptophan standard solution (10 mg/L) (f) tryptophan in cocoa powder (g) nicotinamide standard solution (10 mg/L) (h) nicotinamide in cocoa powder (i) niacin standard solution (10 mg/L) (j) niacin in cocoa powder.

There was a good linearity between the peak areas and concentrations up to 10 mg/L for kynurenine, kynurenic acid and tryptophan. On the other hand, a good linearity was also observed up to the concentration of 1 mg/L for niacin and nicotinamide. Since all of the fermented foods analyzed have complex matrix and matrix effects can strongly effect LC-MS/MS analysis, matrix-matched calibration was used to quantitate the tryptophan and its derivatives in all fermented foods. Linearity equations and determination coefficients for every fermented food were given in Table 4.2.

Table 4.2. Linearity equations and determination coefficients of tryptophan and its derivatives in different food matrices

Food Samples	Kynurenine	Kynurenic acid	Niacin	Nicotinamide	Tryptophan
Bread	y=39.347x+149.35	y=125.1x-495.62	y=28.736x+2892.3	y=28.254x+3481.4	y=61.443x+69695
	R^2=0.99995	R^2=0.99986	R^2=0.99771	R^2=0.99964	R^2=0.99568
Beer	y=27.109x+1389.4	y=74.845x+604.55	y=20.077x+8917.6	y=22.844x+378.07	y=60.876X+67050
	R^2=0.99901	R^2=0.99984	R^2=0.99991	R^2=0.99865	R^2=0.9987
Red Wine	y=38.637x+32.29	y=102.15x+2300.5	y=29.951x+2531.2	y=32.23x-53.959	y=66.555+20672
	R^2=0.99994	R^2=0.99894	R^2=0.99824	R^2=0.99938	R^2=0.99939
White cheese	y=22.332x+172.6	y=94.418x-814.69	y=22.633x+368.51	y=20.28x+42.694	y=70.771x+40316
	R^2=0.99872	R^2=0.99837	R^2=0.99937	R^2=0.99991	R^2=0.97894
Yoghurt	y=33.047x+838.48	y=124.04x-1039.7	y=26.357x+1208.9	y=29.569x+271.51	y=68.426x+18003
	R^2=0.99417	R^2=0.99926	R^2=0.9977	R^2=0.99849	R^2=0.99652
Kefir	y=31.581x+357	y=102.29x-649.55	y=24.971x+507.86	y=26.847x+472.79	y=61.365x+13020
	R^2=0.99948	R^2=0.9994	R^2=0.99841	R^2=0.99869	R^2=0.99753
Cocoa	y=38.184-18.857	y=108.2x+6171	y=26.656x+6369.4	y=30.393+1109.6	y=68.893x+35735
	R^2=1.00	R^2=0.99995	R^2=0.99845	R^2=0.99962	R^2=0.98967

The LOD and LOQ values for the detection of tryptophan derivatives in various fermented foods in this study are demonstrated in Table 4.3. The highest LOD and LOQ values were observed for cacao powder in comparison to other fermented food samples analyzed. The advantage of using tandem MS was to confirm structurally the presence of target molecules by their fragmentation ions in the complex fermented food matrices. However, liquid chromatography coupled with fluorescent detection does not provide structurally confirmative results. According to the studies performed by liquid chromatography coupled with fluorescent detection, different LOD values for tryptophan in food and biological samples were reported depending upon the matrix. The LOD values for tryptophan were 0.01 mg/kg [209], 5-9 µg/kg [152], 0.7 mmol/L [210] in the analysis of flour, honey and serum samples, respectively. There is no study reporting the LOD and LOQ values for kynurenine and kynurenic acid in foods. LOD values for the analysis of kynurenine in biological samples were reported as 0.0245 µmol/L [211] and 2 nmol/L [210]. Also, the LOD values for the analysis of kynurenic acid in rat plasma and human serum were reported as 0.16 nM and 0.08 nM, respectively [212, 213]. Although niacin was analyzed with liquid chromatography coupled to fluorescent detection in a study reported by Tsuruta and Kohashi [214], the analyses of niacin and nicotinamide were generally performed by liquid chromatography mass spectrometry [215-217]. The LOD value for the analysis of nicotinic acid in coffee was reported as 18.5 ng/mL [217]. The LOQ value for the analysis of nicotinic acid and nicotinamide in mouse erythrocytes was 1 pmol [216].

Table 4.3. LOD and LOQ values of tryptophan and its derivatives in different food matrices (µg/kg or µg/L)

	Tryptophan		Kynurenine		Kynurenic acid		Nicotinamide		Niacin	
	LOD	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**
Bread	8.2	27.3	0.7	2.2	0.6	1.9	7.3	24.2	11.8	39.2
Red wine*	1.4	4.7	0.5	1.7	1.7	5.8	1.2	4.0	3.7	12.2
Beer*	12.5	41.8	0.8	2.8	0.1	0.3	0.1	0.3	0.9	3.0
Cacao powder	37.0	123.5	2.0	6.7	4.6	15.2	5.8	19.4	31.4	94.5
Yoghurt	16.5	54.8	1.0	3.3	4.8	15.9	1.0	3.4	6.3	21.0
White cheese	15.2	50.6	0.8	2.8	0.5	1.6	0.9	3.1	0.9	2.9
Kefir	9.3	31.0	1.2	4.2	1.6	5.2	0.6	2.1	3.5	11.6

*LOD and LOQ levels of marked food samples were indicated as µg/L.

Percentage standard deviation of the retention time of kynurenine, kynurenic acid, niacin, nicotinamide and tryptophan was found to be 3.5%, 1.0%, 1.0%, 3.0% and 2.4%, respectively. The mass spectrometric detection reproducibility was also found high with a percentage deviation of 3.6%, 3.8%, 3.2%, 4.9% and 3.0% for the peak area of kynurenine, kynurenic acid, niacin, nicotinamide and tryptophan, respectively. Therefore, day-to-day reproducibility of the ESI-MS signal intensities of the ions was found high with a coefficient of variation of less than 5.0%. Percentage recoveries of tryptophan and its derivatives in beer, red wine, bread, yoghurt, white cheese, kefir and cocoa samples were given in Table 4.4. The recoveries of tryptophan were found to be mostly higher than 88.4%. The high percentage recoveries of kynurenine were found in beer, red wine, bread and yoghurt. However, white cheese had the lowest percentage recovery for kynurenine within the range of 59.3% and 67.5%. The percentage recoveries of kynurenic acid and niacin were found to be within the range of 72.9% and 114.5% and, 69.7% and 99.2%, respectively. The percentage recovery values of nicotinamide ranged from 46.4% to 82.1%.

To determine the extraction efficiency of tryptophan derivatives from freeze-dried yoghurt and bread samples; water, methanol and the mixtures of water-methanol (60:40, v/v and 30:70, v/v) were used. Figure 4.2a illustrated the concentrations of tryptophan extracted with different solvents in bread and yoghurt. Extraction with only methanol was found not to be as effective as either water or water-methanol mixtures ($p<0.05$). However, it should be mentioned that there were no significant differences in the content of tryptophan when water alone and the mixture of water-methanol were used for extraction ($p>0.05$). Figure 4.2b illustrated the concentrations of kynurenine, niacin and nicotinamide extracted with different solvents in bread. As could be clearly seen from Figure 4.2b, when methanol alone was used as the extraction solvent, the concentrations of kynurenine, niacin and nicotinamide were significantly less than their concentrations in case of water alone was used. It was also notable that the concentration of niacin was the highest when extracted with water. Its concentration became significantly less when the ratio of methanol was increased in the extraction solvent ($p<0.05$). Figure 4.2c illustrated the level of kynurenine, niacin and kynurenic acid extracted with different solvents in yoghurt. Level of kynurenine, niacin and kynurenic acid in

yoghurt sample did not change significantly when different solvent mixtures were used for extraction ($p>0.05$). In the view of these results, water was chosen as the most effective solvent in the extraction of kynurenine, kynurenic acid, tryptophan, niacin and nicotinamide in both bread and yoghurt.

4.3.2 Determination of Tryptophan and Its Derivatives in Foods

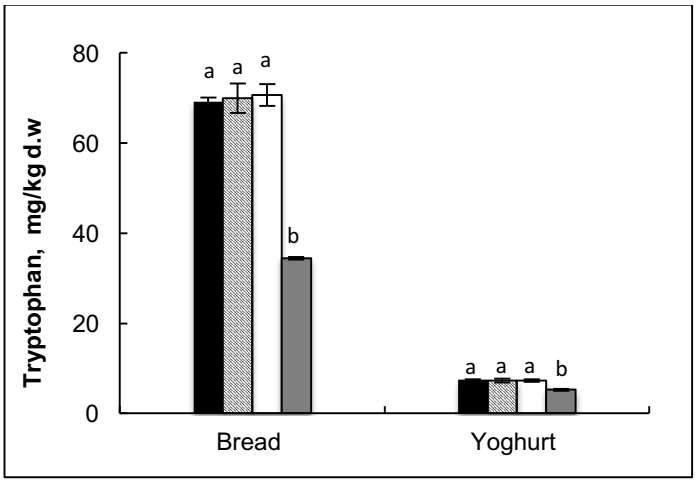
The UPLC-MS/MS method was used to detect tryptophan derivatives in kynurenine pathway in various fermented foods including bread (n=3), beer (n=6), red wine (n=4), white cheese (n=6), yoghurt (n=5), kefir (n=3) and cocoa powder (n=4). It was previously reported that kynurenine pathway was found in yeasts [3, 4, 145]. In addition, NAD^+ synthesis via kynurenine pathway was observed in some prokaryotes [5]. Therefore, fermented foods were considered as potential sources of tryptophan derivatives in kynurenine pathway due to use of yeasts and bacteria in their production.

The concentrations of tryptophan and its derivatives in various food samples are given in Table 4.5. The concentrations of tryptophan and its derivatives were quantified without restoring according to recovery percentages of compounds. When yeast-fermented foods were analyzed to determine their kynurenine content, it was found that one of the bread samples contained 143.5±28.2 µg/kg d.w of kynurenine. All of the beer samples analyzed were found to contain kynurenine within the range of 28.7±0.7 µg/L and 86.3±0.5 µg/L. However, red wine samples did not contain kynurenine. Kynurenine was also not detected in cacao samples analyzed. Among dairy products, white cheese, yoghurt and kefir contained kynurenine within the range of 30.3±5.4 and 321.6±66.9 µg/kg d.w, 274.3±80.3 and 751.9±34.2 µg/kg d.w, 372.8±16.3 and 763.8±106.6 µg/kg d.w, respectively. It was reported by Bertazzo et al [153] that kynurenine was detected in yoghurt and probiotic drinks within the range of approximately 60-80 µg/L in fresh weight.

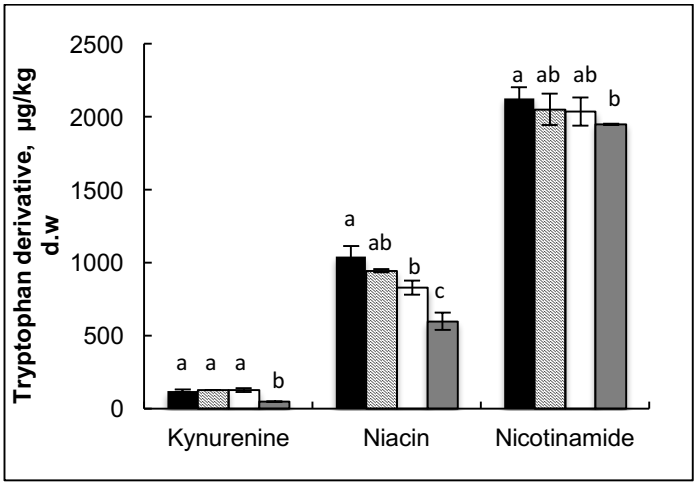
Table 4.4. Percentage recoveries of tryptophan and its derivatives with standard deviations (SD) in different food matrices

Food samples	Spiking level (µg/kg or µg/L)	Recovery ± SD (%)				
		Kynurenine	**Kynurenic acid**	**Niacin**	**Nicotinamide**	**Tryptophan**
Beer	50	107.4±9.1	93.5±5.4	97.2±0.5	77.8±1.8	-*
	100	100.9±6.8	89.9±2.6	72.0±3.0	68.4±2.5	88.4±0.2
	1000	-*	-*	-*	-*	91.6±1.4
Red wine	50	123.0±6.0	109.0±0.9	89.5±7.4	74.2±6.0	-*
	100	124.1±10.6	108.2±2.1	92.6±4.0	72.2±2.6	107.1±1.9
	1000	-*	-*	-*	-*	109.2±5.7
Bread	100	109.9±4.4	86.2±3.7	99.2±5.4	82.1±1.8	109.6±1.1
	1000	120.3±1.7	93.6±0.8	91.7±0.2	62.2±0.5	109.8±3.7
Yoghurt	100	107.4±6.5	87.3±2.1	96.7±4.1	54.7±2.3	104.6±2.2
	1000	119.4±3.6	94.2±5.0	80.1±2.4	63.4±3.1	100.2±5.9
White cheese	100	59.3±3.4	72.9±6.0	77.9±1.6	60.3±3.3	109.0±1.4
	1000	67.5±0.6	77.5±1.3	91.0±0.5	65.0±4.2	98.1±1.3
Kefir	100	78.8±2.5	78.6±3.9	95.9±20.1	58.1±5.3	104.6±6.2
	1000	88.5±0.8	82.4±2.5	97.9±2.2	65.7±1.2	98.4±0.3
Cocoa powder	100	71.2±9.9	111.0±3.9	77.9±0.4	46.4±4.1	103.6±0.2
	1000	81.0±2.4	114.5±1.0	69.7±1.3	55.3±0.0	91.2±7.9

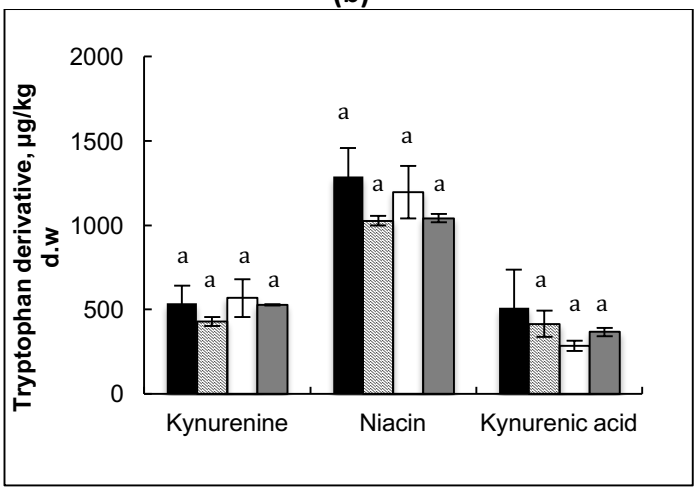
* Spiking at this level was not performed for this sample



(a)



(b)



(c)

Figure 4.2. Effect of extraction with different solvents on the concentration of (a) tryptophan in bread and yoghurt (b) tryptophan derivatives in bread (c) tryptophan derivatives in yoghurt. Black bars: water extracts; striped bars: water-methanol (60:40, v/v) extracts; white bars: water-methanol (30:70, v/v) extracts; grey bars: methanol extracts.

