**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL**

# POTENTIAL OF LACTIC ACID BACTERIA FERMENTATION AS A STRATEGY FOR VALORISATION AND BIOTRANSFORMATION OF MUSHROOMS

**Ph.D. THESIS**

**Eda Nur AYAR SÜMER**

**Department of Food Engineering**

**Food Engineering Programme**

**AUGUST 2024**

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL**

# POTENTIAL OF LACTIC ACID BACTERIA FERMENTATION AS A STRATEGY FOR VALORISATION AND BIOTRANSFORMATION OF MUSHROOMS

**Ph.D. THESIS**

**Eda Nur AYAR SÜMER**
**(506172502)**

**Department of Food Engineering**

**Food Engineering Programme**

**Thesis Advisor: Prof. Dr. Beraat ÖZÇELİK**

**AUGUST 2024**

**İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ**

# MANTARLARIN BİYOTRANSFORMASYONU VE ATIK MANTARLARIN DEĞERLENDİRİLMESİ AMACIYLA LAKTİK ASİT FERMANTASYONUNUN STRATEJİK KULLANIM POTANSİYELİ

**DOKTORA TEZİ**

**Eda Nur AYAR SÜMER**
**(506172502)**

**Gıda Mühendisliği Anabilim Dalı**

**Gıda Mühendisliği Programı**

**Tez Danışmanı: Prof. Dr. Beraat ÖZÇELİK**

**AĞUSTOS 2024**

Eda Nur AYAR SÜMER, a Ph.D. student of ITU Graduate School student ID 506172502, successfully defended the thesis entitled “POTENTIAL OF LACTIC ACID BACTERIA FERMENTATION AS A STRATEGY FOR VALORISATION AND BIOTRANSFORMATION OF MUSHROOMS”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

**Thesis Advisor :** **Prof. Dr. Beraat ÖZÇELİK** .............................
İstanbul Technical University

**Jury Members :** **Prof. Dr. Hatice Funda KARBANCIOĞLU GÜLER**...........
Istanbul Technical University

**Doç. Dr. Aysun YÜCETEPE** .............................
Aksaray University

**Dr. Öğr. Üyesi Evren DEMİRCAN** .............................
Istanbul Technical University

**Dr. Öğr. Üyesi Ümit ALTUNTAŞ** .............................
Gümüşhane University

**Date of Submission : 24 May 2024**
**Date of Defense : 15 Aug 2024**

***To my beloved family,***

# FOREWORD

I am deeply grateful to my supervisor, Prof. Dr. Beraat ÖZÇELIK, for her constant support, guidance and scientific passion that motivated me to complete my Ph.D. She has been a key figure in my academic life and career and will continue to be so for a lifetime. Apart from being a great scientist, she is also a unique and affectionate individual, and I am honoured to have been her student. I would also like to express my sincere gratitude to my supervisor at Gent University, Prof. Dr. Katleen RAES, a key figure in my Ph.D. Her trust and belief in me not only helped me during my Ph.D. study but also prepared me for my future academic journey. Her wisdom, generosity, support, mentorship, and inspiration were invaluable to me, and I am grateful for every moment I spent working with and learning from her. In addition, being a member of the VEG-i-TEC research group was a pleasant and productive experience. Without the contributions of these distinguished women and remarkable scientists, this study would not have been possible.

This thesis was financially supported by The Scientific and Technological Research Council of Turkey with the 2214-A-International Research Fellowship Programme for PhD students (application number 1059B142100530) and by the Erasmus+ Project. I acknowledge that I was supported by 100/2000 Council of Higher Education Doctoral Scholarship in Priority Areas and 2211-A Doctoral Scholarship Program, TÜBİTAK. During my stay at UGENT, all project expenses were covered by EFROFlanders, Hermes Fund, TUA WEST, Province West-Flanders and Ghent University for the OIP Voeding 1173 project. I am grateful for all the support I have received. More than just covering research expenses, feeling this support behind me has strengthened me for the challenges ahead and helped me to believe in myself.

I am grateful to my jury members, Prof. Dr. Funda KARBANCIOĞLU GÜLER and Assoc. Prof. Dr. Aysun YÜCETEPE, for their scientific contributions.

My sincere thanks go to Dr. Aysun YÜCETEPE, who involved me in her research group and broadened my perspective through our collaboration beyond my doctoral research. I would also like to thank Dr. Merve YAVUZ for including me in her own project and allowing me to collaborate. Participating in these collaborations during my doctoral education allowed me to work on various projects in addition to my own, providing me with diverse experiences.

My special thanks to the VEG-i-TEC research group. I would particularly like to thank Yannick and Yang, who worked closely with me on my research and supported me while writing our papers. I would like to express my gratitude to Ellen, Ioanna, Akram, Moustapha, Bashar, Caian, Kyriaki, Ben, Baptiste, Ngan, Tessa, Job, and Lucas. They have certainly made this journey easier and more enjoyable in the lab or in front of a computer while working or on Friday afternoons.

I would also like to thank Hilde DAMMAN for not letting me feel lonely and for making the house a home for me for a year. It was my good fortune to have lived with her.

I am grateful to Dr. Evren DEMIRCAN, Dr. Eda ŞENSU, Elif Feyza AYDAR, and Zehra MERTDINÇ from ITU for their scientific contributions and help in the laboratory when I asked for it.

I am grateful to Dr. Büşra GÜLTEKIN SUBAŞI and Dr. Reyhan KOYUNCU for their teamwork during our lessons and qualification exam period.

I always feel lucky to have you in my life Dr. Zeynep KOÇER, thank you for the all inspration and love you bring.

I am very grateful to friends Melisa TAN and Aysenur TEZEL for their support during the periods when I was overwhelmed with official paperwork about my grants. They were always there for me in any case, no matter what, to support me even in the most stressful moments.

I would also like to thank my friends Yeliz UÇAR and Sinem AYDOĞAN, who particularly supported me during my doctoral qualification exam period. Without you, I would have gone crazy.

I also want to thank the kindest grandmother one could ever have, Fatma İMAMOĞLU who left us during this Ph.D journey, continues to watch over me and lives on in my heart.

Last but not the least, I would like to thank my beloved family, who are my greatest supporters. Without them, I cannot even imagine being where I am today or accomplishing what I have achieved. I would like to express my boundless gratitude and thanks to my idols and my greatest fortune, whom I admire greatly, my mother, Meryem AYAR; my father, Osman AYAR, and my older siblings, Tuba AYAR and Ömer AYAR, whom I am most proud of and admire immensely. Siz olmasaydınız şu an olduğum yerde olamazdım, iyi ki varsınız. Hayatımı bu kadar güzel şekillendirdiğiniz için teşekkür ederim. Siz benim bu hayattaki en büyük şansımsınız, sizi çok seviyorum. Finally, a heartfelt note of appreciation and thanks to my spouse, Ömer Aykut SÜMER, for his encouragement, patience, and love throughout the challenges we have faced together. I am grateful for every day I spend with you and eagerly look forward to living a wonderful life together. Thank you for calming the storms in my mind with your embrace. Thank you for being my safe harbour. I love you.

I am thankful for my life, for being born into a wonderful family and for having the opportunity to create a beautiful family beyond my dreams. I am thankful to life for allowing me to achieve my dreams, ideals and even more. I am thankful to everyone who has nourished the excited, lively little girl still inside me.

May 2024

Eda Nur AYAR SÜMER
(Food Engineer M.Sc.)

## TABLE OF CONTENTS

**Page**

## ABBREVIATIONS

| | |
|---|---|
| **AA** | : Antioxidant Activity |
| **ABTS** | : 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) |
| **AJS** | : Agilent Jet Stream |
| **ANOVA** | : Analysis of variance |
| **BCCM/LMG** | : Belgian Coordinated Collections of Microorganisms / Laboratorium voor Microbiologie Gent (Laboratory of Microbiology Ghent) |
| **Caco-2** | : Human Colorectal Adenocarcinoma Cell Line |
| **CAGR** | : Compound Annual Growth Rate |
| **CFU** | : Colony Forming Unit |
| **CID** | : Collision-Induced Dissociation |
| **$CO_2$** | : Carbon dioxide |
| **CUPRAC** | : Cupric ion reducing antioxidant capacity |
| **Da** | : Dalton |
| **DAD** | : Diode Array Detector |
| **DM** | : Dry Matter |
| **DPPH** | : 2,2-diphenyl-1-picrylhydrazyl |
| **DTGS** | : Deuterated Triglycine Sulfate |
| **DW** | : Dry Weight |
| **ECD** | : Electron Capture Dissociation |
| **EDTA** | : Ethylenediaminetetraacetic acid |
| **EGCG** | : (−)-epigallocatechin-3-gallate |
| **ESI** | : Electrospray Ionization |
| **EtOH** | : Ethanol |
| **FA** | : Formic acid |
| **FAOSAT** | : Food and Agriculture Organization Corporate Statistical Database |
| **FC** | : Folin-Ciocalteau |
| **FDA** | : Food and Drug Administration |
| **FRAP** | : Ferric ion Reducing Antioxidant Power |
| **FTIR** | : Fourier Transform Infrared Spectroscopy |

**GAE** : Gallic Acid Equivalent
**GI** : Gastrointestinal digestion
**GRAS** : Generally Recognized as Safe
**h** : Hour
**HPLC** : High Performance Liquid Chromatography
**HSD** : Honestly Significant Difference
**IBS** : Irritable Bowel Syndrome
**ICP-AES** : Inductively Coupled Plasma Atomic Emission Spectrometer
**LAB** : Lactic Acid Bacteria
**LC** : Liquid Chromatography
**LER** : *Lentinula Edodes* Residue
**LOD** : Limit of Detection
**LOQ** : Limit of Quantitation
**MALDI** : Matrix-Assisted Laser Desorption/Ionization
**MALDI-TOF**: Matrix-Assisted Laser Desorption/Ionization - Time of Flight
**min** : Minute
**MRS** : De Man, Rogosa, and Sharpe
**MS** : Mass Spectrometry
**MS/MS** : Tandem Mass Spectrometry
**NMR** : Nuclear Magnetic Resonance
**OHC** : Oil Holding Capacity
**ORAC** : Oxygen Radical Absorbance Capacity
**PC1** : The First Principal Component
**PC2** : The Second Principal Component
**PCA** : Principal Component Analysis
**PCDL** : Personal Compound Database and Library
**Ppm** : Parts Per Million
**QTOF** : Backpropagation
**ROS** : Reactive Oxygen Species
**RT** : Retantion Time
**SD** : Standard Devioation
**SCFA** : Short-Chain Fatty Acid
**SDS** : Sodium Dodecyl Sulfate
**SDS-PAGE** : Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
**SGF** : Simulated gastric fluid

**SHIME** **:** Simulator of the Human Intestinal Microbial Ecosystem ,
**SIF** **:** Simulated intestinal fluid
**SIMGISI** **:** Simulator of the Gastro-Intestinal tract
**SMS** **:** Spent Mushroom Substrate
**TE** **:** Trolox® Equivalent
**TEAC** **:** Trolox® Equivalent Antioxidant Capacity
**TIM-2** **:** TNO Gastro-Intestinal Model-2
**TNBS** **:** 2,4,6-trinitrobenzene sulfonic acid
**TNO** **:** Netherlands Organisation for Applied Scientific Research
**TPC** **:** Total Phenolic Content
**Trolox** **:** 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
**UPLC** **:** Ultra High Performance Liquid Chromatography
**UPLC** **:** Ultra Performance Liquid Chromatography
**UV** **:** Ultraviyole
**WHC** **:** Water Holding Capacity

## SYMBOLS

**°C** : degree Celsius
**~** : around
**B** : magnetic flux density
***g*** : gravitational force equivalent
**Hz** : hertz
**J** : joule
**m** : milli ($10^{-3}$)
**n** : nano ($10^{-9}$)
***n*** : number (statistical analysis)
***p*** : probability (statistical analysis)
**pH** : power of hydrogen
**pI** : isoelectric point
**R**$^2$ : correlation coefficient (statistical analysis)
**T** : temperature
**t** : time
**V** : volt
**W** : watt
**w** : weight
**α** : alpha (in α-helix)
**β** : beta (in β-sheet)
**γ** : gamma (in γ-peptides)
**ζ** : zeta (in zeta potential)
**µ** : micro ($10^{-6}$)
**Σ** : sum
**ρ** : density
**λ** : wavelength for spectroscopic analysis (nm)

## LIST OF TABLES

## LIST OF FIGURES

**Page**

# POTENTIAL OF LACTIC ACID BACTERIA FERMENTATION AS A STRATEGY FOR VALORISATION AND BIOTRANSFORMATION OF MUSHROOMS

## SUMMARY

Consumers increasingly recognise the importance of healthy eating and the potential benefits of incorporating mushrooms into their diets. Known for their rich nutritional profile, mushrooms provide essential vitamins, minerals, fibre, and antioxidants, making them valuable plant-based food sources. They are also rich in bioactive compounds with anti-inflammatory, antioxidant, antitumor, antiviral, and antimicrobial properties, promoting health and reducing disease risks in humans. Among various bioactive components in mushrooms, phenolic compounds are particularly noteworthy. These compounds are considered significant secondary metabolites in mushrooms and are found in free and bound forms within food matrices. However, the bioaccessibility and bioavailability of bound phenolic compounds are lower than that of free phenolic compounds due to their covalent bonds to cell wall matrices, which prevent absorption in the small intestine. Additionally, mushroom production generates various by-products, which pose environmental and financial challenges due to their disposal.

Innovative processing techniques are required to enhance the bioavailability of phenolic compounds and add value to mushroom by-products. Fermentation with lactic acid bacteria (LAB) is effective in this context. LAB fermentation not only extends the shelf life and improves sensory properties but also breaks down macronutrients such as carbohydrates and proteins. This alters the nutritional composition of the food and facilitates the transformation of bound phenolic compounds into more bioavailable forms, similar to free phenolic compounds. Through LAB fermentation, macronutrients are transformed, antioxidative peptides are released, and phenolic compounds are modified. This process enhances the health benefits of mushrooms by increasing the bioavailability of mushroom phenolics, making them more accessible for absorption and use by the body. Therefore, fermentation techniques can significantly improve mushrooms' use, health benefits, and by-products.

Given this information, a research framework for this doctoral thesis explores modifying industrial mushroom wastes and specific extracted components, as well as mushrooms like *L. edodes* and *La. deliciosus*, which may become waste due to their short shelf life. The research plan is based on processing mushroom waste and mushrooms through LAB fermentation. The objectives of this doctoral thesis are: (i) to valorise the mushroom waste generated from bioactive substance extraction, modifying its structure and nutritional composition through fermentation with lactic acid bacteria; (ii) to ferment the mushrooms with lactic acid bacteria to facilitate the transition of phenolic compounds from bound to free form, altering the structure of *L. edodes* and *La. deliciosus*; (iii) to determine biotransformation of these phenolic

compounds use analytical identification; (iv) to investigate the effects of LAB fermentation on the bioaccessibility and intestinal transport of mushroom phenolics using an in vitro gastrointestinal digestion model.

To achieve these objectives, three different experimental studies (*Chapters 3-5*) were conducted within the scope of this thesis. The first study focused on the fermentation of *L. edodes* mushroom waste, *L. edodes* residue (LER), with lactic acid bacteria compared to *L. edodes* itself (LE), examining changes in its structure and nutritional composition for functional properties (*Chapter 3*). The second study was based on the changes in phenolic components, interactions with other metabolites, and the profiling of phenolic substances in fermented *L. edodes* and *La. deliciosus* (*Chapter 4*). Based on the previous chapter's findings, the third study examined phenolic components' bioaccessibility and antioxidant activity trends in an in vitro gastrointestinal digestion model. These studies included a comparison with unfermented mushrooms to assess the impact of fermentation process steps, presenting the bioaccessibility and metabolite differences of phenolic components in both fermented and unfermented mushrooms in the in vitro gastrointestinal digestion model (*Chapter 5*).

In *Chapter 1*, the scope and objectives of the research were defined. In *Chapter 2*, a detailed literature review on the functional properties and effects of mushrooms and fermentation on bioactive substances was presented. The study primarily focused on the variable compositions of mushrooms and mushroom by-products, as well as the technologies used to extract and modify bioactive components. Additionally, the bioactive components and extracts of *L. edodes* and *La. deliciosus* used in this thesis were thoroughly examined for their bioactive mechanisms. The structures of phenolic compounds, the differences between their free and bound forms, the extraction methods for bound forms, and the identification of phenolic substances through tandem mass spectrometry were explained. Finally, the effects of lactic acid bacteria fermentation on phenolic substances in foods and the usage and impacts of lactic acid fermentation in mushrooms were discussed. These sections outlined the main framework of the thesis and highlighted the connections between topics.

In *Chapter 3*, the structural, nutritional, antioxidant activities, and some technofunctional properties of *L. edodes* mushroom by-products fermented with lactic acid bacteria were investigated. Initially, water-soluble polysaccharides were extracted from the mushrooms, after which the remaining by-products (LER) and mushrooms (LE) were fermented with *Lactiplantibacillus plantarum*. Samples were collected every 24 hours, and fermentation was continued for a total of 72 hours. Changes in *L. edodes* natural components, such as proteins, polysaccharides, and phenolic substances, were studied during this process. It was observed that free sugars decreased and organic acids increased during fermentation, supported by a pH decrease. The degradation of proteins during fermentation led to increased free amino groups and the conversion of high molecular weight proteins to lower molecular weights, as confirmed by SDS-PAGE. This degradation affected technofunctional properties such as oil and water-holding capacities. On the other hand, in LER, the breakdown of peptide bonds during fermentation led to the release of smaller peptides and free amino acids with antioxidant properties, increasing free amino groups. Both fractions showed an increase in total phenolic content (TPC) during the first 24 hours of fermentation but no significant changes after that ($p > 0.05$). These changes in phenolic substances and proteins enhanced the antioxidant activity of both LE and LER, particularly after 24 hours of fermentation. This study has demonstrated for the first time how the valorisation of mushroom by-products through lactic acid fermentation can improve specific functional and structural properties.

In *Chapter 4*, the fermentation of *L. edodes* and *La. deliciosus* mushrooms with *Lb. plantarum* for 72 hours and the effects of this process on bound and free phenolic compounds were examined. The TPC, phenolic profile, and antioxidant activities of the free and bound phenolic fractions (FPs and BPs, respectively) are evaluated. In both types of mushrooms, the TPC of BPs was higher than that of FPs and showed high DPPH and ABTS radical scavenging capabilities. The fermentation by *Lb. plantarum* significantly increased the TPC in the FPs of *L. edodes* and *La. deliciosus*, particularly after 24 hours (%28.48 and %27.29, respectively). During fermentation, the TPC of BPs in mushrooms decreased, indicating their release from the matrix. Additionally, 30 bioactive compounds were tentatively identified using UPLC-Q-TOF-MS/MS, considering molecule formulas, errors in ppm (<10 ppm), and MS/MS fragment ions. The principal component analysis score and volcano plots from the UPLC-Q-TOF-MS analysis showed apparent differences between fermented mushroom samples during fermentation.