Table 4.5. Concentrations of tryptophan (mg/kg d.w or mg/L) and its derivatives (µg/kg d.w or µg/L) found in various fermented food samples

Food Samples	Kynurenine	Kynurenic acid	Niacin	Nicotinamide	Tryptophan
Bread					
1	143.5±28.2	n.d.	1153.0±140.0	1949.0±155.5	72.5±0.6
2	n.d.	n.d.	2693.0±283.7	3537.9±248.6	31.7±0.5
3	n.d.	n.d.	970.0±13.6	2534.0±148.5	69.2±1.5
Beer*					
1	53.2±1.5	17.2±2.2	564.9±31.9	141.8±24.1	31.1±0.1
2	72.2±0.1	52.0±18.3	354.5±0.7	58.4±16.7	16.6±0.0
3	85.2±4.6	28.8±4.2	897.8±10.5	24.1±10.3	15.0±0.0
4	86.3±0.5	23.7±1.8	709.4±16.5	28.3±0.1	16.4±0.5
5	28.7±0.7	27.3±1.0	333.7±26.4	n.d.	4.8±0.0
6	46.6±1.1	16.9±0.8	1056.0±62.2	14.6±0.3	13.8±0.0
Red wine*					
1	n.d.	108.4±23.3	562.4±25.2	206.7±16.2	1.0±0.0
2	n.d.	179.7±5.1	367.2±40.8	36.3±10.5	1.4±0.0
3	n.d.	82.4±6.1	446.9±33.4	424.4±8.3	1.6±0.0
4	n.d.	105.9±4.4	407.2±46.9	88.5±15.3	1.8±0.0
White Cheese					
1	182.9±24.0	47.5±4.9	224.1±42.0	n.d.	5.7±0.0
2	30.3±5.4	76.6±25.1	625.9±89.4	n.d.	13.3±0.8
3	166.1±32.9	52.0±0.3	383.5±11.3	n.d.	12.6±2.2
4	64.6±13.3	26.7±9.0	481.4±12.2	n.d.	3.8±0.1
5	321.6±66.9	27.5±3.8	n.d.	n.d.	37.6±0.9
6	195.0±11.1	64.5±1.0	397.8±23.8	n.d.	24.2±1.2
Yoghurt					
1	751.9±34.2	286.8±35.9	1516.5±23.3	n.d.	10.6±0.4
2	288.8±66.3	178.8±46.8	1112.9±191.7	n.d.	6.7±0.2
3	411.2±87.3	172.7±31.7	896.2±39.7	n.d.	13.4±0.3
4	624.9±33.8	231.6±6.4	912.9±22.22	n.d.	12.5±0.1
5	274.3±80.3	67.5±14.6	895.8±132.4	n.d.	3.2±0.0
Kefir					
1	763.8±106.6	241.7±4.6	1560.5±191.6	n.d.	5.1±0.2
2	406.6±36.4	150.7±29.7	314.1±4.6	n.d.	5.4±0.0
3	372.8±16.3	113.4±13.5	1036.7±25.8	n.d.	2.9±0.0
Cocoa powder					
1	n.d.	4390.5±456.0	22971.5±6.4	2281.0±29.7	21.5±0.5
2	n.d.	4409.9±333.4	18009.9±532.1	2534.0±162.8	37.5±3.3
3	n.d.	4000.3±120.5	22207.5±162.1	2565.5±220.3	12.9±0.0
4	n.d.	4486.2±165.6	22804.2±868.8	2583.9±472.1	18.4±1.2

* Concentrations of tryptophan derivatives in marked food samples (liquid samples) were given as µg/L; n.d.: not detected

Kynurenic acid was not detected in bread samples analyzed. Beer samples were found to contain kynurenic acid within the range of 16.9±0.8 and 52.0±18.3 µg/L. Besides, red wine samples contained kynurenic acid within the range of 82.4±6.1 and 179.7±5.1 µg/L. Shin et al [148] reported that *Saccharomyces uvarum* could synthesize kynurenic acid when tryptophan was found in the culture medium. It was a remarkable result that cacao powder (within the range of 4000.3±120.5 and 4486.2±165.6 µg/kg d.w) had the highest amount of kynurenic acid among food samples analyzed. Kynurenic acid was also detected in all dairy products analyzed. White cheese and yoghurt contained kynurenic acid within the range of 26.7±9.0 and 76.6±25.1 µg/kg d.w, and 67.5±14.6 and 286.8±35.9 µg/kg d.w, respectively. Kynurenic acid level in kefir was found to be averagely 168.6 µg/kg d.w. It was reported that hard cheese, kefir and yoghurt contained 44.4 pmol/g, 56.8 pmol/g, 90.1 pmol/g of kynurenic acid in fresh weight, respectively [149].

Bread contained niacin within the range of 970.0±13.6 and 2693.0±283.7 µg/kg d.w. The niacin concentrations in beer and wine samples were found to be within the range of 333.7±26.4 and 1056.0±62.2 µg/L and, 367.2±40.8 and 562.4±25.2 µg/L, respectively. It was reported that beer contained niacin within the range of 3 and 8 mg/L [218]. Hall et al [219] reported that sweet red wines contained 2420, 1900 and 1840 µg/L of niacin. Differences in the concentration of niacin in the beer or wine samples may be related to the characteristics of malt and grapes, fermentation conditions and yeast strains. To date, several studies about synthesis of niacin by *Saccharomyces cerevisiae* were carried out [220, 221]. However, investigations about the effect of tryptophan-NAD^+ pathway on the niacin synthesis in yeasts are limited. Shin et al [148] reported that when kynurenine was added in the culture medium of *Saccharomyces uvarum*, niacin content increased.

In addition to yeasts, some bacteria such as microflora in human colon could synthesize vitamins [222]. In our study, yoghurt, white cheese (except one sample) and kefir, which were fermented with bacteria strains, contained niacin. The concentrations of niacin in yoghurt samples ranged from 895.8±132.4 to 1516.5±23.3 µg/kg d.w. The highest concentration of niacin in cheeses was found to be 625.9±89.4 µg/kg d.w. Combs Jr and McClung [223] reported that yoghurt and cheese contained 2 and 0.2-10 mg/kg of niacin, respectively.

All of the yeast-fermented foods analyzed except one beer sample were found to contain nicotinamide. The highest nicotinamide concentrations were observed in bread and cacao samples with the mean values of 2673.6 and 2491.1 μg/kg d.w, respectively. On the other hand, dairy products (white cheese, yoghurt and kefir) did not contain nicotinamide.

Tryptophan concentrations of bread samples were found to be within the range of 31.7±0.5 and 72.5±0.6 mg/kg d.w. It was revealed in a study that free tryptophan concentration of wheat flour was 35 mg/kg [224]. It was found that beer and red wine contained tryptophan with the mean concentrations of 16.3 and 1.5 mg/L, respectively. White cheese contained tryptophan in a wide range (3.8±0.1-37.6±0.9 mg/kg d.w). Yoghurt, kefir and cocoa powder contained tryptophan within the range of 3.2±0.0 and 13.4±0.3 mg/kg d.w, 2.9±0.0 and 5.4±0.0 mg/kg d.w and, 12.9±0.0 and 37.5±3.3 mg/kg d.w, respectively. Free tryptophan content appeared to be low in red wine and cocoa powder as compared to previous studies [199, 225]. It was reported that wine and cocoa contained 18.6 mg/kg and 180 mg/kg of free tryptophan, respectively [199, 225].

Analyses of tryptophan derivatives in foods have importance in human health due to the fact that most of them have neuroactive properties. In this study, we reported that one of the bread sample and beer samples as yeast fermented foods contained kynurenine. However, it is not known whether presence of wheat flour/malt/baker's yeast or metabolism of yeast during fermentation is responsible for the presence of kynurenine in bread and beer. Therefore, probability of synthesis of tryptophan derivatives in kynurenine pathway by yeasts during bread and beer fermentation needs to be clarified. Moreover, the food processes and the type of yeast fermentation should be taken into consideration in further studies. It was also found that dairy products contained kynurenine, kynurenic acid and niacin. Therefore, the fermentation effect on tryptophan derivatives in dairy products needs to be investigated in detail.

In this study, it was found that beer and red wine contained kynurenic acid, which is an important result as they consumed widely all over the world. Moreover, it was remarkable that cacao has very high content of kynurenic acid. These results should be taken into consideration due to the fact that kynurenic acid is a neuroprotective compound as revealed in the Chapter 1. Kynurenic acid also has

positive effects on gastrointestinal diseases [138]. Besides, kynurenic acid blocks quinolinic acid induced neurotoxicity [122]. When it is thought that cacao is consumed widely all over the world, presence of kynurenic acid in cacao has an importance. Therefore, the processing effects such as roasting, fermentation and alkalization on kynurenic acid content of cacao should be investigated. Furthermore, when tryptophan derivatives are consumed with diet, their bioavailability needs to be also clarified.

5 INVESTIGATION OF TRYPTOPHAN DERIVATIVES IN KYNURENINE PATHWAY DURING WORT FERMENTATION

5.1 Introduction

Tryptophan derivatives in kynurenine pathway have important roles on human health as mentioned in Chapter 1. Beside humans, kynurenine pathway is found in yeasts [3, 4]. Kucharczyk et al [4] demonstrated evidence of the existence of the kynurenine pathway in yeast and characterized the 3-hydroxyanthranilic acid dioxygenase. Panozzo et al [3] identified the genes encoding the different enzymes of the kynurenine pathway in *Saccharomyces cerevisiae*. It was reported that Bna3p is the yeast kynurenine aminotransferase [143]. Furthermore, it was also reported that biosynthesis of NAD^+ and niacin via kynurenine pathway is present in *Saccharomyces cerevisiae* and *Saccharomyces uvarum*, respectively [147, 148].

Formation of tryptophan derivatives in kynurenine pathway during beer fermentation has not been investigated to date. For this purpose, *Saccharomyces pastorianus* NCYC 203 and *Saccharomyces cerevisiae* NCYC 88 were used in this study. Effect of tryptophan content on the formation of tryptophan derivatives during fermentation was also evaluated by adding different concentrations of tryptophan to the worts.

5.2 Material and Methods

5.2.1 Chemicals and Consumables

Methanol (HPLC grade), L-kynurenine (≥98%), kynurenic acid (≥98%) and nicotinic acid (niacin) (≥98%) were obtained from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%) and L-tryptophan (Trp) were purchased from Merck Co. (Darmstadt, Germany). YPD and Agar were supplied from Lab M (Lancashire, UK). *Saccharomyces pastorianus* NCYC 203 and *Saccharomyces cerevisiae* NCYC 88 were supplied from The National Collection of Yeast Cultures (Norwich, UK). Syringe filters (nylon, 0.45 µm) were supplied by Waters Corp. (Milford, MA, USA). Hypersil GOLD PFP column (100 x 2.1 mm i.d., 1.9 µm) was obtained from Thermo Scientific (Waltham, MA, USA). Deionized water (5.6 µS/m) was used throughout the experiments.

5.2.2 Preparation of Wort

Malt was obtained from the Anadolu Efes Beverage Company (Ankara, Turkey). Wort was prepared according to the method described previously in Chapter 2 [226]. After preparation of wort, different concentrations of tryptophan (100, 150 and 300 mg/L) were added into the some wort samples to investigate the effect of tryptophan on the formation of tryptophan derivatives during fermentation. Then, the glass tubes were filled with the wort samples (5 mL) and autoclaving was carried out.

5.2.3 Fermentation

Saccharomyces pastorianus NCYC 203 and *Saccharomyces cerevisiae* NCYC 88 were propagated in wort (10 mL) at 28 °C for 48 h. After incubation, wort containing propagated yeast (100 µL) was added into the tubes containing wort samples (5 mL), which had been autoclaved. The initial counts of *Saccharomyces pastorianus* NCYC 203 and *Saccharomyces cerevisiae* NCYC 88 were 6.00±0.09 and 5.81±0.24 log cfu/mL in worts, respectively. Fermentation was performed at 28 °C for 8 days and 15 °C for 12 days in wort samples fermented with *Saccharomyces cerevisiae* NCYC 88 and *Saccharomyces pastorianus* NCYC 203, respectively. Fermentation was carried out in duplicates. The sample in which tryptophan was not added and fermented with *Saccharomyces cerevisiae* NCYC 88 or *Saccharomyces pastorianus* NCYC 203 was termed as “control” sample.

5.2.4 Analysis of Tryptophan Derivatives in Kynurenine Pathway

One milliliter of fermented wort sample was mixed with one mililiter of methanol and stored - 80 °C to prevent yeast activation. The samples were mixed by vortexing for 5 min just before analysis. After vortexing, the samples were centrifuged at 3000x*g* for 5 min and the supernatant was collected. The samples were filtered through a 0.45 µm syringe filter into an autosampler vial prior to analyses of tryptophan derivatives. Tryptophan derivatives were determined according to the method described previously in Chapter 4 [227].

5.2.5 Yeast Count

Fermented wort samples collected every 4 days of the fermentation were inoculated by spread plate technique to YPD Agar. Yeast count was performed by using YPD Agar after incubation of the plates for 48 h at 28 °C.

5.2.6 Kinetic Modeling

The formation of kynurenine and kynurenic acid in worts fermented with *Saccharomyces cerevisiae* NCYC 88 and *Saccharomyces pastorianus* NCYC 203 was modeled according to the Gompertz model modified by Zwietering et al [228].

$$y = A \exp\left(-\exp\left(\frac{\mu_m \, e}{A}\,(\lambda - t) + 1\right)\right)$$

Where A is the asymptotic concentration defined as maximum kynurenine or kynurenic acid production (μg/L), μ_m is the maximum kynurenine or kynurenic acid production rate (μg/L.day), e is the Euler number (2.718), λ is the lag period (day), t is the time (day). The experimental data were modelled using the curve-fitting module in MATLAB (MathWorks, 2011b).