In *Chapter 5*, the bioaccessibility and metabolite differences of high-bound phenolic content in the in vitro gastrointestinal digestion model were investigated. In this context, the effects of lactic acid fermentation and in vitro digestion of bioactive compounds in *L. edodes* and *La. deliciosus* were evaluated, with *Lb. plantarum* used for the fermentation process. *Lb. plantarum* fermentation significantly increased the mushrooms' total phenolic content and improved phenolics' bioaccessibility during in vitro digestion. Although fermentation did not considerably affect ABTS radical scavenging activity, it increased DPPH radical scavenging capacity; this indicates how fermentation can affect various aspects of antioxidant capacity. Additionally, fermentation, mainly through protein metabolism, improved the peptide profiles of mushrooms before and after in vitro digestion. This facilitated the transformation of proteins into smaller, more bioactive peptides, enhancing both digestibility and potential health benefits. Comparative analyses using UPLC-Q-TOF-MS identified significant component differences between fermented and unfermented mushroom metabolites, visualised through principal component analysis score plots and volcano plots.

Finally, in *Chapter 6*, based on the data obtained from previous chapters, a general discussion on the effects of fermentation with lactic acid bacteria on phenolic substances and other components, their antioxidant properties, and bioaccessibility was provided, along with conclusions and recommendations for future research. The results obtained from this thesis discussed the revaluation of mushroom by-products/wastes, the biotransformation of phenolic compounds through fermentation, and the effects of fermentation on the bioaccessibility of mushroom phenolics during in vitro digestion. Compared to other non-thermal food processing methods, the positive and negative aspects of fermentation with lactic acid bacteria were examined. Suggestions for improving the study and various recommendations for future research were also discussed.

# MANTARLARIN BİYOTRANSFORMASYONU VE ATIK MANTARLARIN DEĞERLENDİRİLMESİ AMACIYLA LAKTİK ASİT FERMANTASYONUNUN STRATEJİK KULLANIM POTANSİYELİ

## ÖZET

Sağlıklı beslenmenin öneminin giderek daha fazla fark edilmeye başlamasının yanı sıra insanlar potansiyel sağlık faydaları sebebiyle mantarları diyetlerine eklemektedirler. Zengin besin profili ile bilinen mantarlar, gerekli vitaminler, mineraller, lif ve antioksidanlar sağlayan değerli bitki bazlı gıda kaynaklarıdır. Ayrıca, anti-enflamatuar, antioksidan, antitümör, antiviral ve antimikrobiyal özellikler içeren biyoaktif bileşikler açısından zengindirler, bu bileşikler sağlığı teşvik eder ve insan vücudunda hastalık riskini azaltır. Mantarların çeşitli biyoaktif bileşenleri arasında, özellikle fenolik bileşikler mantarlarda önemli ikincil metabolitler olarak kabul edilir ve gıda matrislerinde hem serbest hem de bağlı formlarda bulunurlar. Ancak, bağlı fenolik bileşikler hücre duvarı matrisleriyle kovalent bağlarla bağlandığından, ince bağırsakta emilemezler ve serbest fenolik bileşiklere kıyasla daha düşük biyoyararlanıma sahiptirler. Mantar üretimi sırasında çeşitli yan ürünler ve atıklar oluşur, bunların yönetimi hem çevresel hem de mali zorluklar yaratır.

Fenolik bileşiklerin biyoyararlanımını artırmak, mantar yan ürünlerine ve atıklarlarına değer katmak için yenilikçi işleme teknikleri uygulanabilmektedir. Bu bağlamda, laktik asit bakterileri (LAB) ile fermentasyon, etkili bir yöntem olarak öne çıkar. LAB fermentasyonu sadece raf ömrünü uzatıp duyusal özellikleri iyileştirmekle kalmaz, aynı zamanda karbonhidratlar ve proteinler gibi makro besin öğelerini parçalar. Bu, gıdanın besin kompozisyonunu değiştirir ve bağlı fenolik bileşiklerin, serbest fenolikler gibi daha biyoyararlanabilir formlara dönüşümünü sağlar. LAB fermentasyonu ile makro besin öğeleri dönüştürülür, antioksidatif peptitler salınır ve fenolik bileşikler modifiye edilir. Bu süreç, mantarların sağlık faydalarını artırarak, mantar fenoliklerinin biyoyararlanımını artırır ve böylece vücut tarafından emilim ve kullanım için daha erişilebilir hale getirir.

Verilen bu bilgilerin ışığında, bu doktora tezinin temel amacı, endüstriyel mantar atıklarının ve belirli bileşenlerin ekstraksiyonu sonrası oluşan mantar atıklarının yanı sıra, kısa raf ömrü nedeniyle potansiyel olarak atık haline gelebilecek *Lentinula edodes* ve *Lactarius deliciosus* mantarlarının modifikasyonunu incelemektir. Araştırma planı, laktik asit fermentasyonu kullanarak hem mantar atıklarının hem de mantarların modifikasyonu üzerine kurulmuştur. Bu doktora tezi hedefleri (i) biyoaktif madde ekstraksiyonu sonucu oluşan mantar atığının karakterizasyonu, yapısının ve besin değeri/kompozisyonunun değiştirilmesi amacıyla laktik asit bakterileri ile fermente edilmesi; (ii) fermente edilen mantarlardan fenolik bileşiklerin *L. edodes* ve *La. deliciosus* mantarlarının yapısını değiştirerek, bağlı formdaki fenolik bileşenlerin serbest forma geçişini sağlamak amacıyla laktik asit bakterileri ile fermentasyonu; (iii) bu fenolik bileşenlerin biyotransformasyonunu inceleyebilmek için tanımlama analizlerinin uygulanması; (iv) laktik asit fermentasyonunun mantar fenoliklerinin

biyoerişilebilirliği ve bağırsak taşınımı üzerindeki etkilerini in vitro mide-bağırsak sindirim modelini kullanarak araştırmaktır.

Bu tez kapsamında belirlenen hedeflere ulaşmak üzere üç farklı deneysel çalışma (*Bölüm 3-5*) yürütülmüştür. İlk çalışma, suda çözünebilen polisakkaritlerin ekstraksiyonu sonucu oluşan *L. edodes* mantar atığının *L. edodes* ile kıyaslanarak laktik asit bakterileri ile fermentasyonu üzerine odaklanmıştır. Bu aşamada, mantar atığının değisen yapısı ve besin kompozisyonu fonksiyonel özellikleri incelenmiştir (*Bölüm 3*). İkinci çalışma, fermente edilen *L.edodes* ve *La. deliciosus* mantarlarının fenolik bileşenlerindeki değişimler, diğer metabolitlerle olan etkileşimleri ve fenolik madde profillerinin tanımlanması üzerine kurulmuştur (*Bölüm 4*). Üçüncü çalışmada ise, bir önceki bölümden elde edilen bulgular ışığında, fenolik bileşenlerin in vitro mide-bağırsak sindirim modelindeki biyoerişilebilirliği ve antioksidan aktivite trendleri incelenmiştir. Bu çalışmalar, fermente edilmeyen mantarların da dahil edildiği bir kıyaslama ile fermentasyon işlem basamaklarının etkisini değerlendirerek, fermente edilen ve edilmeyen mantarların fenolik bileşenlerinin in vitro mide-bağırsak sindirim modelindeki biyoerişilebilirliği ve metabolit farklılıkları ortaya konulmuştur (*Bölüm 5*).

Bu doktora tezinin *birinci bölümünde*, araştırmanın kapsamı ve hedefleri tanımlanmıştır. *İkinci bölümde* ise, mantarlar ve fermentasyonun fonksiyonel özellikler ile biyoaktif maddeler üzerine etkilerine dair detaylı bir literatür derlemesi sunulmuştur. Araştırma, öncelikle mantarların ve mantar atıklarının değişken kompozisyonlarına odaklanmış, bioaktif bileşenlerin ekstraksiyonu ve modifikasyonu için kullanılan teknolojiler incelenmiştir. Ayrıca, bu tezde kullanılan *L. edodes* ve *La. deliciosus* mantarlarının biyoaktif bileşenlerinin ve ekstrelerinin biyoaktif mekanizmaları detaylı bir şekilde irdelenmiştir. Fenolik bileşenlerin yapıları, serbest veya bağlı formdaki farklılıkları, bağlı formdakilerin ekstraksiyon yöntemleri ve fenolik maddelerin tandem kütle spektrometrisi ile tanımlanması açıklanmıştır. Son olarak, gıdalarda laktik asit bakteri fermentasyonunun fenolik maddeler üzerine etkisi ve laktik asit fermentasyonunun mantarlarda kullanımı ve etkileri ele alınmıştır. Bu bölümler, tezin ana hatlarını oluşturmakta ve konular arasındaki bağlantıları vurgulamaktadır.