5.2.7 Statistical Analysis

The results were reported as mean ± standard deviation. Significant differences ($p < 0.05$) were evaluated by Duncan test via analysis of variance (ANOVA) by using SPSS 17.0 (Chicago, IL, USA).

5.3 Results and Discussion

Fermentation of wort was carried out using *Saccharomyces cerevisiae* NCYC 88 and *Saccharomyces pastorianus* NCYC 203 in this study. Changes in the counts of *S. cerevisiae* and *S. pastorianus* of wort samples during fermentation were given in Table 5.1. The initial count of *S. cerevisiae* in wort was 5.81±0.24 log cfu/mL. After 8 days of fermentation, count of *S. cerevisiae* in control sample increased 1.5 log units. Furthermore, after 8 days of fermentation, the count of *S. cerevisiae* increased 1.7, 1.4 and 1.5 log units in worts added 100, 150 and 300 mg/L of tryptophan, respectively. It could be said that adding 100 mg/L of tryptophan into the wort increased the count of *S. cerevisiae.* The initial count of *S. pastorianus* in wort was 6.00±0.09 log cfu/mL. After 12 days of fermentation, increase in *S. pastorianus* count was 1.9 log units in wort sample. When 100, 150 and 300 mg/L of tryptophan were added into the wort, increases in *S. pastorianus* count were found to be 1.8, 1.6 and 1.4 log units after fermentation, respectively.

Table 5.1. Changes in the counts of *S. cerevisiae* and *S. pastorianus* (log cfu/mL) of wort samples during fermentation*

	Fermentation time (days)	Control	100 mg/L Trp	150 mg/L Trp	300 mg/L Trp
Saccharomyces cerevisiae	0	5.81±0.24^{a}	5.81±0.24^{a}	5.81±0.24^{a}	5.81±0.24^{a}
	4	7.43±0.03^{b}	7.31±0.29^{b}	7.35±0.02^{b}	7.45±0.06^{b}
	8	7.30±0.00^{b}	7.48±0.01^{b}	7.25±0.35^{b}	7.31±0.01^{b}
Saccharomyces pastorianus	0	6.00±0.09^{a}	6.00±0.09^{a}	6.00±0.09^{a}	6.00±0.09^{a}
	4	7.79±0.17^{b}	7.78±0.05^{bc}	7.70±0.07^{b}	7.73±0.06^{b}
	8	7.48±0.26^{c}	7.69±0.01^{b}	7.89±0.08^{c}	7.70±0.14^{b}
	12	7.90±0.00^{b}	7.84±0.00^{c}	7.55±0.00^{b}	7.41±0.00^{c}

*Different letters within the same column in every panel indicate statistical significance ($p < 0.05$).

Changes in the concentrations of tryptophan of worts fermented with *S. cerevisiae* and *S. pastorianus* were given in Table 5.2 and Table 5.3**,** respectively. Initial concentration of tryptophan was found 46.2±0.8 mg/L in control sample fermented with *S. cerevisiae*. After 8 days of fermentation, the control sample fermented with *S. cerevisiae* contained 3.7±0.3 mg/L of tryptophan. When 100, 150 and 300 mg/L of tryptophan were added into the wort, 57%, 57% and 69% of tryptophan were used by *S. cerevisiae,* respectively. In control sample fermented with *S. pastorianus*, the concentration of tryptophan decreased from 52.0±0.5 to 34.1±1.8 mg/L. In worts added 100, 150 and 300 mg/L of tryptophan, *S. pastorianus* used 12%, 12% and 6% of tryptophan, respectively. From this data, it could be seen that more tryptophan was utilized by *S. cerevisiae* NCYC 88 during fermentation as compared to *S. pastorianus* NCYC 203. It was reported that tryptophan concentrations of wort and beer were 11.9 and 5.8 mg/100 mL, respectively [14].

The changes in kynurenic acid concentration of worts fermented with *S. cerevisiae* and *S. pastorianus* during fermentation were demonstrated in Figure 5.1. The initial concentration of kynurenic acid was found to be 23.5±0.6 µg/L in control sample, which can be related to malt or yeast. In Figure 5.1a, there is a clear trend of increasing kynurenic acid concentration in wort fermented with *S. cerevisiae*. The kynurenic acid concentration of control sample fermented with *S. cerevisiae* increased from 23.5±0.6 to 333.1±108.3 µg/L after 8 days of fermentation. When 100, 150 and 300 mg/L of tryptophan were added into the wort, the concentrations of the kynurenic acid were found to be 955.5±2.1 µg/L, 1420±137.9 µg/L and 2410±282.8 µg/L after fermentation, respectively. Therefore, it can be concluded from these data that the concentration of tryptophan in the medium has a critical importance for the formation of kynurenic acid in the beer.

Figure 5.1b illustrated the changes in kynurenic acid content of wort fermented with *S. pastorianus* during fermentation. It was found that the kynurenic acid content of control sample increased from 24.2±5.7 µg/L to 94.0±17.3 µg/L after 12 days of fermentation. When 100, 150 and 300 mg/L of tryptophan were added into the wort, the kynurenic acid contents of worts were found to be 111.7±8.8 µg/L, 157.8±33.7 µg/L and 253.1±14.3 µg/L of kynurenic acid, respectively. These data outlined that kynurenic acid concentration increased in wort fermented with *S. pastorianus* NCYC 203 when tryptophan concentration was higher in the wort.

This is a significant result due to the fact that kynurenic acid is a neuroprotective compound as mentioned in the Chapter 1. Kynurenic acid also has positive effects on gastrointestinal diseases [138]**.** Besides, it was found in this study that *Saccharomyces cerevisiae* NCYC 88 synthesized more kynurenic acid than *Saccharomyces pastorianus* NCYC 203. This result could be attributed to more utilization of tryptophan by *Saccharomyces cerevisiae* NCYC 88. According to our previous study [227], beer samples obtained from market contained kynurenic acid within the range of 16.9±0.8 and 52.0±18.3 µg/L. However, kynurenic acid content in wort samples after fermentation was found higher in this study. This difference might be due to different fermentation conditions, yeast strains and processing conditions.

The formation of kynurenic acid in worts fermented with *Saccharomyces cerevisiae* NCYC 88 and *Saccharomyces pastorianus* NCYC 203 during fermentation was modelled by using modified Gompertz model (Figure 5.1). As shown in Table 5.4, the regression coefficients ranged from 0.957 to 0.999 for kynurenic acid, which showed that the model fitted the experimental data. Maximum kynurenic acid production rates increased in worts fermented with both *Saccharomyces cerevisiae* NCYC 88 and *Saccharomyces pastorianus* NCYC 203 when tryptophan concentration was higher in the medium. Besides, maximum kynurenic acid production rates were higher in the worts fermented with *Saccharomyces cerevisiae* NCYC 88 when compared to *Saccharomyces pastorianus* NCYC 203. Lag time for formation of kynurenic acid ranged from 1.34 and 4.27 days in worts fermented with *Saccharomyces cerevisiae* NCYC 88. On the other hand, lag time was not observed for the formation of kynurenic acid in worts fermented with *Saccharomyces pastorianus* NCYC 203. Maximum kynurenic acid production was highly affected by adding tryptophan in the wort. The maximum kynurenic acid production was observed in the wort added 150 mg/L of tryptophan and fermented with *Saccharomyces cerevisiae* NCYC 88. Besides, the maximum kynurenic acid production was detected in the wort added 300 mg/L of tryptophan and fermented with *Saccharomyces pastorianus* NCYC 203.

Table 5.2. Changes in the concentrations of tryptophan, kynurenine and niacin of worts fermented with *S. cerevisiae* during fermentation*

	Fermentation time (days)	Control	100 mg/L Trp	150 mg/L Trp	300 mg/L Trp
Tryptophan (mg/L)	0	46.2±0.8^{a}	125.1±1.0^{a}	185.8±1.9^{a}	341.4±2.4^{a}
	1	34.3±2.0^{b}	109.1±7.6^{a}	165.9±11.2^{ab}	298.2±11.7^{b}
	2	18.8±8.5^{c}	85.5±4.8^{b}	146.6±8.1^{b}	282.2±2.2^{b}
	4	22.9±0.4^{c}	79.1±12.2^{b}	135.1±0.0^{b}	244.8±20.9^{c}
	6	18.7±5.9^{c}	64.5±16.4^{bc}	96.8±34.1^{c}	167.0±21.6^{d}
	8	3.7±3.0^{d}	54.2±0.3^{c}	78.8±2.8^{c}	105.1±17.0^{e}
Kynurenine (µg/L)	0	48.1±5.7^{ab}	52.2±0.1^{a}	59.8±2.0^{a}	66.3±0.8^{a}
	1	46.1±9.4^{ab}	65.9±2.5^{ab}	65.2±10.2^{ab}	79.9±4.0^{ab}
	2	54.3±2.3^{a}	70.8±6.2^{b}	68.9±6.8^{ab}	114.2±6.6^{bc}
	4	60.0±1.4^{a}	75.8±11.2^{b}	83.9±14.1^{b}	99.0±7.8^{abc}
	6	55.2±9.3^{a}	76.8±4.8^{b}	83.1±9.5^{ab}	120.9±4.8^{bc}
	8	29.8±10.2^{b}	71.6±10.3^{b}	84.1±7.6^{b}	141.1±42.8^{c}
Niacin (µg/L)	0	754.9±17.3^{a}	657.7±11.3^{a}	643.4±26.7^{a}	632.3±30.5^{a}
	1	350.9±14.1^{b}	326.2±46.2^{b}	387.9±87.9^{b}	292.3±83.8^{b}
	2	181.8±69.3^{c}	187.9±27.1^{c}	241.6±82.0^{b}	254.0±25.3^{b}
	4	313.9±53.1^{bc}	213.9±112.0^{bc}	349.7±39.3^{b}	211.3±11.7^{b}
	6	347.6±62.9^{b}	290.4±19.4^{bc}	311.5±40.7^{b}	298.1±84.5^{b}
	8	192.1±98.5^{c}	325.2±29.9^{b}	299.6±106.7^{b}	328.9±49.7^{b}

*Different letters within the same column in every panel indicate statistical significance ($p < 0.05$).

Table 5.3. Changes in the concentrations of tryptophan, kynurenine and niacin of worts fermented with *S. pastorianus* during fermentation*

	Fermentation time (days)	Control	100 mg/L Trp	150 mg/L Trp	300 mg/L Trp
Tryptophan (mg/L)	0	52.0±0.5^{a}	125.8±0.2^{a}	191.2±0.9^{a}	334.3±5.1^{a}
	4	34.0±0.1^{b}	116.4±2.1^{ab}	165.2±4.0^{b}	320.4±5.7^{a}
	8	34.6±2.4^{b}	104.5±9.8^{b}	158.9±8.8^{b}	309.2±19.0^{a}
	12	34.1±1.8^{b}	109.8±2.4^{b}	167.8±2.3^{b}	314.9±8.0^{a}
Kynurenine (µg/L)	0	43.1±3.9^{a}	53.0±3.7^{a}	56.4±3.6^{a}	67.6±0.4^{a}
	4	53.3±7.2^{a}	54.0±1.9^{a}	57.4±0.6^{a}	74.4±0.1^{a}
	8	50.6±11.0^{a}	72.7±8.1^{b}	74.3±2.3^{a}	90.0±2.6^{a}
	12	59.2±3.8^{a}	76.4±7.5^{b}	70.0±14.1^{a}	86.5±15.8^{a}
Niacin (µg/L)	0	658.5±24.6^{a}	611.8±0.4^{a}	649±17.1^{a}	704.8±11.0^{a}
	4	20.8±0.3^{b}	82.9±29.1^{b}	106.2±80.1^{b}	110.1±38.9^{b}
	8	61.6±14.6^{bc}	63.2±14.1^{b}	91.9±8.5^{b}	118.3±25.2^{b}
	12	65.6±9.5^{c}	55.9±14.1^{b}	98.9±0.4^{b}	107.7±7.2^{b}

*Different letters within the same column in every panel indicate statistical significance ($p < 0.05$).

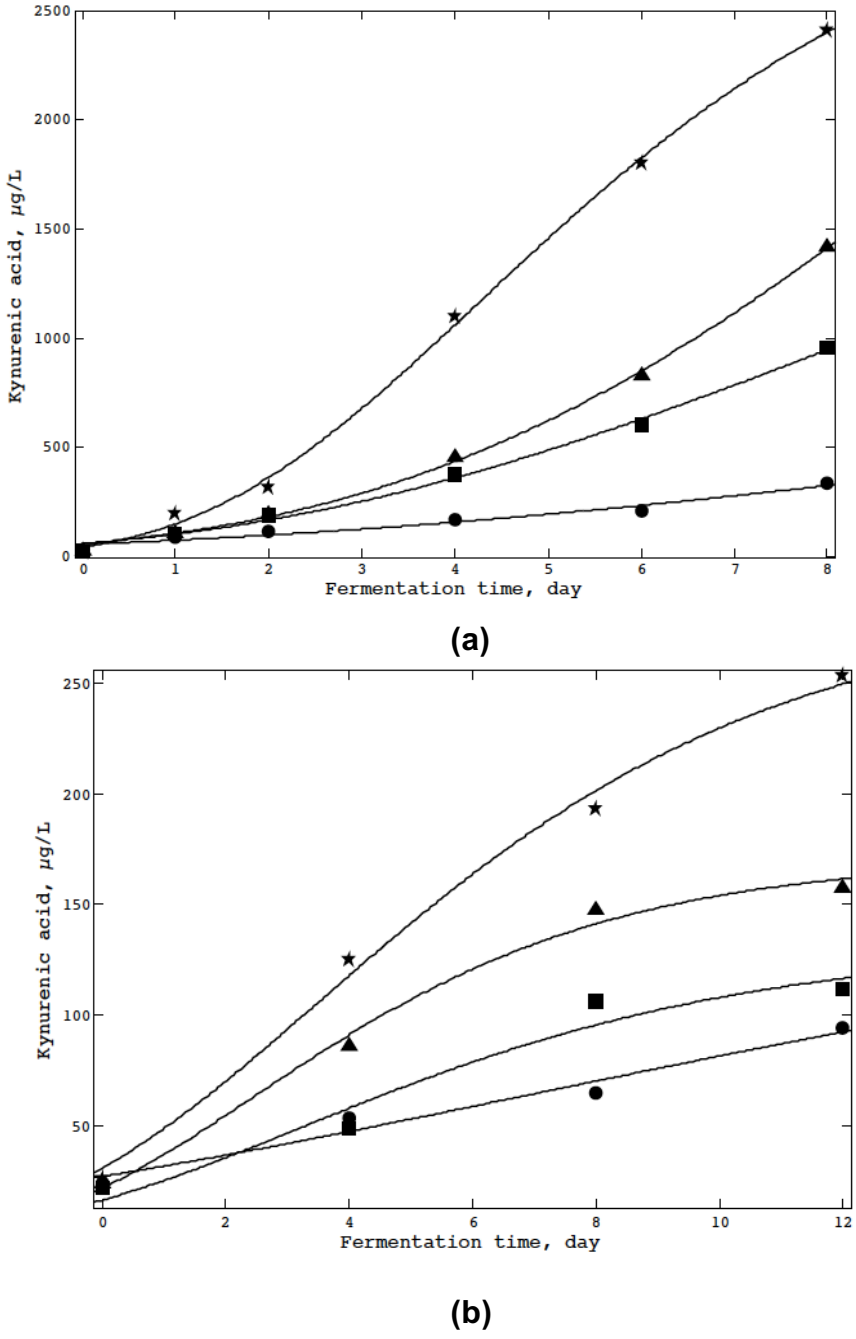


Figure 5.1. The changes in kynurenic acid concentration of control (•) and 100 mg/L of Trp (■), 150 mg/L of Trp (▲), 300 mg/L of Trp (★) added worts fermented with **(a)** *S. cerevisiae* and **(b)** *S. pastorianus* during fermentation with Gompertz model fits.

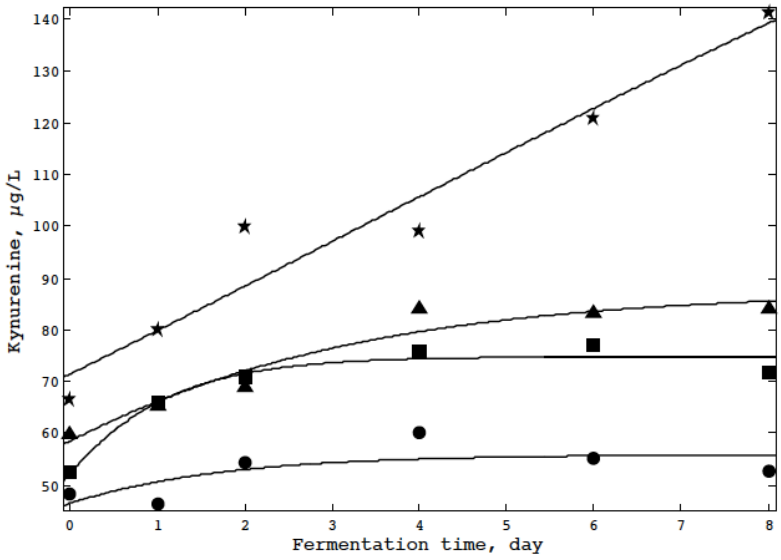


Figure 5.2. The changes in kynurenine concentration of control (•) and 100 mg/L of Trp (■), 150 mg/L of Trp (▲), 300 mg/L of Trp (★) added worts fermented with *S. cerevisiae* during fermentation with Gompertz model fits.

Figure 5.2 showed the changes in kynurenine concentration of worts fermented with *S. cerevisiae* during fermentation with Gompertz model fits. The concentration of kynurenine in control sample fermented with *S. cerevisiae* did not change significantly at the end of the fermentation ($p>0.05$). In spite of that, the kynurenine contents of worts added 100, 150 and 300 mg/L of tryptophan increased significantly at the end of 8 days of fermentation ($p<0.05$). The concentrations of kynurenine were found to be 71.6±10.3 µg/L, 84.1±7.6 µg/L and 141.1±42.8 µg/L in the worts added 100, 150 and 300 mg/L of tryptophan after 8 days of fermentation, respectively. These results showed that *S. cerevisiae* could produce kynurenine when enough tryptophan was present in the medium. This result was also evaluated with the parameters of Gompertz model. Maximum kynurenine production increased when tryptophan was higher in the medium (Table 5.4). However, maximum kynurenine production rates were changeable in worts fermented with *Saccharomyces cerevisiae* NCYC 88. Moreover, lag time was not observed for the formation of kynurenine in worts fermented with *Saccharomyces cerevisiae* NCYC 88. Except control sample, regression coefficients were found high in Gompertz model for kynurenine formation. Since kynurenine content did not change significantly in the control sample fermented with *Saccharomyces cerevisiae* NCYC 88, regression parameter was found lower.

Table 5.4. Gompertz parameters for the formation of kynurenic acid and kynurenine in worts fermented with *S. cerevisiae* and *S. pastorianus*

		Gompertz model parameters			
		A	μ_m	λ	R^2
S. cerevisiae					
Kynurenic acid	Control	1662.0	56.3	2.46	0.962
	100 mg/L Trp	2839.0	165.1	2.26	0.995
	150 mg/L Trp	7300.0	363.1	4.27	0.999
	300 mg/L Trp	3175.0	399.3	1.34	0.999
Kynurenine	Control	55.8	12.8	-	0.525
	100 mg/L Trp	74.8	28.6	-	0.960
	150 mg/L Trp	87.4	11.7	-	0.943
	300 mg/L Trp	269.1	8.7	-	0.943
S. pastorianus					
Kynurenic acid	Control	174.9	5.7	-	0.969
	100 mg/L Trp	132.8	11.3	-	0.957
	150 mg/L Trp	171.4	18.7	-	0.992
	300 mg/L Trp	287.7	24.3	-	0.984

A, asymptotic concentration (µg/L); μ_m, maximum production rate (µg/L.day); λ, lag time (day); R^2, regression coefficient

(-) indicates no lag time

Control sample fermented with *S. pastorianus* was found to contain 43.1±3.9 µg/L kynurenine (Table 5.3). After 12 days of fermentation, the concentration of kynurenine did not change significantly in control sample ($p>0.05$). Moreover, the kynurenine concentration did not also change in the worts added 150 and 300 mg/L of tryptophan during fermentation. However, kynurenine concentration increased significantly from 53.0±3.7 to 76.4±7.5 µg/L in wort added 100 mg/L of tryptophan after 12 days of fermentation ($p<0.05$).

Concentrations of niacin decreased significantly in worts fermented with *S. cerevisiae* and *S. pastorianus* ($p<0.05$) (Table 5.2 and Table 5.3). The worts fermented with *S. cerevisiae* contained niacin ranging between 632.3±30.5 µg/L and 754.9±17.3 µg/L. When 100, 150 and 300 mg/L of tryptophan were added into the wort, 51%, 53% and 48% of niacin were used by *S. cerevisiae* after 8 days of fermentation, respectively. In control sample fermented with *S. pastorianus*, the niacin concentration decreased from 658.5±24.6 to 65.6±9.5 µg/L. When 100, 150 and 300 mg/L of tryptophan were added into the wort, 91%, 85% and 85% of niacin were used by *S. pastorianus* at the end of 12 days of fermentation, respectively. It can be resulted from these data that *S. pastorianus* used more niacin than *S. cerevisiae* during beer fermentation. Bamforth [218] reported that beer contained niacin ranging from 3 to 8 mg/L. It was also reported that yeasts could take up niacin found in the environment to synthesize NAD^+ [144].

GENERAL CONCLUSION AND DISCUSSION

The principal focus of this thesis was to understand changes of amino acids and formation of bioactive amines and tryptophan derivatives in different fermented food products. In **Chapter 2**, the effect of *S. cerevisiae* on the formation of GABA and the other bioactive amines during wort fermentation was evaluated. As result of this study, it was found that *S. cerevisiae* has ability to produce GABA during wort fermentation. Therefore, the present study showed that GABA is not an exact indicator of microbial contamination in beers differently from tyramine and histamine. This study also indicated that total amino acid concentration in both spoiled and unspoiled worts increased after a certain fermentation time. Hence, future studies are essential to clarify the impact of protease activity in fermented foods on bioactive amine formation.

The formation of tyramine during yoghurt fermentation with the focus on interaction between *Streptococcus thermophilus* RSKK 04082, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 and *Lactobacillus plantarum* RSKK 02030 was revealed in **Chapter 3**. This study demonstrated that *L. delbrueckii* subsp. *bulgaricus* DSM 20081 strain did not produce tyramine in yoghurt. However, *S. thermophilus* RSKK 04082 and *L. plantarum* RSKK 02030 produced tyramine depending on the fermentation conditions. Tyramine formation was not only caused by decarboxylase-positive bacteria but also by microbial interactions. Relationship between *S. thermophilus* RSKK 04082 and *L. plantarum* RSKK 02030 in terms of tyramine production could be explained as antagonism. Antagonistic interaction between microorganisms could be an effective way to reduce the amount of tyramine in yoghurt. On the other hand, interaction in tyramine production between *S. thermophilus* RSKK 04082 and *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and, *L. delbrueckii* subsp. *bulgaricus* DSM 20081 and *L. plantarum* RSKK 02030 was determined as synergism. This interaction was due to the fact that *L. delbrueckii* subsp. *bulgaricus* DSM 20081 provides nitrogen sources for *S. thermophilus* RSKK 04082 and *L. plantarum* RSKK 02030. Therefore, it should not be ignored that microbial interactions could be effective on accumulation of bioactive amines in foods. The dairy industry must take into account both microbial interactions and decarboxylase negative strains when it is

considered to add a new strain to the yoghurt. Thus, bioactive amine concentrations are kept under control.

In **Chapter 4**, a LC-MS/MS method was developed for the determination of tryptophan derivatives in kynurenine pathway in various types of fermented foods. The results indicated the occurrence of varying amounts of neuroactive tryptophan derivatives in kynurenine pathway in different fermented foods largely consumed in all over the world. Therefore, the method described here could be a reliable analytical tool to investigate the effects of fermentation on formation of neuroactive tryptophan derivatives in different food matrices.

The formation of tryptophan derivatives in kynurenine pathway by *S. cerevisiae* NCYC 88 and *S. pastorianus* NCYC 203 during wort fermentation was evaluated in **Chapter 5**. The results revealed formation of kynurenic acid and kynurenine by yeasts during beer fermentation. As beer is mostly consumed food product in the world, these results should be taken into consideration. *S. cerevisiae* NCYC 88 increased the kynurenine and kynurenic acid concentration in wort depending on tryptophan concentration. Besides, the effect of malt properties, fermentation conditions and yeast strains on the formation of tryptophan derivatives in beer needs to be clarified in the future.

Overall, all investigations in this thesis enlightened the formation of bioactive amines and some neuroactive compounds, which have an important roles on human heath and mood, during different fermentation conditions. Based on this thesis, relationship between fermented foods and human mood can be evaluated in the future studies.

REFERENCES

[1] Nuñez, M., del Olmo, A., Calzada, J., Biogenic Amines. *Encyclopedia of Food and Health*, (eds: Cabellero, B., Finglas, P. M., Toldra, F.), Academic Press, Oxford, 416-423, **2016**.

[2] Colín-González, A. L., Maldonado, P. D., Santamaría, A., 3-Hydroxykynurenine: An intriguing molecule exerting dual actions in the Central Nervous System, *NeuroToxicology*, 34, 189-204, **2013**.

[3] Panozzo, C., Nawara, M., Suski, C., Kucharczyka, R., Skoneczny, M.,Bécam, A.-M., Rytka, J., Herbert, C. J., Aerobic and anaerobic NAD+ metabolism in Saccharomyces cerevisiae, *FEBS Letters*, 517, 97-102, **2002**.

[4] Kucharczyk, R., Zagulski, M., Rytka, J., Herbert, C. J., The yeast gene YJR025c encodes a 3-hydroxyanthranilic acid dioxygenase and is involved in nicotinic acid biosynthesis, *FEBS Letters*, 424, 127-130, **1998**.

[5] Kurnasov, O., Goral, V., Colabroy, K., Gerdes, S., Anantha, S., Osterman, A.,Begley, T. P., NAD Biosynthesis: Identification of the Tryptophan to Quinolinate Pathway in Bacteria, *Chemistry & Biology*, 10, 1195-1204, **2003**.

[6] El-Mansi, E. M. T., Bryce, C., Hartley, B., Demain, A., Fermentation Microbiology and Biotechnology. *Fermentation Microbiology and Biotechnology, Third Edition*, (eds: El-Mansi, E. M. T., Bryce, C., Demain, A. L., Allman, A. R.), CRC Press, 1-8, **2011**.

[7] Gest, H., The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellows of The Royal Society, *Notes and Records of the Royal Society of London*, 58, 187-201, **2004**.

[8] Nair, B., Prajapati, J., The History of Fermented Foods. *Handbook of Fermented Functional Foods*, (eds: Farnworth, E. R.), CRC Press, 1-25, **2003**.

[9] Santer, M., Joseph Lister: first use of a bacterium as a 'model organism' to illustrate the cause of infectious disease of humans, *Notes and Records of the Royal Society*, 64, 59-65, **2010**.

[10] Guizani, N., Mothershaw, A., Fermentation. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*, (eds: Hui, Y. H., Sherkat, F.), CRC Press, **2005**.

[11] Adams, M., Nout, M. J. R., Fermentation and Food Safety. Springer US: **2001**.

[12] Ansorena, D., Astiasarán, I., Fermented Foods: Composition and Health effects. *Encyclopedia of Food and Health*, (eds: Cabellero, B., Finglas, P. M., Toldra, F.), Academic Press, Oxford, 649-655, **2016**.

[13] Bamforth, C. W., The Science Underpinning Food Fermentations. *Food, Fermentation and Micro-organisms*, (eds: Bamforth, C. W.), Blackwell Publishing Ltd, 1-39, **2007**.

[14] Boulton, C., Quain, D., The Brewing Process. *Brewing Yeast and Fermentation*, (eds: Boulton, C., Quain, D.), Blackwell Science Ltd, 19-68, **2007**.

[15] Munroe, J., Fermentation. *Handbook of Brewing, Second Edition*, (eds: Stewart, G. G., Priest, F. G.), CRC Press, 487-524, **2006**.

[16] Tamime, A. Y., Robinson, R. K., Yoghurt Science and Technology. CRC Press, New York, **2000**.

[17] Chandan, R. C., O'Rell, K. R., Principles of Yogurt Processing. *Manufacturing Yogurt and Fermented Milks*, (eds: Chandan, R. C., Kilara, A.), Blackwell Publishing, 195-210, **2007**.

[18] Özer, B., Strategies for Yogurt Manufacturing. *Development and Manufacture of Yogurt and Other Functional Dairy Products*, (eds: Yildiz, F.), CRC Press, 47-96, **2009**.