*Üçüncü bölümde, L. edodes* mantar atıklarının laktik asit bakterileri ile fermente edilmesi sonucunda yapısal, besinsel, antioksidan aktiviteleri ve bazı teknofonksiyonel özelliklerindeki değişiklikler incelenmiştir. Başlangıçta, mantarlardan suda çözünebilen polisakkaritler ekstrakte edilmiş, ardından kalan atıklar (LER) ve mantarlar (LE) *Lactiplantibacillus plantarum* ile fermente edilmiştir. Her 24 saatte bir örnekler alınmış ve toplam 72 saat süresince fermantasyon devam ettirilmiştir. Bu süreçte, *L.edodes*'in doğal bileşenleri olan proteinler, polisakkaritler ve fenolik maddelerde meydana gelen değişiklikler araştırılmıştır. Fermantasyon sırasında, serbest şekerlerin azaldığı ve organik asitlerin arttığı gözlemlenmiş, bu durum pH'taki düşüşle desteklenmiştir. Fermantasyon sırasında proteinlerin degredasyonu, serbest amino gruplarında artışa ve yüksek molekül ağırlıklı proteinlerin daha düşük molekül ağırlıklarına dönüşümüne neden olmuştur, bu değişimler SDS-PAGE ile doğrulanmıştır. Bu parçalanma, yağ ve su tutma kapasiteleri gibi teknofonksiyonel özellikleri etkilemiştir. Öte yandan, LER'de fermantasyon boyunca peptit bağlarının kırılması sonucu antioksidan özelliklere sahip daha küçük peptitlerin ve serbest amino asitlerin salınımıyla serbest amino gruplarında artış gözlemlenmiştir. Her iki fraksiyon da fermantasyonun ilk 24 saatinde toplam fenolik madde miktari (TPC) artışı göstermiş, ancak sonrasında önemli bir değişiklik olmamıştır ($p > 0.05$). Fenolik maddeler ve proteinlerdeki bu değişimler, hem LE hem de LER'in antioksidan aktivitesini, özellikle 24 saatlik fermantasyon sonrasında artırmıştır. Bu çalışma, mantar atıklarının laktik asit fermantasyonu ile değerlendirilmesinin, bazı fonksiyonel ve yapısal özellikleri nasıl iyileştirdiğini ilk kez göstermiştir.

Dördüncü bölümde, *L. edodes* ve *La. deliciosus* mantarlarının 72 saat boyunca *Lb. plantarum* ile fermentasyonu ve bu işlemin bağlı ve serbest fenolik bileşikler üzerindeki etkileri incelenmiştir. Serbest ve bağlı fenolik fraksiyonlar (sırasıyla FPs ve BPs) açısından TPC, fenolik profil ve antioksidan aktiviteler değerlendirilmiştir. Her iki mantar türünde de, bağlı fenoliklerin toplam fenolik içeriği, serbest fenoliklere göre daha yüksek bulunmuş ve yüksek DPPH ve ABTS radikal süpürme kapasiteleri göstermiştir. *Lb. plantarum*'un fermentasyonu, özellikle 24 saat sonunda *L. edodes* ve *La. deliciosus*'taki serbest fenoliklerin toplam fenolik içeriğini önemli ölçüde artırmıştır (%28.48 ve %27.29). Fermentasyon sırasında mantarlardaki bağlı fenoliklerin toplam fenolik mikrarı azalmış, bu da fenoliklerin mantar matrisinden serbest bırakıldığını göstermiştir. Ayrıca, UPLC-Q-TOF-MS/MS kullanılarak 30 biyoaktif bileşen molekül formülleri ile tanımlanmış, ppm düzeyindeki hatalar (<10 ppm) ve MS/MS fragment iyonları dikkate alınarak incelenmiştir. UPLC-Q-TOF-MS/MS analizinden elde edilen başlıca bileşen analizi skor grafikleri ve volkan grafikleri, fermente mantar örnekleri arasında belirgin farklar olduğunu ortaya koymuştur. Çalışmamız, yüksek fenolik içeriğe sahip mantarların laktik asit fermentasyonu sonucunda, bağlı fenoliklerin serbest bırakılmasının biyoaktivitelerini ve biyoerişilebilirliklerini nasıl artırdığını ilk kez göstermiştir.

Beşinci bölümde, yüksek bağlı fenolik madde içeriğinin in vitro mide-bağırsak sindirim modelindeki biyoerişilebilirliği ve metabolit farklılıkları incelenmiştir. Bu kapsamda, *L. edodes* ve *La. deliciosus*'taki biyoaktif bileşikler üzerinde laktik asit fermentasyonunun ve in vitro sindiriminin etkileri değerlendirilmiş, *Lb. plantarum* bu süreçte ferementasyon için kullanılmıştır. *Lb. plantarum* fermentasyonu, mantarların toplam fenolik içeriğinde önemli bir artış sağlamış ve fenoliklerin in vitro sindirim sırasında biyoerişilebilirliğini iyileştirmiştir. Fermentasyon, ABTS radikal süpürme aktivitesi üzerinde belirgin bir etki göstermese de, DPPH radikal süpürme kapasitesini artırmıştır; bu da fermentasyonun antioksidan kapasitenin çeşitli yönlerini nasıl etkileyebileceğini göstermektedir. Ayrıca, fermentasyon, özellikle protein metabolizması sayesinde, in vitro sindirim öncesi ve sonrasında mantarların peptid profillerini iyileştirmiştir. Bu durum, proteinlerin daha küçük ve daha biyoaktif peptitlere dönüşümünü sağlayarak, hem sindirilebilirliği hem de potansiyel sağlık faydalarını artırmıştır. UPLC-Q-TOF-MS analizleriyle yapılan karşılaştırmalarda, fermente edilen ve edilmeyen mantar metabolitleri arasında belirgin bileşen farklılıkları tespit edilmiş, bu farklar başlıca bileşen analizi skor grafikleri ve volkan grafikleri ile görselleştirilmiştir.

Son olarak, altıncı bölümde, önceki bölümlerde elde edilen veriler ışığında laktik asit bakterileri ile fermentasyonun fenolik maddeler ve diğer bileşenler üzerindeki etkileri, antioksidan aktivite özellikleri ve biyoerişilebilirlik üzerine genel bir tartışma yapılmıştır ve gelecekteki araştırmalar için önerilerde bulunulmuştur. Bu tezden elde edilen sonuçlar, mantar yan ürünleri/atıklarının yeniden değerlendirilmesi, fermentasyonla fenolik bileşenlerin biyotransformasyonu ve fermentasyonun mantar fenoliklerinin in vitro sindirim sırasında biyoerişilebilirliği üzerine etkilerini tartışmıştır.

# 1. INTRODUCTION

The quote by Hippocrates, "Let food be thy medicine and medicine be thy food," highlights the importance of functional foods in maintaining a healthy diet. With an increased global awareness of the connection between diet and health, there is a growing effort to consume nutritious, functional foods to promote wellness and prevent diseases. In this regard, edible mushrooms have been a vital component of human culture since ancient times and are valued globally for their nutritional and therapeutic properties (Kour et al., 2022). Furthermore, although not yet fully explored, mushrooms have recently been recognised as the new superfood essential in enhancing health and well-being within the human diet (Thakur, 2020). The mushrooms are known for their various health benefits, such as antioxidant, anti-inflammatory, antiviral, antibacterial, antidiabetic, anticancer, antiaging and antiallergenic activities and properties (Thakur, 2020). These benefits are attributed to a wide range of bioactive components found in mushrooms, such as proteins, polysaccharides, terpenes, and lipids, and secondary metabolites, such as phenolic compounds, have gained significant interest for their potential therapeutic properties (M. Wang & Zhao, 2023).

However, the effectiveness of these compounds, mainly phenolic compounds, can be limited because of their natural structure, interaction with the food matrix and digestion phase (Kashyap et al., 2022). Furthermore, the phenolic compounds may be lost during the post-harvest handling, processing, and storage of food products (Krahe et al., 2021). These processes and the short shelf life of food products also cause the production of food by-products (Jafarzadeh et al., 2021). In this sense, nonthermal, economical, traditional, green food processing technology such as fermentation has been getting attention and being used as a strategy. Fermentation involves the metabolic processing of organic substrates by microorganisms, such as lactic acid bacteria, to achieve beneficial biochemical changes. Lactic acid fermentation not only extends the shelf life of food through the production of inhibitory metabolites and the elimination of pathogens but also enhances the nutritional and sensory qualities of fermented foods, promoting human health (Mandha et al., 2021).

The global mushroom market is expanding, with demand expected to rise from 15.25 million tonnes in 2021 to 24.05 million tonnes in 2028, representing a compound annual growth rate (CAGR) of 6.74% during the forecast period. Consequently, the economic potential of mushrooms in the future appears to be significant (Naher et al., 2024). However, the rapid growth of the mushroom industry has led to the production of approximately 60 million tons of mushroom waste annually (Guo et al., 2022). Additionally, mushrooms are highly perishable and are at risk of being wasted before consumption (Castellanos-Reyes et al., 2021). It is, therefore, crucial to explore the processing technologies of mushroom by-products to assess the feasibility of their utilisation.

## 1.1 Purpose of Thesis

Considering the above, in this PhD thesis, several novel perspectives are offered to bridge existing knowledge of phenolic compound modification of mushrooms and the resultant health impacts. *Chapter 2* provides an introductory overview of mushrooms and mushroom by-products, phenolic compounds, structure, interactions, and bioaccessibility, all within a comprehensive literature review. This chapter also delves into the latest findings on fermented mushrooms, mushroom phenolics, and the associated health effects of mushrooms. The subsequent chapters (3, 4, and 5) present the fresh insights from doctoral research.