[19] Josephsen, J.,Jespersen, L., Fermented Food and Starter Cultures. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*, (eds: Hui, Y. H., Sherkat, F.), CRC Press, **2005**.

[20] Camilli, A., Bassler, B. L., Bacterial Small-Molecule Signaling Pathways, *Science (New York, N.Y.)*, 311, 1113-1116, **2006**.

[21] Sieuwerts, S., de Bok, F. A. M., Hugenholtz, J., van Hylckama Vlieg, J. E. T., Unraveling Microbial Interactions in Food Fermentations: from Classical to Genomics Approaches, *Applied and Environmental Microbiology*, 74, 4997-5007, **2008**.

[22] Smid, E. J., Lacroix, C., Microbe–microbe interactions in mixed culture food fermentations, *Current Opinion in Biotechnology*, 24, 148-154, **2013**.

[23] Herve-Jimenez, L., Guillouard, I.,Guedon, E., Boudebbouze, S., Hols, P., Monnet, V., Maguin, E., Rul, F., Postgenomic Analysis of Streptococcus thermophilus Cocultivated in Milk with Lactobacillus delbrueckii subsp. bulgaricus: Involvement of Nitrogen, Purine, and Iron Metabolism, *Applied and Environmental Microbiology*, 75, 2062-2073, **2009**.

[24] Sieuwerts, S., Molenaar, D., van Hijum, S. A. F. T., Beerthuyzen, M., Stevens, M. J. A., Janssen, P. W. M., Ingham, C. J., de Bok, F. A. M., de Vos, W. M., van Hylckama Vlieg, J. E. T., Mixed-Culture Transcriptome Analysis Reveals the Molecular Basis of Mixed-Culture Growth in Streptococcus thermophilus and Lactobacillus bulgaricus, *Applied and Environmental Microbiology*, 76, 7775-7784, **2010**.

[25] Crittenden, R. G., Martinez, N. R., Playne, M. J., Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria, *International Journal of Food Microbiology*, 80, 217-222, **2003**.

[26] Courtin, P., Monnet, V., Rul, F., Cell-wall proteinases PrtS and PrtB have a different role in Streptococcus thermophilus/Lactobacillus bulgaricus mixed cultures in milk, *Microbiology*, 148, 3413-3421, **2002**.

[27] Bouzar, F.,Cerning, J.,Desmazeaud, M., Exopolysaccharide Production and Texture-Promoting Abilities of Mixed-Strain Starter Cultures in Yogurt Production, *Journal of Dairy Science*, 80, 2310-2317, **1997**.

[28] Hamdan, I. Y., Kunsman, J. E., Jr., Deanne, D. D., Acetaldehyde Production by Combined Yogurt Cultures¹, *Journal of Dairy Science*, 54, 1080-1082,

[29] Heard, G. M., Fleet, G. H., The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice, *Journal of Applied Bacteriology*, 65, 23-28, **1988**.

[30] Liu, Y., Rousseaux, S., Tourdot-Maréchal, R., Sadoudi, M., Gougeon, R., Schmitt-Kopplin, P., Alexandre, H., Wine microbiome: A dynamic world of microbial interactions, *Critical Reviews in Food Science and Nutrition*, 57, 856-873, **2017**.

[31] Fleet, G. H., Yeast interactions and wine flavour, *International Journal of Food Microbiology*, 86, 11-22, **2003**.

[32] Mounier, J., Monnet, C., Vallaeys, T., Arditi, R., Sarthou, A.-S., Hélias, A., Irlinger, F., Microbial Interactions within a Cheese Microbial Community, *Applied and Environmental Microbiology*, 74, **2008**.

[33] Irlinger, F., Mounier, J., Microbial interactions in cheese: implications for cheese quality and safety, *Current Opinion in Biotechnology*, 20, 142-148, **2009**.

[34] Russell, I., Yeast. *Handbook of Brewing, Second Edition*, (eds: Stewart, G. G., Priest, F. G.), CRC Press, 281-332, **2006**.

[35] Boulton, C., Quain, D., The Biochemistry of Fermentation. *Brewing Yeast and Fermentation*, (eds: Boulton, C., Quain, D.), Blackwell Science Ltd, 69-142, **2007**.

[36] Dennis E. Briggs, Chris A. Boulton, Brookes, P. A., Stevens, R., Brewing Science and Practice. Woodhead Publishing Limited and CRC Press, **2004**.

[37] Tamime, A. Y.,Robinson, R. K., 7 - Biochemistry of fermentation. *Tamime and Robinson's Yoghurt (Third Edition)*, (eds: Tamime, A. Y.,Robinson, R. K.), Woodhead Publishing, 535-607, **2007**.

[38] Tunail, N., Mikrobiyoloji. Pelin Ofset: Ankara, **2009**; p 448.

[39] Willaert, R., Biochemistry and Fermentation of Beer. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*, eds: (Hui, Y. H., Sherkat, F.), CRC Press, **2005**.

[40] Özer, B., Microbiology and Biochemistry of Yogurt and Other Fermented Milk Products. *Dairy Microbiology and Biochemistry*, (eds: Ozer, B., Akdemir-Evrendilek, G.), CRC Press,167-213, **2014**.

[41] Gürakan, G., Altay, N., Yogurt Microbiology and Biochemistry. *Development and Manufacture of Yogurt and Other Functional Dairy Products*, (eds: Yildiz, F.), CRC Press, 97-121, **2009**.

[42] Rao, D. R., Reddy, J. C., Effects of Lactic Fermentation of Milk on Milk Lipids, *Journal of Food Science*, 49, 748-750, **1984**.

[43] Alvarez, M. A., Moreno-Arribas, M. V., The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading

microorganisms as a solution, *Trends in Food Science & Technology*, 39, 146-155, **2014**.

[44] Guo, X., Guan, X., Wang, Y., Li, L., Wu, D., Chen, Y., Pei, H., Xiao, D., Reduction of biogenic amines production by eliminating the PEP4 gene in Saccharomyces cerevisiae during fermentation of Chinese rice wine, *Food Chemistry*, 178, 208-211, **2015**.

[45] Sentellas, S., Núñez, Ó., Saurina, J., Recent Advances in the Determination of Biogenic Amines in Food Samples by (U)HPLC, *Journal of Agricultural and Food Chemistry*, 64, 7667-7678, **2016**.

[46] Restuccia, D., Spizzirri, U., Puoci, F., Parisi, O., Curcio, M., Picci, N., Accumulation of Biogenic Amines in Foods: Hazard Identification and Control Options. *Microbial Food Safety and Preservation Techniques*, (eds: Rai, V. R., Bai, J. A.), CRC Press, 53-74, **2014**.

[47] Kumar, A., Biogenic Amine Neurotransmitters. *Separation Techniques in Clinical Chemistry*, (eds: Aboul-Enein, H. Y.), CRC Press, **2003**.

[48] Hazards, E. P. O. B., Scientific Opinion on risk based control of biogenic amine formation in fermented foods, *EFSA Journal*, 9, 2393-n/a, **2011**.

[49] Pereira, C. I., Barreto Crespo, M. T., San Romão, M. V., Evidence for proteolytic activity and biogenic amines production in Lactobacillus curvatus and L. homohiochii, *International Journal of Food Microbiology*, 68, 211-216, **2001**.

[50] Esselen, M., Schrenk, D., 11 - Toxicants in foods generated by non-thermal processes. *Chemical Contaminants and Residues in Food*, (eds: Schrenk, D.), Woodhead Publishing, 250-285, **2012**.

[51] Linares, D. M., Martín, M., Ladero, V., Alvarez, M. A., Fernández, M., Biogenic Amines in Dairy Products, *Critical Reviews in Food Science and Nutrition*, 51, 691-703, **2011**.

[52] Victor, L., Marina, C.-E., Maria, F., Miguel, A. A., Toxicological Effects of Dietary Biogenic Amines, *Current Nutrition & Food Science*, 6, 145-156, **2010**.

[53] Commission, E., Commission Regulation N° 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, *Official Journal of the European Union, L338*, 1-26, **2005**.

[54] del Rio, B., Redruello, B., Linares, D. M., Ladero, V., Fernandez, M., Martin, M. C., Ruas-Madiedo, P., Alvarez, M. A., The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture, *Food Chemistry*, 218, 249-255, **2017**.

[55] Stadnik, J., Significance of Biogenic Amines in Fermented Foods and Methods of Their Control. *Beneficial Microbes in Fermented and Functional Foods*, (eds: Rai, V. R., Bai, J. A.) CRC Press, 149-164, **2014**.

[56] Benkerroum, N., Biogenic Amines in Dairy Products: Origin, Incidence, and Control Means, *Comprehensive Reviews in Food Science and Food Safety*, 15, 801-826, **2016**.

[57] Chung, K.-T., Gadupudi, G. S., Possible roles of excess tryptophan metabolites in cancer, *Environmental and Molecular Mutagenesis*, 52, 81-104, **2011**.

[58] Distler, M. G., Plant, L. D., Sokoloff, G., Hawk, A. J., Aneas, I., Wuenschell, G. E., Termini, J., Meredith, S. C., Nobrega, M. A., Palmer, A. A., Glyoxalase 1 increases anxiety by reducing GABA(A) receptor agonist methylglyoxal, *The Journal of Clinical Investigation*, 122, 2306-2315, **2012**.

[59] Padgett, C. L., Lalive, A. L., Tan, K. R., Terunuma, M., Munoz, M. B., Pangalos, M. N., Martínez-Hernández, J., Watanabe, M., Moss, S. J., Luján, R., Lüscher, C., Slesinger, P. A., Methamphetamine-evoked depression of GABA(B) receptor signaling in GABA neurons of the VTA, *Neuron*, 73, 978-989, **2012**.

[60] Gottesmann, C., GABA mechanisms and sleep, *Neuroscience*, 111, 231-239, **2002**.

[61] Ting Wong, C. G., Bottiglieri, T., Snead, O. C., GABA, γ-hydroxybutyric acid, and neurological disease, *Annals of Neurology*, 54, S3-S12, **2003**.

[62] Islam, J., Shirakawa, H., Nguyen, T. K., Aso, H., Komai, M., Simultaneous analysis of serotonin, tryptophan and tryptamine levels in common fresh fruits and vegetables in Japan using fluorescence HPLC, *Food Bioscience*, 13, 56-59, **2016**.

[63] Watanabe, H., Akasaka, D., Ogasawara, H., Sato, K., Miyake, M., Saito, K., Takahashi, Y., Kanaya, T., Takakura, I., Hondo, T., Chao, G., Rose, M. T., Ohwada, S., Watanabe, K., Yamaguchi, T., Aso, H., Peripheral Serotonin Enhances Lipid Metabolism by Accelerating Bile Acid Turnover, *Endocrinology*, 151, 4776-4786, **2010**.

[64] Kot, M., Pilc, A., Daniel, W. A., Simultaneous alterations of brain and plasma serotonin concentrations and liver cytochrome P450 in rats fed on a tryptophan-free diet, *Pharmacological Research*, 66, 292-299, **2012**.

[65] Voigt, J.-P., Fink, H., Serotonin controlling feeding and satiety, *Behavioural Brain Research*, 277, 14-31, **2015**.

[66] Young, S. N., Leyton, M., The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels, *Pharmacology Biochemistry and Behavior*, 71, 857-865, **2002**.

[67] Watts, S. W., Morrison, S. F., Davis, R. P., Barman, S. M., Serotonin and Blood Pressure Regulation, *Pharmacological Reviews*, 64, 359-388, **2012**.

[68] Meltzer, C. C., Smith, G., DeKosky, S. T., Pollock, B. G., Mathis, C. A., Moore, R. Y., Kupfer, D. J., Reynolds, C. F., Serotonin in Aging, Late-Life Depression, and Alzheimer's Disease: The Emerging Role of Functional Imaging, *Neuropsychopharmacology*, 18, 407-430, **1998**.

[69] Spadaro, P. A., Naug, H. L., Du Toit, E. F., Donner, D., Colson, N. J., A refined high carbohydrate diet is associated with changes in the serotonin pathway and visceral obesity, *Genetics Research*, 97, **2015**.

[70] Bleich, A., Brown, S.-L., Kahn, R., van Praag, H. M., The Role of Serotonin in Schizophrenia, *Schizophrenia Bulletin*, 14, 297-315, **1988**.

[71] Vidal-Carou, M., Latorre-Moratalla, M., Bover-Cid, S., Biogenic Amines. *Safety Analysis of Foods of Animal Origin*, (eds: Nollet, L. M. L., Toldra, F.), CRC Press, 399-420, **2010**.

[72] Beatriz, M.,Gloria, A., Bioactive Amines. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*, (eds: Hui, Y. H., Sherkat, F.), CRC Press, **2005**.

[73] Baranowska, I., Płonka, J., Simultaneous Determination of Biogenic Amines and Methylxanthines in Foodstuff—Sample Preparation with HPLC-DAD-FL Analysis, *Food Analytical Methods*, 8, 963-972, **2015**.

[74] Płonka, J., Michalski, A., The influence of processing technique on the catecholamine and indolamine contents of fruits, *Journal of Food Composition and Analysis*, 57, 102-108, **2017**.

[75] Hernández-Orte, P., Lapeña, A. C., Peña-Gallego, A., Astrain, J., Baron, C., Pardo, I., Polo, L., Ferrer, S., Cacho, J., Ferreira, V., Biogenic amine determination in wine fermented in oak barrels: Factors affecting formation, *Food Research International*, 41, 697-706, **2008**.

[76] Løvaas, E., Antioxidative effects of polyamines, *Journal of the American Oil Chemists' Society*, 68, 353-358, **1991**.

[77] Moreno-Arribas, M. V., Polo, M. C., Jorganes, F., Muñoz, R., Screening of biogenic amine production by lactic acid bacteria isolated from grape must and wine, *International Journal of Food Microbiology*, 84, 117-123, **2003**.

[78] Zuljan, F. A., Mortera, P., Alarcón, S. H., Blancato, V. S., Espariz, M., Magni, C., Lactic acid bacteria decarboxylation reactions in cheese, *International Dairy Journal*, 62, 53-62, **2016**.

[79] Gardini, F., Özogul, Y., Suzzi, G., Tabanelli, G.,Özogul, F., Technological Factors Affecting Biogenic Amine Content in Foods: A Review, *Frontiers in Microbiology*, 7, **2016**.

[80] Aflaki, F., Ghoulipour, V., Saemian, N., Sheibani, S., Biogenic Amine Contents in Non-alcoholic Beers: Screening and Optimization of Derivatization, *Food Analytical Methods*, 7, 713-720, **2014**.

[81] Buňka, F., Budinský, P., Čechová, M., Drienovský, V., Pachlová, V., Matoulková, D., Kubáň, V., Buňková, L., Content of biogenic amines and polyamines in beers from the Czech Republic, *Journal of the Institute of Brewing*, 118, 213-216, **2012**.

[82] Izquierdo-Pulido, M., Albalá-Hurtado, S., Mariné-Font, A., Carmen Vidal-Carou, M., Biogenic amines in Spanish beers: differences among breweries, *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 203, 507-511, **1996**.

[83] Kalač, P., Hlavatá, V., Křížek, M., Concentrations of five biogenic amines in Czech beers and factors affecting their formation, *Food Chemistry*, 58, 209-214, **1997**.

[84] Slomkowska, A., Ambroziak, W., Biogenic amine profile of the most popular Polish beers, *European Food Research and Technology*, 215, 380-383, **2002**.

[85] Izquierdo-Pulido, M., Hernández-Jover, T., Mariné-Font, A., Vidal-Carou, M. C., Biogenic Amines in European Beers, *Journal of Agricultural and Food Chemistry*, 44, 3159-3163, **1996**.

[86] Daniel, D., dos Santos, V. B., Vidal, D. T. R., do Lago, C. L., Determination of biogenic amines in beer and wine by capillary electrophoresis–tandem mass spectrometry, *Journal of Chromatography A*, 1416, 121-128, **2015**.

[87] Kalac, P., Krízek, M., A Review of Biogenic Amines and Polyamines in Beer, *Journal of the Institute of Brewing*, 109, 123-128, **2003**.

[88] Zee, J. A., Simard, R. E., Vaillancourt, R., Boudreau, A., Effect of Lactobacillus brevis, Saccharomyces uvarum and Grist Composition on Amine Formations in Beers, *Canadian Institute of Food Science and Technology Journal*, 14, 321-325, **1981**.

[89] Izquierdo-Pulido, M., Carceller-Rosa, J.-M., Mariné-Font, A., Vidal-Carou, M. C., Tyramine Formation by Pediococcus spp. during Beer Fermentation, *Journal of Food Protection*, 60, 831-836, **1997**.

[90] Izquierdo-Pulido, M., Font-Fábregas, J., Vidal-Carou, C., Influence of Saccharomyces cerevisiae var. uvarum on histamine and tyramine formation during beer fermentation, *Food Chemistry*, 54, 51-54, **1995**.

[91] Preti, R.,Vinci, G., Biogenic Amine Content in Red Wines from Different Protected Designations of Origin of Southern Italy: Chemometric Characterization and Classification, *Food Analytical Methods*, 9, 2280-2287, **2016**.

[92] López, R., Tenorio, C., Gutiérrez, A. R., Garde-Cerdán, T., Garijo, P., González-Arenzana, L., López-Alfaro, I., Santamaría, P., Elaboration of Tempranillo wines at two different pHs. Influence on biogenic amine contents, *Food Control*, 25, 583-590, **2012**.

[93] Nalazek-Rudnicka, K., Wasik, A., Development and validation of an LC–MS/MS method for the determination of biogenic amines in wines and beers, *Monatshefte für Chemie - Chemical Monthly*, 148, 1685-1696, **2017**.

[94] Guo, Y.-Y., Yang, Y.-P., Peng, Q., Han, Y., Biogenic amines in wine: a review, *International Journal of Food Science & Technology*, 50, 1523-1532, **2015**.

[95] Smit, I., Pfliehinger, M., Binner, A., Großmann, M., Horst, W. J., Löhnertz, O., Nitrogen fertilisation increases biogenic amines and amino acid concentrations in Vitis vinifera var. Riesling musts and wines, *Journal of the Science of Food and Agriculture*, 94, 2064-2072, **2014**.

[96] Landete, J. M., Ferrer, S., Polo, L., Pardo, I., Biogenic Amines in Wines from Three Spanish Regions, *Journal of Agricultural and Food Chemistry*, 53, 1119-1124, **2005**.

[97] Ancín-Azpilicueta, C., González-Marco, A., Jiménez-Moreno, N., Current Knowledge about the Presence of Amines in Wine, *Critical Reviews in Food Science and Nutrition*, 48, 257-275, **2008**.

[98] Martín-Álvarez, P. J., Marcobal, Á., Polo, C., Moreno-Arribas, M. V., Influence of technological practices on biogenic amine contents in red wines, *European Food Research and Technology*, 222, 420-424, **2006**.

[99] Vidal-Carou, M. C., Ambatlle-Espunyes, A., Ulla-Ulla, M. C., Mariné-Font, A., Histamine and Tyramine in Spanish Wines: Their Formation During the Winemaking Process, *American Journal of Enology and Viticulture*, 41, 160-167, **1990**.

[100] Stratton, J. E., Hutkins, R. W., Taylor, S. L., Biogenic Amines in Cheese and other Fermented Foods: A Review, *Journal of Food Protection*, 54, 460-470, **1991**.

[101] Vale, S., Glória, M. B. A., Biogenic amines in Brazilian cheeses, *Food Chemistry*, 63, 343-348, **1998**.

[102] Torracca, B., Pedonese, F., López, M. B., Turchi, B., Fratini, F., Nuvoloni, R., Effect of milk pasteurisation and of ripening in a cave on biogenic amine content and sensory properties of a pecorino cheese, *International Dairy Journal*, 61, 189-195, **2016**.

[103] Pachlová, V., Buňka, F., Buňková, L., Purkrtová, S., Havlíková, Š., Němečková, I., Biogenic amines and their producers in Akawi white cheese, *International Journal of Dairy Technology*, 69, 386-392, **2016**.

[104] Poveda, J. M., Molina, G. M., Gómez-Alonso, S., Variability of biogenic amine and free amino acid concentrations in regionally produced goat milk cheeses, *Journal of Food Composition and Analysis*, 51, 85-92, **2016**.

[105] Halász, A., Baráth, Á., Simon-Sarkadi, L., Holzapfel, W., Biogenic amines and their production by microorganisms in food, *Trends in Food Science & Technology*, 5, 42-49, **1994**.

[106] Shalaby, A. R., Significance of biogenic amines to food safety and human health, *Food Research International*, 29, 675-690, **1996**.

[107] Bodmer, S., Imark, C., Kneubühl, M., Biogenic amines in foods: Histamine and food processing, *Inflammation Research*, 48, 296-300, **1999**.

[108] Min, J.-S., Lee, S.-O., Jang, A., Lee, M., Kim, Y., Quantitative Analysis of Biogenic Amines in Raw and Processed Foods of Animal Origin on Korean Domestic Market, *Asian-Australasian Journal of Animal Sciences*, 17, 1764-1768, **2004**.

[109] Zhang, Q. Q., Jiang, M., Rui, X., Li, W., Chen, X. H., Dong, M. S., Effect of rose polyphenols on oxidation, biogenic amines and microbial diversity in naturally dry fermented sausages, *Food Control*, 78, 324-330, **2017**.

[110] Van Ba, H., Seo, H.-W., Kim, J.-H., Cho, S.-H., Kim, Y.-S., Ham, J.-S., Park, B.-Y., Kim, H.-W., Kim, T.-B., Seong, P.-N., The effects of starter culture types on the technological quality, lipid oxidation and biogenic amines in fermented sausages, *LWT - Food Science and Technology*, 74, 191-198, **2016**.

[111] Sun, X., Zhou, K., Gong, Y., Zhang, N., Yang, M., Qing, D., Li, Y., Lu, J., Li, J., Feng, C., Li, C., Yang, Y., Determination of Biogenic Amines in Sichuan-Style Spontaneously Fermented Sausages, *Food Analytical Methods*, 9, 2299-2307, **2016**.

[112] Oracz, J., Nebesny, E., Influence of roasting conditions on the biogenic amine content in cocoa beans of different Theobroma cacao cultivars, *Food Research International*, 55, 1-10, **2014**.

[113] do Carmo Brito, B. d. N., Campos Chisté, R., da Silva Pena, R., Abreu Gloria, M. B., Santos Lopes, A., Bioactive amines and phenolic compounds in cocoa beans are affected by fermentation, *Food Chemistry*, 228, 484-490, **2017**.

[114] Diana, M., Rafecas, M., Quílez, J., Free amino acids, acrylamide and biogenic amines in gamma-aminobutyric acid enriched sourdough and commercial breads, *Journal of Cereal Science*, 60, 639-644, **2014**.

[115] Erhardt, S., Olsson, S. K., Engberg, G., Pharmacological Manipulation ofKynurenic Acid Potential in the Treatment of Psychiatric Disorders, *CNS Drugs*, 23, 91-101, **2009**.

[116] Lovelace, M. D., Varney, B., Sundaram, G., Franco, N. F., Ng, M. L., Pai, S., Lim, C. K., Guillemin, G. J., Brew, B. J., Current Evidence for a Role of the Kynurenine Pathway of Tryptophan Metabolism in Multiple Sclerosis, *Frontiers in Immunology*, 7, 246, **2016**.

[117] Botting, N. P., Chemistry and neurochemistry of the kynurenine pathway of tryptophan metabolism, *Chemical Society Reviews*, 24, 401-412, **1995**.

[118] Fuertig, R., Ceci, A., Camus, S. M., Bezard, E., Luippold, A. H., Hengerer, B., LC–MS/MS-based quantification of kynurenine metabolites, tryptophan, monoamines and neopterin in plasma, cerebrospinal fluid and brain, *Bioanalysis*, 8, 1903-1917, **2016**.

[119] Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., von Deimling, A., Wick, W., Platten, M., An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor, *Nature*, 478, 197-203, **2011**.

[120] O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., Cryan, J. F., Serotonin, tryptophan metabolism and the brain-gut-microbiome axis, *Behavioural Brain Research*, 277, 32-48, **2015**.

[121] Lugo-Huitrón, R., Blanco-Ayala, T., Ugalde-Muñiz, P., Carrillo-Mora, P., Pedraza-Chaverrí, J., Silva-Adaya, D., Maldonado, P. D., Torres, I., Pinzón, E., Ortiz-Islas, E., López, T., García, E., Pineda, B., Torres-Ramos, M., Santamaría, A., La Cruz, V. P.-D., On the antioxidant properties of kynurenic acid: Free radical scavenging activity and inhibition of oxidative stress, *Neurotoxicology and Teratology*, 33, 538-547, **2011**.

[122] Chen, Y., Guillemin, G. J., Kynurenine Pathway Metabolites in Humans: Disease and Healthy States, *International Journal of Tryptophan Research : IJTR*, 2, 1-19, **2009**.

[123] Stoy, N., Mackay, G. M., Forrest, C. M., Christofides, J., Egerton, M., Stone, T. W., Darlington, L. G., Tryptophan metabolism and oxidative stress in patients with Huntington's disease, *Journal of Neurochemistry*, 93, 611-623, **2005**.

[124] Guillemin, G. J., Kerr, S. J., Brew, B. J., Involvement of quinolinic acid in aids dementia complex, *Neurotoxicity Research*, 7, 103-123, **2005**.

[125] Guillemin, G. J., Brew, B. J., Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease, *Redox Report*, 7, 199-206, **2002**.

[126] Schwarcz, R., Rassoulpour, A., Wu, H.-Q., Medoff, D., Tamminga, C. A., Roberts, R. C., Increased cortical kynurenate content in schizophrenia, *Biological Psychiatry*, 50, 521-530, **2001**.

[127] Heng, B., Lim, C. K., Lovejoy, D. B., Bessede, A., Gluch, L., Guillemin, G. J., Understanding the role of the kynurenine pathway in human breast cancer immunobiology, *Oncotarget*, 7, 6506-6520, **2016**.

[128] Schwarcz, R.,Bruno, J. P., Muchowski, P. J., Wu, H.-Q., Kynurenines in the mammalian brain: when physiology meets pathology, *Nature reviews. Neuroscience*, 13, 465-477, **2012**.

[129] Ogawa, T., Matson, W., Beal, M., Myers, R.,Bird, E., Milbury, P., Saso, S., Kynurenine pathway abnormalities in Parkinson's disease, *Neurology*, 42, 1702-1706, **1992**.

[130] Guillemin, G. J., Brew, B. J., Noonan, C. E., Takikawa, O., Cullen, K. M., Indoleamine 2,3 dioxygenase and quinolinic acid Immunoreactivity in Alzheimer's disease hippocampus, *Neuropathology and Applied Neurobiology*, 31, 395-404, **2005**.

[131] Guidetti, P., Bates, G. P., Graham, R. K., Hayden, M. R., Leavitt, B. R., MacDonald, M. E., Slow, E. J., Wheeler, V. C., Woodman, B., Schwarcz, R., Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice, *Neurobiology of Disease*, 23, 190-197, **2006**.

[132] Lapin, I. P., Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice, *Journal of Neural Transmission*, 42, 37-43, **1978**.

[133] Erhardt, S., Schwieler, L., Engberg, G., Kynurenic Acid And Schizophrenia. *Developments in Tryptophan and Serotonin Metabolism*, (eds: Allegri, G., Costa, C. V. L., Ragazzi, E., Steinhart, H., Varesio, L.), Springer US, Boston, MA, 155-165, **2003**.

[134] Carlborg, A., Jokinen, J., Jönsson, E. G., Erhardt, S., Nordström, P., CSF kynurenic acid and suicide risk in schizophrenia spectrum psychosis, *Psychiatry Research*, 205, 165-167, **2013**.

[135] Erhardt, S., Lim, C. K., Linderholm, K. R., Janelidze, S., Lindqvist, D., Samuelsson, M., Lundberg, K., Postolache, T. T., Traskman-Bendz, L., Guillemin, G. J., Brundin, L., Connecting inflammation with glutamate agonism in suicidality, *Neuropsychopharmacology*, 38, 743-752, **2013**.

[136] Christmas, D. M., Badawy, A. A. B., Hince, D., Davies, S. J. C., Probert, C., Creed, T., Smithson, J., Afzal, M., Nutt, D. J., Potokar, J. P., Increased serum free tryptophan in patients with diarrhea-predominant irritable bowel syndrome, *Nutrition Research*, 30, 678-688, **2010**.

[137] Clarke, G., Fitzgerald, P., Cryan, J. F., Cassidy, E. M., Quigley, E. M., Dinan, T. G., Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort, *BMC Gastroenterology*, 9, 6-6, **2009**.