*Chapter 3* introduces new data regarding the potential of mushroom by-products for the fermentation of lactic acid bacteria, observing structural changes and phenolic-protein interactions in *L. edodes.*

*Chapter 4* introduces comparative research on the impact of lactic acid bacteria fermentation on *L. edodes* and *La. deliciosus* phenolic compounds. The identification and characterisation of phenolic compounds and the trend on the bound phenolic compounds.

*Chapter 5* introduces new evidence on the effects of lactic acid bacteria fermentation on phenolic bioaccessibility in mushrooms and mushroom-phenolic interactions during in vitro digestion.

The concluding chapter, *Chapter 6*, synthesises the overall findings with recommendations. Figure 1.1 graphically presents the structure of this thesis.

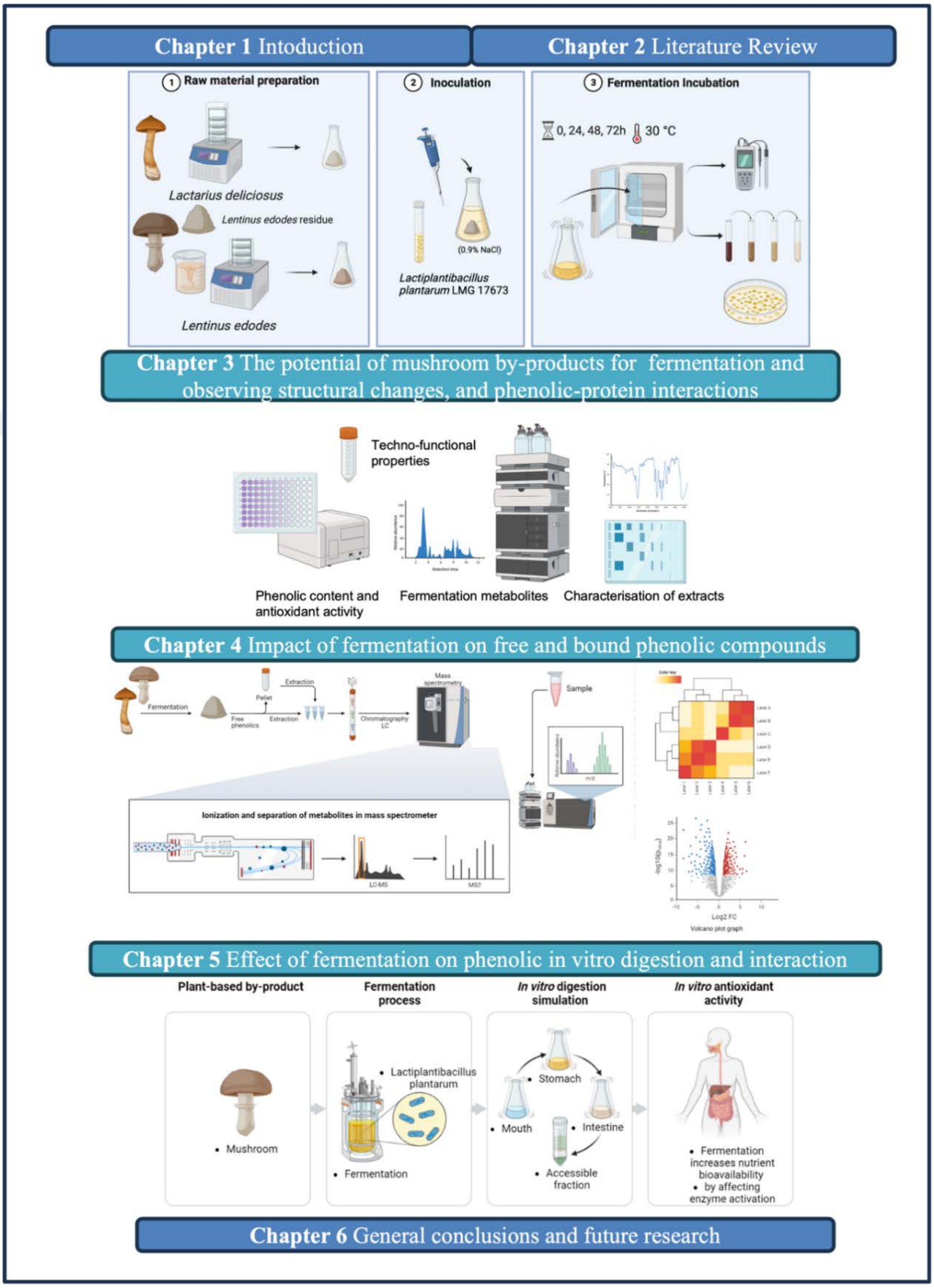


**Figure 1.1:** Schematic overview of the Ph.D. thesis.

# 2. LITERATURE REVIEW

## 2.1 Mushrooms and Mushrooms By-Products Bioactive Compounds

### 2.1.1 Mushrooms

Mushrooms have been an important part of the human diet and medicine for centuries, providing nutritional and therapeutic benefits. Scientific studies have confirmed that mushrooms produce compounds with therapeutic properties, which have been utilised in traditional and modern medical practices (Assemie & Abaya, 2022). Within the macrofungi classification, *Basidiomycetes encompasses* species that proliferate either hypogeous, beneath the earth, or epigeous, upon its surface. Globally revered as a sophisticated culinary element, mushrooms adorn the gastronomic traditions of virtually every culture, highlighting their universal appeal. Historically, the therapeutic benefits of mushrooms have been documented, notably in ancient Chinese medicine, where their use spans thousands of years. However, it is only in contemporary times that their health-promoting attributes have garnered widespread acknowledgement. Rich in essential amino acids, minerals, proteins, and bioactive polysaccharides, mushrooms offer significant contributions to dietary health and wellness (Chun et al., 2021).

Traditionally, medicinal mushrooms were harvested from forests and used in various forms, including hot water extracts and powders, to exploit their medical effects. Different parts of the mushroom, such as the stem, cap, spores, and mycelium, have been leveraged for their health benefits (Chang & Buswell, 1999). Despite approximately 1.5 million fungal species, only about 10% are currently known, with 5,000 of these being edible (Assemie & Abaya, 2022; Suwannarach et al., 2022).

Mushrooms synthesize and accumulate many nutrients and secondary metabolites, like vitamins, minerals, organic acids, alkaloids, phenols, and terpenoids. Mushrooms are also a rich source of new antimicrobial compound constituents, such as terpenes, steroids, anthraquinones, quinolones, benzoic acid derivatives, and oxalic acid, together with peptides and proteins (primary metabolites) (Bains et al., 2021). They are, therefore, of nutritional importance, containing significant amounts of vitamins B1, B2, B12, C, D, E, tannins, and other biologically active substances, serving as an important reserve and sources of different nutraceuticals providing synergistic health defence (Chun et al., 2021). With the sample of *L.edodes*, the main active mushroom components are present in Figure 2.1 (Roszczyk et al., 2022).

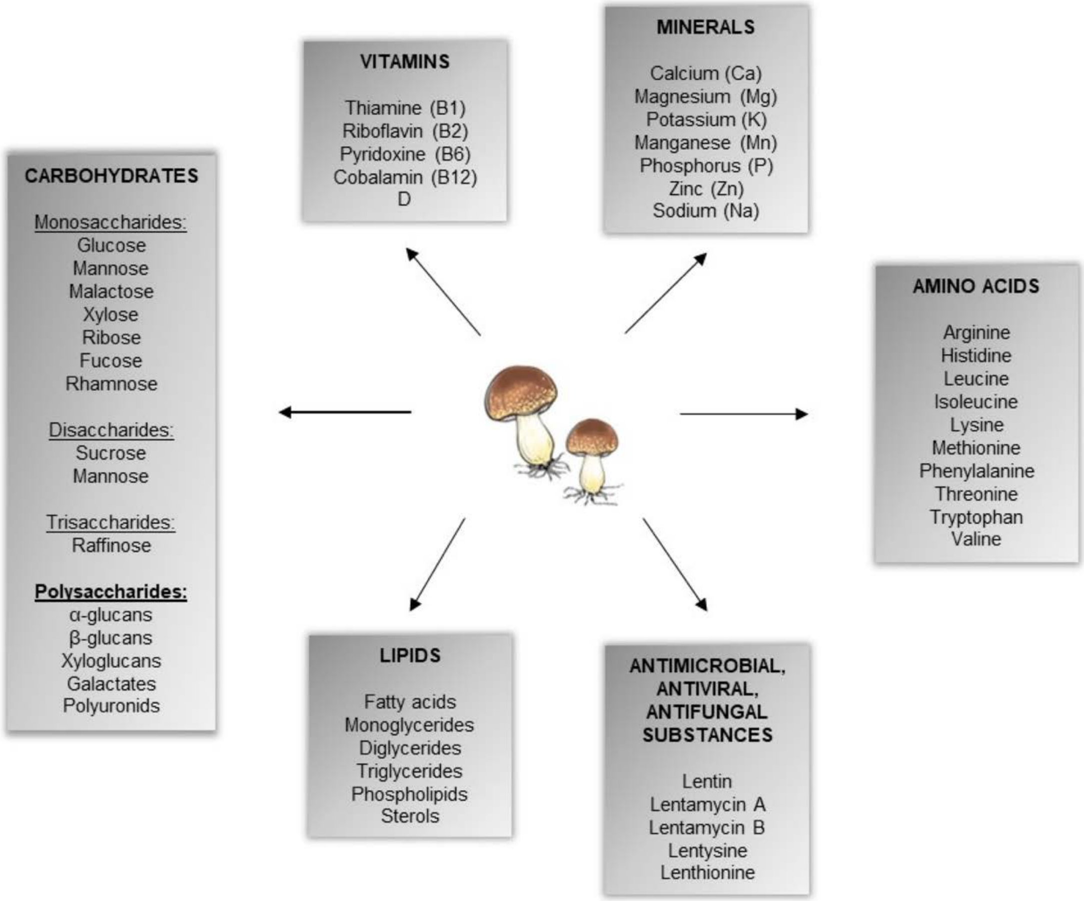


**Figure 2.1 :** Representation of active compounds of *Lentinula edodes* (Roszczyk et al., 2022).

The pharmaceutical value of mushrooms has increased so much that they can now be regarded as genuine mini-pharmaceutical factories. The level and amount of those biologically active substances within mushrooms do differ, and indeed, it may vary considerably due to many factors such as strain variation, differences in the substrate used, cultivation method differences, stage of development, age, conditions of storage, and culinary processing (Bains et al., 2021). Mushrooms are, therefore, a good source of the aforementioned diverse bioactive substances (Chun et al., 2021).

### 2.1.2 Mushroom and mushroom by-product production

Mushroom production is increasing worldwide, with China being one of the top producers of this crop, as shown in Figure 2.2. In addition, Turkey produced 56 thousand tons mushrom, 86% of which were *Agaricus bisporus* (TÜİK, 2024). Market research suggests that the global mushroom cultivation industry is projected to grow by 57% by 2028, reaching an estimated 24 million tons (FAOSTAT, 2016). This growth indicates a growing demand for mushrooms, but it also highlights various environmental challenges. The perishable nature of mushrooms is one of the major issues, which necessitates their consumption or processing quickly to prevent spoilage. These challenges underscore the complexity of the industry's expansion.

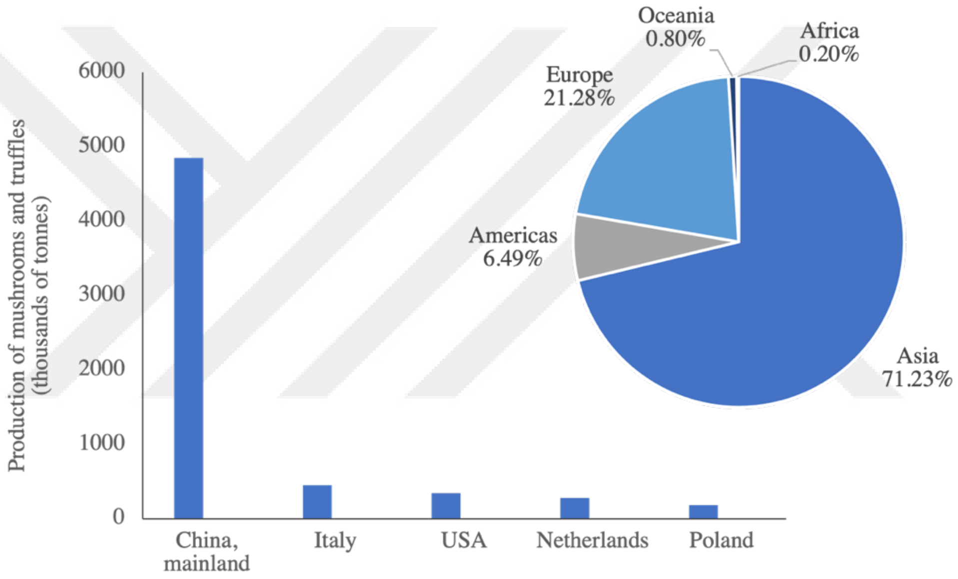


**Figure 2.2 :** Worldwide production of mushrooms and truffles, from 2004 to 2014 (FAOSTAT, 2016).

Unfortunately, the rapid growth of the edible mushroom industry also leads to the production of approximately 60 million tons of mushroom waste annually. The commercial mushroom production process consists of three primary steps: cultivation, trimming, and processing, which generate significant by-products categorized into three types. The first and most abundant category, generated during the cultivation phase, is known as spent mushroom substrate (SMS) or mushroom compost. Research indicates that about 5 kg of spent mushroom substrate by-products are generated per kilogram of fresh mushroom produced (Leong et al., 2022). The second category includes misshapen mushrooms and stems that, although edible, do not meet commercial standards. The third category comprises residues left after extracting specific substances from mushrooms. As shown in Figure 2.3 (Roszczyk et al., 2022)., mushrooms contain several active compounds, making their residues valuable for further research.

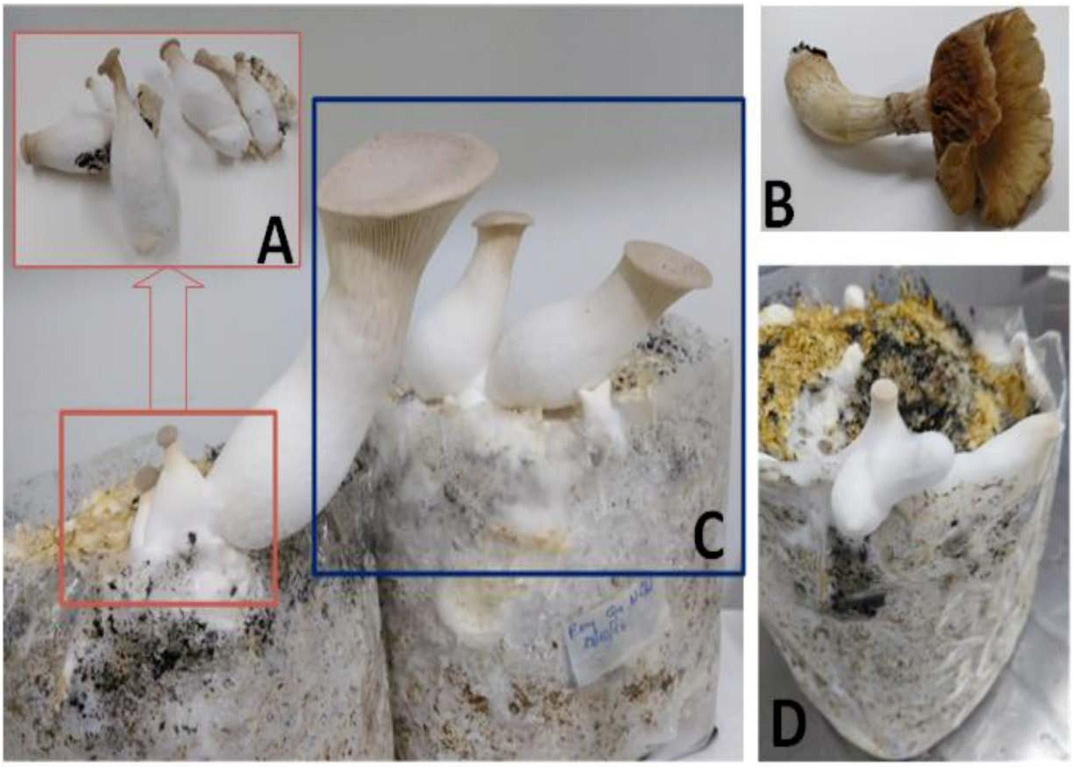


**Figure 2.3 :** Mushroom by-products derived from solid substrate fermentation. (A) and (B), which are wastes from mushroom cultivation (such as stripes and mushrooms not meeting commercial standards in terms of size, shape, or calibre, accounting for 5-20% of total production weight); (C) refers to excess production by-products (less than 5%); and (D) is the spent mushroom substrate (Antunes et al., 2020).

Interestingly, 20% of fresh mushroom production volume is attributed to misshaped mushroom by-products (Umaña et al., 2020). These by-products constitute a significant source of bioactive compounds across diverse mushroom species. The misshapen mushrooms have basically the same chemical composition as that of the normal mushroom (Antunes et al., 2020). Therefore, applying food processing to mushrooms may not only serve as an effective means of valorisation to by-product mushrooms but also contribute to extending the shelf life of all mushrooms. In addition, the fruiting body of mushrooms, utilized predominantly for food and medicinal purposes, presents another significant area of by-product utilization. Notably, stipes are recognized for their potential in the extraction of value-added compounds. A case in point is the shiitake mushroom's stipe, which, despite being commonly discarded due to its fibrous texture, can be repurposed as an alternative nitrogen source in alcoholic fermentation owing to its rich protein content (Lin et al., 2010). Table 2.1 lists several studies that focus on the bioactive compounds found in mushroom by-products.

Several studies have reviewed the use of SMS in producing biogas, bio-fertilizers, and animal feed, as well as its application in biomaterials (Grimm & Wösten, 2018; Mohd Hanafi et al., 2018; Pérez-Chávez et al., 2019). However, despite the acknowledged value of the second and third categories of by-products have received relatively limited attention and warrant further research. This Ph.D. research aims to broaden the understanding of the applications of these categories of mushroom by-products and evaluate the potential opportunities and challenges associated with these by-products. In the context of this study, although misshapen mushroom by-products were initially considered for this research, experimental results indicated no significant difference in using either the mushrooms or their misshapen by-products, given their identical nutritional compositions. For practical reasons, research proceeded directly with mushrooms, deemed more feasible than sourcing them from mushroom by-products. This decision underscores the need for pragmatic approaches in leveraging mushroom by-products, potentially enhancing the sustainability and efficiency of mushroom production and by-product utilization. In conclusion, this discussion offers valuable insights and practical strategies for the comprehensive utilization of mushroom by-products.