[138] Turski, M. P., Chwil, S., Turska, M., Chwil, M., Kocki, T., Rajtar, G., Parada-Turska, J., An exceptionally high content of kynurenic acid in chestnut honey and flowers of chestnut tree, *Journal of Food Composition and Analysis*, 48, 67-72, **2016**.

[139] Giles, G. I., Collins, C. A., Stone, T. W., Jacob, C., Electrochemical and in vitro evaluation of the redox-properties of kynurenine species, *Biochemical and Biophysical Research Communications*, 300, 719-724, **2003**.

[140] Goldstein, L. E., Leopold, M. C., Huang, X., Atwood, C. S., Saunders, A. J., Hartshorn, M., Lim, J. T., Faget, K. Y., Muffat, J. A., Scarpa, R. C., Chylack, L. T., Bowden, E. F., Tanzi, R. E., Bush, A. I., 3-Hydroxykynurenine and 3-Hydroxyanthranilic Acid Generate Hydrogen Peroxide and Promote α-Crystallin Cross-Linking by Metal Ion Reduction, *Biochemistry*, 39, 7266-7275, **2000**.

[141] Rios, C., Santamaria, A., Quinolinic acid is a potent lipid peroxidant in rat brain homogenates, *Neurochemical Research*, 16, 1139-1143, **1991**.

[142] Ahmad, F., Moat, A. G., Nicotinic Acid Biosynthesis in Prototrophs and Tryptophan Auxotrophs of Saccharomyces cerevisiaee, *The Journal of Biological Chemistry*, 241, 775-780, **1965**.

[143] Wogulis, M., Chew, E. R., Donohoue, P. D., Wilson, D. K., Identification of Formyl Kynurenine Formamidase and Kynurenine Aminotransferase from Saccharomyces cerevisiae Using Crystallographic, Bioinformatic and Biochemical Evidence, *Biochemistry*, 47, 1608-1621, **2008**.

[144] Sporty, J., Lin, S.-J., Kato, M., Ognibene, T., Stewart, B., Turteltaub, K., Bench, G., Quantitation of NAD(+) biosynthesis from the salvage pathway in Saccharomyces cerevisiae, *Yeast (Chichester, England)*, 26, 363-369, **2009**.

[145] Rongvaux, A., Andris, F., Van Gool, F., Leo, O., Reconstructing eukaryotic NAD metabolism, *BioEssays*, 25, 683-690, **2003**.

[146] Li, Y.-F., Bao, W.-G., Why do some yeast species require niacin for growth? Different modes of NAD synthesis, *FEMS Yeast Research*, 7, 657-664, **2007**.

[147] Ohashi, K., Kawai, S., Murata, K., Secretion of Quinolinic Acid, an Intermediate in the Kynurenine Pathway, for Utilization in NAD(+) Biosynthesis in the Yeast Saccharomyces cerevisiae, *Eukaryotic Cell*, 12, 648-653, **2013**.

[148] Shin, M., Sano, K., Umezawa, C., Metabolism of tryptophan to niacin in Saccharomyces uvarum., *Journal of Nutritional Science and Vitaminology*, 37, 269-283, **1991**.

[149] Turski, M. P., Turska, M., Zgrajka, W., Kuc, D., Turski, W. A., Presence of kynurenic acid in food and honeybee products, *Amino Acids*, 36, 75-80, **2009**.

[150] Turski, M. P., Kamiński, P., Zgrajka, W., Turska, M., Turski, W. A., Potato-An Important Source of Nutritional Kynurenic Acid, *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, 67, 17-23, **2012**.

[151] Turski, M. P., Turska, M., Kocki, T., Turski, W. A., Paluszkiewicz, P., Kynurenic Acid Content in Selected Culinary Herbs and Spices, *Journal of Chemistry*, 2015, 6, **2015**.

[152] Soto, M. E., Ares, A. M., Bernal, J., Nozal, M. J., Bernal, J. L., Simultaneous determination of tryptophan, kynurenine, kynurenic and xanthurenic acids in honey by liquid chromatography with diode array, fluorescence and tandem mass spectrometry detection, *Journal of Chromatography A*, 1218, 7592-7600, **2011**.

[153] Bertazzo, A., Ragazzi, E., Visioli, F., Evolution of tryptophan and its foremost metabolites' concentrations in milk and fermented dairy products, *PharmaNutrition*, 4, 62-67, **2016**.

[154] Möller, M., Du Preez, J. L., Harvey, B. H., Development and validation of a single analytical method for the determination of tryptophan, and its kynurenine metabolites in rat plasma, *Journal of Chromatography B*, 898, 121-129, **2012**.

[155] Hervé, C., Beyne, P., Jamault, H., Delacoux, E., Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection, *Journal of Chromatography B: Biomedical Sciences and Applications*, 675, 157-161, **1996**.

[156] Vaarmann, A., Kask, A., Mäeorg, U., Novel and sensitive high-performance liquid chromatographic method based on electrochemical coulometric array detection for simultaneous determination of catecholamines, kynurenine and indole derivatives of tryptophan, *Journal of Chromatography B*, 769, 145-153, **2002**.

[157] Galarce, O., Henríquez-Aedo, K., Peterssen, D., Peña-Farfal, C., Aranda, M., A Selective Chromatographic Method to Determine the Dynamic of Biogenic Amines During Brewing Process, *Food Analytical Methods*, 9, 3385-3395, **2016**.

[158] Lee, S., Yoo, M., Shin, D., The identification and quantification of biogenic amines in Korean turbid rice wine, Makgeolli by HPLC with mass spectrometry detection, *LWT - Food Science and Technology*, 62, 350-356, **2015**.

[159] Diana, M., Quílez, J., Rafecas, M., Gamma-aminobutyric acid as a bioactive compound in foods: a review, *Journal of Functional Foods*, 10, 407-420, **2014**.

[160] Diana, M., Rafecas, M., Arco, C., Quílez, J., Free amino acid profile of Spanish artisanal cheeses: Importance of gamma-aminobutyric acid (GABA) and ornithine content, *Journal of Food Composition and Analysis*, 35, 94-100, **2014**.

[161] Rizzello, C. G., Cassone, A., Di Cagno, R., Gobbetti, M., Synthesis of Angiotensin I-Converting Enzyme (ACE)-Inhibitory Peptides and γ-Aminobutyric Acid (GABA) during Sourdough Fermentation by Selected Lactic Acid Bacteria, *Journal of Agricultural and Food Chemistry*, 56, 6936-6943, **2008**.

[162] Komatsuzaki, N., Shima, J., Kawamoto, S., Momose, H., Kimura, T., Production of γ-aminobutyric acid (GABA) by Lactobacillus paracasei isolated from traditional fermented foods, *Food Microbiology*, 22, 497-504, **2005**.

[163] Hudec, J., Kobida, Ľ., Čanigová, M., Lacko-Bartošová, M., Ložek, O., Chlebo, P., Mrázová, J., Ducsay, L., Bystrická, J., Production of γ-aminobutyric acid by microorganisms from different food sources, *Journal of the Science of Food and Agriculture*, 95, 1190-1198, **2015**.

[164] Yılmaz, C., Kocadağlı, T., Gökmen, V., Formation of Melatonin and Its Isomer during Bread Dough Fermentation and Effect of Baking, *Journal of Agricultural and Food Chemistry*, 62, 2900-2905, **2014**.

[165] Kalač, P., Šavel, J., Křížek, M., Pelikánová, T., Prokopová, M., Biogenic amine formation in bottled beer, *Food Chemistry*, 79, 431-434, **2002**.

[166] Loret, S., Deloyer, P., Dandrifosse, G., Levels of biogenic amines as a measure of the quality of the beer fermentation process: Data from Belgian samples, *Food Chemistry*, 89, 519-525, **2005**.

[167] Hu, Z., Li, L., Yuan, Y., Yue, T., Ultrasensitive and simultaneous determination of twenty-one amino acids and amines in culture media, red wine and beer, *Food Chemistry*, 158, 56-65, **2014**.

[168] Deetae, P., Perello, M.-C., de Revel, G., Occurrence of ochratoxin A and biogenic amines in Asian beers sold in French markets, *Journal of the Institute of Brewing*, 119, 57-63, **2013**.

[169] Kocadağlı, T., Özdemir, K. S., Gökmen, V., Effects of infusion conditions and decaffeination on free amino acid profiles of green and black tea, *Food Research International*, 53, 720-725, **2013**.

[170] Bach, B., Meudec, E., Lepoutre, J.-P., Rossignol, T., Blondin, B., Dequin, S., Camarasa, C., New Insights into γ-Aminobutyric Acid Catabolism: Evidence for γ-Hydroxybutyric Acid and Polyhydroxybutyrate Synthesis in Saccharomyces cerevisiae, *Applied and Environmental Microbiology*, 75, 4231-4239, **2009**.

[171] De Barber, C. B., Prieto, J. A., Collar, C., Reversed-Phase High-Performance Liquid Chromatography Analysis of Changes in Free Amino Acids During Wheat Bread Dough Fermentation, *Cereal Chemistry*, 66, 283-288, **1989**.

[172] Geissler, A. J., Behr, J., von Kamp, K., Vogel, R. F., Metabolic strategies of beer spoilage lactic acid bacteria in beer, *International Journal of Food Microbiology*, 216, 60-68, **2016**.

[173] Lekkas, C., Stewart, G. G., Hill, A. E., Taidi, B., Hodgson, J., Elucidation of the Role of Nitrogenous Wort Components in Yeast Fermentation, *Journal of the Institute of Brewing*, 113, 3-8, **2007**.

[174] Ormrod, I. H. L., Lalor, E. F., Sharpe, F. R., The release of yeast proteolytic enzymes into beer, *Journal of the Institute of Brewing*, 97, 441-443, **1991**.

[175] Maddox, I. S., Hough, J. S., Proteolytic enzymes of Saccharomyces carlsbergensis, *Biochemical Journal*, 117, 843-852, **1970**.

[176] Nouadje, G., Siméon, N., Dedieu, F., Nertz, M., Puig, P., Couderc, F., Determination of twenty eight biogenic amines and amino acids during wine aging by micellar electrokinetic chromatography and laser-induced fluorescence detection, *Journal of Chromatography A*, 765, 337-343, **1997**.

[177] Soufleros, E. H., Bouloumpasi, E., Zotou, A., Loukou, Z., Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration, *Food Chemistry*, 101, 704-716, **2007**.

[178] Boulton, C., Quain, D., Beer and Brewing. *Brewing Yeast and Fermentation*, (eds: Boulton, C., Quain, D.) Blackwell Science Ltd, 1-18, **2007**.

[179] Shimwell, J. L., On the relation between the staining properties of bacteria and their reaction towards hop antiseptic, *Journal of the Institute of Brewing*, 43, 111-118, **1937**.

[180] Sakamoto, K., Konings, W. N., Beer spoilage bacteria and hop resistance, *International Journal of Food Microbiology*, 89, 105-124, **2003**.

[181] Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., Sanders, M. E., Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic, *Nature Reviews Gastroenterology & Hepatology*, 11, 506-514, **2014**.

[182] Sidira, M., Santarmaki, V., Kiourtzidis, M., Argyri, A. A., Papadopoulou, O. S., Chorianopoulos, N., Tassou, C., Kaloutsas, S., Galanis, A., Kourkoutas, Y., Evaluation of immobilized Lactobacillus plantarum 2035 on whey protein as adjunct probiotic culture in yoghurt production, *LWT - Food Science and Technology*, 75, 137-146, **2017**.

[183] Lomer, M. C. E., Parkes, G. C., Sanderson, J. D., Review article: lactose intolerance in clinical practice – myths and realities, *Alimentary Pharmacology & Therapeutics*, 27, 93-103, **2008**.

[184] Sazawal, S., Hiremath, G., Dhingra, U., Malik, P., Deb, S., Black, R. E., Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials, *The Lancet Infectious Diseases*, 6, 374-382, **2006**.

[185] Zhang, L., Li, N., Caicedo, R., Neu, J., Alive and Dead Lactobacillus rhamnosus GG Decrease Tumor Necrosis Factor-α–Induced Interleukin-8 Production in Caco-2 Cells, *The Journal of Nutrition*, 135, 1752-1756, **2005**.

[186] Tripathi, M. K., Giri, S. K., Probiotic functional foods: Survival of probiotics during processing and storage, *Journal of Functional Foods*, 9, 225-241, **2014**.

[187] Garcia, E. F., de Oliveira, M. E. G., Queiroga, R. d. C. R. D. E., Machado, T. A. D., de Souza, E. L., Development and quality of a Brazilian semi-hard goat cheese (coalho) with added probiotic lactic acid bacteria, *International Journal of Food Sciences and Nutrition*, 63, 947-956, **2012**.

[188] Shoji, A. S., Oliveira, A. C., Balieiro, J. C. C., Freitas, O., Thomazini, M., Heinemann, R. J. B., Okuro, P. K., Favaro-Trindade, C. S., Viability of L. acidophilus microcapsules and their application to buffalo milk yoghurt, *Food and Bioproducts Processing*, 91, 83-88, **2013**.

[189] Brinques, G. B., Ayub, M. A. Z., Effect of microencapsulation on survival of Lactobacillus plantarum in simulated gastrointestinal conditions, refrigeration, and yogurt, *Journal of Food Engineering*, 103, 123-128, **2011**.

[190] Bezerra, T. K. A., Arcanjo, N. M. d. O., Araújo, A. R. R. d., Queiroz, A. L. M. d., Oliveira, M. E. G. d.,Gomes, A. M. P., Madruga, M. S., Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria, *LWT - Food Science and Technology*, 76, 209-215, **2017**.

[191] dos Santos Cruxen, C. E., Hoffmann, J. F., Zandoná, G. P., Fiorentini, Â. M., Rombaldi, C. V., Chaves, F. C., Probiotic butiá (Butia odorata) ice cream: Development, characterization, stability of bioactive compounds, and viability of Bifidobacterium lactis during storage, *LWT - Food Science and Technology*, 75, 379-385, **2017**.

[192] CODEX STAN 243-2003, Standard for Fermented Milks. In 2003.

[193] Linares, D. M., O'Callaghan, T. F., O'Connor, P. M., Ross, R. P., Stanton, C., Streptococcus thermophilus APC151 Strain Is Suitable for the Manufacture of Naturally GABA-Enriched Bioactive Yogurt, *Frontiers in Microbiology*, 7, 1876, **2016**.

[194] Li, C., Wang, C.-L., Sun, Y.,Li, A.-L., Liu, F., Meng, X.-C., Microencapsulation of Lactobacillus rhamnosus GG by Transglutaminase Cross-Linked Soy Protein Isolate to Improve Survival in Simulated Gastrointestinal Conditions and Yoghurt, *Journal of Food Science*, 81, M1726-M1734, **2016**.