**Table 2.1 :** Innovative and traditional technologies for extracting/modificating components in mushroom by-products.

| Technology | Mushroom Species | Type of By-Product | Extracted Compounds | Characteristics/Health Benefit | References |
|---|---|---|---|---|---|
| Sequential solvent extraction | *Pleurotus Ostreatus* | Wasted mushroom stems | Chitin-glucan complex | Advantages of using recyclable solvents and ability to extract high-value products other than chitin | (Ayser et al., 2023) |
| Microwave-Assisted Extraction | Various species reviewed | SMS | Polysaccharides | Enhanced recovery efficiency; immunomodulatory, anticancer, antibacterial effects | (C. Y. Wang, 2020) |
| Ultrasound-assisted extraction | Various species reviewed | Stipes, broken fruiting bodies | Polysaccharides, triterpenes | Increases extraction efficiency; potential for food and medicine due to immunoregulatory, cardiovascular benefits | (Leong et al., 2021) |
| Pulsed Electrical Fields | Various species reviewed | SMS | Polysaccharides | Improved extraction rates; health-promoting effects like protection of intestinal health | (Leong et al., 2021) |
| High Pressure | Various species reviewed | Discarded space bags, stipes | Ergosterol, glycoproteins | Higher extraction yields; ergosterol for vitamin D2 production, glycoproteins for immune support | (Papoutsis et al., 2020) |
| Supercritical Fluid Technology | *Agaricus Bisporus, Lentinus edodes* | Misshapen mushrooms, stems | Bioactive substances | Enhanced content of bioactive substances; applicable in food, pharmaceutical industries for quality improvement | (Guo et al., 2022) |

**Table 2.1 (continued) :** Innovative and traditional technologies for extracting/modificating components in mushroom by-products.

| Technology | Mushroom Species | Type of By-Product | Extracted Compounds | Characteristics/Health Benefit | References |
|---|---|---|---|---|---|
| Ultrasound-assisted extraction | *Agaricus Blazei* | Misshapen mushrooms | Ergesterol | Ultrasound technology saved time and energy compored to traditional heat extraction and purity of ergosterol | (Taofiq et al., 2019) |
| Ultrasound-assisted extraction | *Auricularia Auricula* | Mushroom residue after polysaccharide extraction | Melanin | Saved time and enhanced the antioxidant activity of melanin | (X. Liu et al., 2019) |
| Microwave-assisted extraction | *Agaricus bispours* | Stems and misshapen mushroom | Ergosterol | Saved time significantly while simplifying the process | (Heleno et al., 2016) |
| Supercritical fluid extraction | *Agaricus bispours* | Stems | Sterol | Shortened extraction time | (Gil-Ramírez et al., 2013) |
| Supercritical fluid extraction | *Lentinula edodes* | Stems | Aroma compounds | Decrease odour compounds in soybean oil and increase aromatic compounds in mushroom | (X. Li et al., 2020) |
| Supercritical fluid extraction | *Lentinula edodes* | Mushroom residue after polysaccharide extraction | Sterol | Has no significant impact | (Morales et al., 2018) |
| Enzyme-Assisted Extraction | Various species reviewed | Stipes, discarded fruiting bodies | Polysaccharides, dietary fiber | Selective extraction of compounds; enhances nutritional value and functional properties of extracts | (Leong et al., 2021) |

### 2.1.3 Mushroom composition

Mushrooms are mainly composed of water, which typically makes up 80% to 95% of their total weight. This high water content affects their texture, shelf life, and nutrient concentration per gram of fresh weight. As a result, mushrooms are low in calories, which makes them an excellent food option for maintaining heart and vascular health due to their nutritional profile (Kalogeropoulos et al., 2013).

Mushrooms contain various bioactive compounds like polysaccharides, proteoglycans, terpenes, phenolic compounds, vitamins, lectins, essential amino acids, peptides, and proteins. These components provide a wide range of health benefits, including antioxidant, antimicrobial, antitumor, anti-inflammatory, immunomodulatory, and cholesterol-lowering effects. Thus, mushrooms are valuable in nutrition, traditional medicine, and as functional foods (Kumar et al., 2021). The crude composition, energy content, and fatty acid composition of two mushrooms, *La. deliciosus* and *L. edodes,* which are examined in this PhD, are detailed in Table 2.2.

**Table 2.2 :** The crude composition, energy content and fatty acid composition of *La. deliciosus* and *L. edodes.*

| Composition | *La. deliciosus* | *L. edodes* |
|---|---|---|
| Water content (g per 100 g) | 92.00** | 91.23* |
| Ash (g per 100 g dw) | 8.62** | 6.73* |
| Lipids (g per 100 g dw) | 4.82** | 1.73* |
| Crude protein (g per 100 g dw) | 17.19** | 21.42* |
| Carbohydrates (g per 100 g dw) | 66.61** | 87.14* |
| Energy (g per 100 g dw) | 378.60** | 359.9* |
| Total dietary fiber (g per 100 g dw) | 31.81*** | 54.23**** |
| Insoluble dietary fiber (g per 100 g dw) | 26.51*** | 35.34**** |
| Soluble dietary fiber (g per 100 g dw) | 5.30*** | 18.88**** |
| C16:0 (% of total fatty acids) | 5.17*** | 2.24***** |
| C16:0 (% of total fatty acids) | 16.96*** | 0.22***** |
| C16:0 (% of total fatty acids) | 48.37*** | 0.38***** |
| C18:2 (% of total fatty acids) | 29.49*** | 13.46***** |

*(Perveen et al., 2023), **(Kalogeropoulos et al., 2013), ***(Xu et al., 2019) ****(Y. Zhang et al., 2024) *****(Chung et al., 2020).

### 2.1.4 Mushroom bioactive compounds

*Lentinula edodes*, commonly referred to as shiitake mushrooms, is the second most cultivated edible mushroom worldwide (Lu et al., 2022). These mushrooms are highly esteemed for their nutritional value, which includes polysaccharides, antioxidants, dietary fibre, ergosterol, minerals, and vitamins B1, B2, and C. The unique and robust flavour profile of shiitake mushrooms has made them a popular ingredient in various consumer products (Z. Chen, Fang, et al., 2021).

*Lactarius deliciosus*, commonly known as saffron milkcap, is one of the top five best-selling varieties in Europe where 268 distinct edible wild mushroom species are officially sanctioned for commercialization. *La. deliciosus* is a popular and extensively consumed wild edible mushroom (Dogan et al., 2022; X. H. Wang et al., 2022). Various extraction methods are listed to isolate bioactive compounds from mushrooms in Table 2.3 and Table 2.4.

## 2.2 Phenolic Compounds

One of the most attractive and widely present bioactive compounds in mushrooms is phenolic compounds (M. Y. Kim et al., 2008). The concentration of these compounds can vary significantly between different species and even within different parts of the mushroom (such as the cap, stem, and mycelium). Studies have shown that the content of these compounds can also be influenced by growth conditions, the substrate used, and post-harvest handling (Büntgen et al., 2012).

Phenolic compounds in mushrooms exhibit various activities, including antioxidant, anti-inflammatory, and antimicrobial activities. Antioxidant activity is one of the most crucial activities of phenolic compounds, as they can neutralize free radicals, which can cause oxidative stress and related diseases such as cardiovascular disorders and cancer (Taofiq et al., 2015). Phenolic compounds also have anti-inflammatory activity and reduce inflammation in the body, which is beneficial in managing chronic inflammatory diseases (Ruiz-Ruiz et al., 2017). Additionally, these compounds exhibit antimicrobial activity against a wide range of pathogens, making them potential candidates for natural antimicrobial agents (Muszyńska et al., 2018).

**Table 2.3 :** Bioactive compounds extracted from *Lentinula edodes*.

| Bioactive compound or extract | Activity and mechanism | References |
|---|---|---|
| Polysaccharides | Immunomodulatory, anticancer | (Roszczyk et al., 2022) |
| Polyacetylenes, sulfur compounds | Antimicrobial activity | (Fukushima-Sakuno, 2020) |
| Oxalic acid | Antimicrobial against phytopathogenic bacteria | (Kwak et al., 2016) |
| Phenolic compounds | Antioxidant, antihyperglycemic activities | (Nam et al., 2021a) |
| Antimicrobial metabolites | Inhibition of pathogenic microbes | (Ekowati et al., 2011) |
| Selenopolysaccharide | Immunomodulation, enhancement of ifn-γ synthesis | (Roszczyk et al., 2022) |
| Proteome analysis | Antiviral, anticancer, antioxidant capacities | (Szliszka et al., 2009) |
| Latcripin protein | Suppression of various cancer cell proliferation | (Gao et al., 2018) |
| Polysaccharide-calcium complex | Immunoactivity, cytokine production enhancement | (Cheong et al., 2023) |
| Spirobicyclo terpene | Unique scaffold, potential biotechnological applications | (Cheong et al., 2023) |
| Hot water extract / β glucan | Anti-tumour effects on mcf-7 and s180 cells with apoptosis-inducing activity in s180 tumor-bearing mice demonstrated anti-tumor effects. | (Ren et al., 2018) |
| Ergosterol | Anti-inflammatory properties and shows potential to inhibit the main protease of the sars-cov-2 virus | (Sillapachaiyaporn et al., 2019) |
| Water extract | Antioxidant activity and inhibition of proliferation against human laryngeal carcinoma (hep-2) and cervical adenocarcinoma (hela) cell lines. | (Finimundy et al., 2013) |
| Mannoglucan, polysaccharide-protein complex, glucan, lentinan | Antiviral activity<br>Against hsv-1; hnv | (Qian et al., 2012) |
| Lentinan | Antiviral activity against Sars-cov-2 | (Geller & Yan, 2020) |
| Lentinan | Antiviral activity against Covid-19 | (Rahi & Malik, 2016) |
| Polysaccharide | Significant antioxidant potential | (Chun et al., 2021) |
| Cdp-like polysaccharide | Anti-inflammatory | (Chopra et al., 2021) |
| Polysaccharide isolated from mycelia | Enhance the immune system's response against salmonella bacteria, which cause two types of diseases: endotoxemia and salmonellosis | (S. P. Kim et al., 2013) |
| Mycelia extracts | Favourably alter the course of cancer in patients undergoing chemotherapy | (Suzuki et al., 2013) |