[195] Settachaimongkon, S., van Valenberg, H. J. F., Winata, V., Wang, X., Nout, M. J. R., van Hooijdonk, T. C. M., Zwietering, M. H., Smid, E. J., Effect of sublethal preculturing on the survival of probiotics and metabolite formation in set-yoghurt, *Food Microbiology*, 49, 104-115, **2015**.

[196] Vijayendra, S. V. N., Gupta, R. C., Associative growth behavior of dahi and yoghurt starter cultures with Bifidobacterium bifidum and Lactobacillus acidophilus in buffalo skim milk, *Annals of Microbiology*, 63, 461-469, **2013**.

[197] Settachaimongkon, S., van Valenberg, H. J. F., Gazi, I., Nout, M. J. R., van Hooijdonk, T. C. M., Zwietering, M. H., Smid, E. J., Influence of Lactobacillus plantarum WCFS1 on post-acidification, metabolite formation and survival of starter bacteria in set-yoghurt, *Food Microbiology*, 59, 14-22, **2016**.

[198] Linares, D. M., del Río, B., Ladero, V., Martínez, N., Fernández, M., Martín, M. C., Álvarez, M. A., Factors Influencing Biogenic Amines Accumulation in Dairy Products, *Frontiers in Microbiology*, 3, 180, **2012**.

[199] Hashim, P., Selamat, J., Syed Muhammad, S. K., Ali, A., Changes in free amino acid, peptide-N, sugar and pyrazine concentration during cocoa

fermentation, *Journal of the Science of Food and Agriculture*, 78, 535-542, **1998**.

[200] Ashraf, R., Shah, N. P., Selective and differential enumerations of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt — A review, *International Journal of Food Microbiology*, 149, 194-208, **2011**.

[201] Süle, J., Kõrösi, T., Hucker, A., Varga, L., Evaluation of culture media for selective enumeration of bifidobacteria and lactic acid bacteria, *Brazilian Journal of Microbiology*, 45, 1023-1030, **2014**.

[202] Buňková, L., Buňka, F., Hlobilová, M., Vaňátková, Z., Nováková, D., Dráb, V., Tyramine production of technological important strains of Lactobacillus, Lactococcus and Streptococcus, *European Food Research and Technology*, 229, 533-538, **2009**.

[203] Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., Vidal-Carou, M. C., Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages, *International Journal of Food Microbiology*, 66, 185-189, **2001**.

[204] Rajagopal, S. N., Sandine, W. E., Associative Growth and Proteolysis of Streptococcus thermophilus and Lactobacillus bulgaricus in Skim Milk1, *Journal of Dairy Science*, 73, 894-899, **1990**.

[205] Georgieva, R., Iliev, I., Haertlé, T., Chobert, J.-M., Ivanova, I., Danova, S., Technological properties of candidate probiotic Lactobacillus plantarum strains, *International Dairy Journal*, 19, 696-702, **2009**.

[206] Beshkova, D. M., Simova, E. D., Frengova, G. I., Simov, Z. I., Adilov, E. F., Production of Amino Acids by Yogurt Bacteria, *Biotechnology Progress*, 14, 963-965, **1998**.

[207] Fernández, M., Linares, D. M., del Río, B., Ladero, V., Alvarez, M. A., HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms, *Journal of Dairy Research*, 74, 276-282, **2007**.

[208] Ladero, V., Martínez, N., Martín, M. C., Fernández, M., Alvarez, M. A., qPCR for quantitative detection of tyramine-producing bacteria in dairy products, *Food Research International*, 43, 289-295, **2010**.

[209] Morales, F. J., Açar, Ö. Ç., Serpen, A., Arribas-Lorenzo, G., Gökmen, V., Degradation of Free Tryptophan in a Cookie Model System and Its Application in Commercial Samples, *Journal of Agricultural and Food Chemistry*, 55, 6793-6797, **2007**.

[210] Vignau, J., Jacquemont, M. C., Lefort, A., Imbenotte, M., Lhermitte, M., Simultaneous determination of tryptophan and kynurenine in serum by HPLC with UV and fluorescence detection, *Biomedical Chromatography*, 18, 872-874, **2004**.

[211] Xiang, Z.-Y., Tang, A.-G., Ren, Y.-P., Zhou, Q.-X., Luo, X.-B., Simultaneous determination of serum tryptophan metabolites in patients with systemic lupus erythematosus by high performance liquid chromatography with fluorescence detection, *Clinical Chemistry and Laboratory Medicine*, 48, 513, **2010**.

[212] Mitsuhashi, S., Fukushima, T., Kawai, J., Tomiya, M., Santa, T., Imai, K., Toyo'oka, T., Improved method for the determination of kynurenic acid in rat plasma by column-switching HPLC with post-column fluorescence detection, *Analytica Chimica Acta*, 562, 36-43, **2006**.

[213] Fukushima, T., Mitsuhashi, S., Tomiya, M., Iyo, M., Hashimoto, K., Toyo'oka, T., Determination of kynurenic acid in human serum and its correlation with the concentration of certain amino acids, *Clinica Chimica Acta*, 377, 174-178, **2007**.

[214] Tsuruta, Y., Kohashi, K., Ishida, S., Ohkura, Y., Determination of nicotinic acid in serum by high-performance liquid chromatography with fluorescence detection, *Journal of Chromatography B: Biomedical Sciences and Applications*, 309, 309-315, **1984**.

[215] Lang, R., Yagar, E. F., Eggers, R., Hofmann, T., Quantitative Investigation of Trigonelline, Nicotinic Acid, and Nicotinamide in Foods, Urine, and Plasma by Means of LC-MS/MS and Stable Isotope Dilution Analysis, *Journal of Agricultural and Food Chemistry*, 56, 11114-11121, **2008**.

[216] Yamada, K., Hara, N., Shibata, T., Osago, H., Tsuchiya, M., The simultaneous measurement of nicotinamide adenine dinucleotide and related compounds by liquid chromatography/electrospray ionization tandem mass spectrometry, *Analytical Biochemistry*, 352, 282-285, **2006**.

[217] Perrone, D., Donangelo, C. M., Farah, A., Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry, *Food Chemistry*, 110, 1030-1035, **2008**.

[218] Bamforth, C. W., Nutritional aspects of beer—a review, *Nutrition Research*, 22, 227-237, **2002**.

[219] Hall, A. P., Brinner, L., Amerine, M. A., Morgan, A. F., The B vitamin content of grapes, musts, and wines, *Journal of Food Science*, 21, 362-371, **1956**.

[220] Tseng, M. M. C., Phillips, C. R., The kinetics of yeast growth and nicotinic acid production, *Biotechnology and Bioengineering*, 24, 1319-1325, **1982**.

[221] Wihervaara, K., Production of nicotinic acid by baker's yeast growing in nitrogen-rich and nitrogen-poor media, *Journal of the Institute of Brewing*, 73, 167-171, **1967**.

[222] LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., Ventura, M., Bacteria as vitamin suppliers to their host: a gut microbiota perspective, *Current Opinion in Biotechnology*, 24, 160-168, **2013**.

[223] Combs Jr, G. F., McClung, J. P., Chapter 13 - Niacin. *The Vitamins (Fifth Edition)*, (eds: Combs Jr, G. F.), Academic Press, 331-350, **2017**.

[224] Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., Allegri, G., The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours, *Food Chemistry*, 100, 1350-1355, **2007**.

[225] Özcan, S., Şenyuva, H. Z., Improved and simplified liquid chromatography/atmospheric pressure chemical ionization mass

spectrometry method for the analysis of underivatized free amino acids in various foods, *Journal of Chromatography A*, 1135, 179-185, **2006**.

[226] Yılmaz, C., Gökmen, V., Comparative evaluation of the formations of gamma-aminobutyric acid and other bioactive amines during unhopped wort fermentation, *Journal of Food Processing and Preservation*, e13405-n/a,

[227] Yılmaz, C., Gökmen, V., Determination of Tryptophan Derivatives in Kynurenine Pathway in Fermented Foods Using Liquid Chromatography Tandem Mass Spectrometry, *Food Chemistry*, **2017**.

[228] Zwietering, M. H., Jongenburger, I., Rombouts, F. M., van 't Riet, K., Modeling of the Bacterial Growth Curve, *Applied and Environmental Microbiology*, 56, 1875-1881, **1990**.

CURRICULUM VITAE

Credentials

Name, Surname : Cemile YILMAZ

Place of Birth : Üsküdar, Turkey

Marital Status : Single

E-mail : cemileyilmaz@hacettepe.edu.tr

Address : Hacettepe University, Department of Food Engineering, 06800, Beytepe, Ankara, Turkey

Education

BSc. : Ankara University, Department of Food Engineering (2005-2010)

MSc. : Hacettepe University, Department of Food Engineering (2010-2013)

PhD. : Hacettepe University, Department of Food Engineering (2013-2017)

Foreign Languages

English (fluent), German (beginner)

Work Experience

Research Assistant, Hacettepe University, Department of Food Engineering, Ankara, Turkey (2011-Current)

Areas of Experiences

Food chemistry, food fermentation, biogenic amines, tryptophan derivatives

Projects and Budgets

-

Publications

(Publications within this PhD thesis are indicated with an asterisk.)

[1]* Yılmaz, C., Gökmen, V., Determination of Tryptophan Derivatives in Kynurenine Pathway in Fermented Foods Using Liquid Chromatography Tandem Mass Spectrometry, *Food Chemistry*, 243, 420-427, **2018**.

[2]* Yılmaz, C., Gökmen, V., Comparative evaluation of the formations of gamma-aminobutyric acid and other bioactive amines during unhopped wort fermentation, *Journal of Food Processing and Preservation,* doi: 10.1111/jfpp.13405, **2017**. (under press)

[3]* Yılmaz, C., Gökmen, V., Formation of tyramine in yoghurt during fermentation – Interaction between yoghurt starter bacteria and Lactobacillus plantarum, *Food Research International,* 97, 288-295, **2017**.

[4] Yılmaz, C., Kocadağlı, T., Gökmen, V., Formation of melatonin and its isomer during bread dough fermentation and effect of baking, *Journal of Agricultural and Food Chemistry,* 62, 2900-2905, **2014**.

[5] Kocadağlı, T., Yılmaz, C., Gökmen, V., Determination of melatonin and its isomer in foods by liquid chromatography tandem mass spectrometry, *Food Chemistry*, 153, 151-156, **2014**.

[6] Özdemir, K.S., Yılmaz, C., Durmaz, G., Gökmen, V., Hazelnut skin powder: A new brown colored functional ingredient, *Food Research International*, 65, 291-297, **2014**.

[7] Yılmaz, C., Gökmen, V., Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions, *Industrial Crops and Products*, 49, 130-135, **2013**.

Book Chapters

[1] Yilmaz C., Gökmen V., Chlorophyll. *The Encyclopedia of Food and Health,* (eds: Caballero, B., Finglas, P., Toldrá, F.), Academic Press, Oxford, 37-41, **2016**.

Oral and Poster Presentations

(Presentations within this PhD thesis are indicated with an asterisk.)

Oral Presentations

(The presenter author underlined.)

[1] Yılmaz, C., Gökmen, V., Sour Cherry Seed Kernel: A Valuable Source of Nutrients from a Wasted Stream of Juice Industry, *CEFood 2012: 6th Central European Congress on Food*, 23-26 May, Novi Sad, Serbia, **2012**.

Poster Presentations

(The presenter author underlined.)

[1] Yılmaz, C., Gökmen, V., Tryptophan derivatives in kynurenine pathway in cocoa and coffee: Effect of roasting and alkalization, *4th International Congress on Cocoa Coffee and Tea*, 25-28 June, Turin, Italy, **2017**.

[2] Kocadağlı, T., Yılmaz, C., Gökmen, V., Formation of melatonin and its isomer during bred dough fermentation and effect of baking, *7th International Conference and Exhibition on Nutraceuticals and Functional Foods*, 14-17 October, Istanbul, Turkey, **2014**.

[3] Özdemir, K.S., Yılmaz, C., Gökmen, V., Hazelnut Skin Powder: A New Brown Colored Functional Ingredient, *1st Congress on Food Structure Design*, 15-17 October, Porto, Portugal, **2014**.

[4] Özdemir, K.S., Yılmaz, C., Gökmen, V., Preparation of brown-coloured submicron-sized hazelnut skin fiber with high antioxidant capacity using high shear homogenization, *7th International Congress on Pigments in Foods*, 18-21 June, Novara, Italy, **2012**.

[5] Yılmaz, C., Gökmen, V., Effect of extraction conditions on the compositional characteristics of sour cherry seed kernel oil as fruit juice processing waste, *EuroFoodChem XVII*, 7-10 May, Istanbul, Turkey, **2013**.

[6] Yılmaz, C., Gökmen, V., Roasting Effect on Chemical Composition of Sour Cherry Kernel Oil, *Chemical Reactions in Foods VII*, 14-16 November, Prague, Czech Republic , **2012**.

HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
THESIS/DISSERTATION ORIGINALITY REPORT

HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
TO THE DEPARTMENT OF FOOD ENGINEERING

Date: 02/01/2018

Thesis Title / Topic: **INVESTIGATION OF CHANGES IN AMINO ACIDS DURING DIFFERENT FERMENTATION CONDITIONS**

According to the originality report obtained by myself/my thesis advisor by using the *Turnitin* plagiarism detection software and by applying the filtering options stated below on 02/01/2018 for the total of 114 pages including the a) Title Page, b) Introduction, c) Main Chapters, d) Conclusion sections of my thesis entitled as above, the similarity index of my thesis is 7 %.

Filtering options applied:

1. Bibliography/Works Cited excluded
2. Quotes excluded
3. Match size up to 5 words excluded

I declare that I have carefully read Hacettepe University Graduate School of Sciene and Engineering Guidelines for Obtaining and Using Thesis Originality Reports; that according to the maximum similarity index values specified in the Guidelines, my thesis does not include any form of plagiarism; that in any future detection of possible infringement of the regulations I accept all legal responsibility; and that all the information I have provided is correct to the best of my knowledge.

I respectfully submit this for approval.

02/01/2018

Name Surname:	Cemile Yılmaz
Student No:	N12249110
Department:	Department of Food Engineering
Program:	Food Engineering
Status:	☐ Masters ☒ Ph.D. ☐ Integrated Ph.D.

ADVISOR APPROVAL

APPROVED.

Prof. Dr. Vural Gökmen