**Table 2.4 :** Bioactive compounds extracted from *Lactarius delicious.*

| Bioactive compound or extract | Activity and mechanism | References |
|---|---|---|
| Ethanol and aqueous extracts | Antioxidant, antihyperglycemic activities | (Xu et al., 2019) |
| Volatile flavor compounds | Flavor profile influence | (Huang & Chen, 2016) |
| Ethanol extracts | Antimicrobial activity, phenolic content | (Onbaşili et al., 2015) |
| Azulene-type sesquiterpenoids | Antibacterial activity | (Feussi Tala et al., 2017) |
| Polysaccharide fraction (ldg-b) | Immunostimulation, macrophage activation | (Hou et al., 2016) |
| Methanolic extract | Antioxidant and free radical scavenging activity | (Kosanić et al., 2016) |
| Methanolic extract | Antimicrobial activity against bacteria and fungi species | (Kosanić et al., 2016) |
| Aqueous and/or ethanol extract | Antioxidative and Antihyperglycemic activity.<br>Ability to inhibit α- amylase and α-glucosidase | (Xu et al., 2019) |
| Polysaccharides | Significant antitumor activity in mice in vivo, and immunomodulatory effects in vitro, inducing the proliferation of both b cells and macrophages | (Kosanić et al., 2016) |

Phenolic compounds present in mushrooms in two forms such as free and bound. The former are solvent-extractable and bioavailable, while the latter are attached to cell wall components and require hydrolysis for release, thus affecting their bioavailability, absorption and biological activities (Gonzales, Smagghe, et al., 2014). However, bound phenolics are attached to cellular components such as fibres and proteins, which must be broken down through digestion or industrial processes before they can be absorbed. The structure of these compounds determines their interaction with the body. Free phenolics can directly interact with metabolic and cellular processes, while bound phenolics may offer sustained release of activities due to their gradual breakdown, which affects their biological activity (Abdelshafy et al., 2022).

Bound phenolics are mostly found in the cell walls of plants and are typically bound to structural carbohydrates and proteins, leading to the formation of complex molecules (B. Zhang et al., 2020). Extracting bound phenolics typically involves harsher conditions than for free phenolics, such as alkaline or enzymatic hydrolysis to release the compounds from the plant matrix (Figure 2.4) (Gulsunoglu et al., 2019; Naczk & Shahidi, 2004).

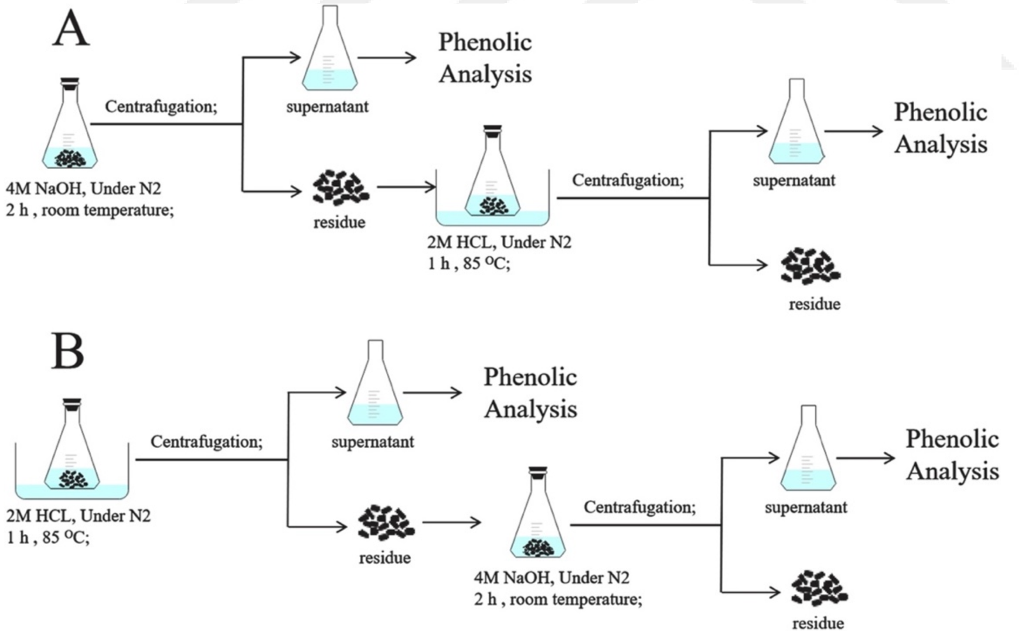
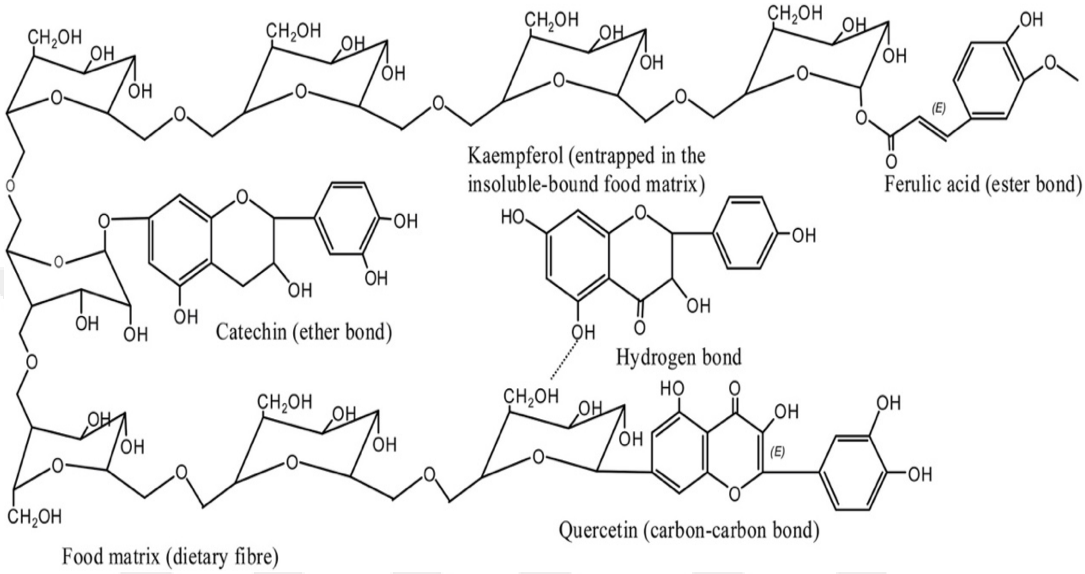
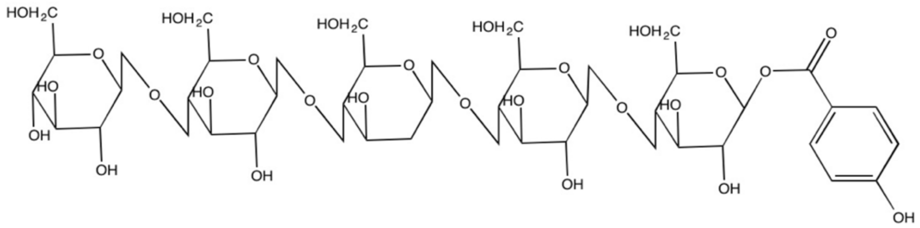


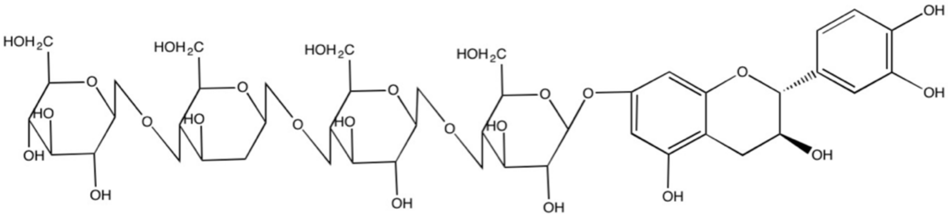
**Figure 2.4 :** A methodological schematic is being used to extract insoluble-bound phenolics through sequential (A) acid/alkaline or (B) alkaline/acid hydrolysis (Bing Zhang, 2020).

BPs are bound to macromolecules through covalent bonds, hydrogen bonding, and hydrophobic interactions, which strengthen the cell wall and provide cells with antibacterial, antifungal, and antioxidant protection (Hashemi Gahruie & Niakousari, 2017; Siemińska-Kuczer et al., 2022). Phenolic acids such as hydroxybenzoic and hydroxycinnamic acids form ester and ether linkages with structural proteins and carbohydrates through their carboxylic and hydroxyl groups in the aromatic ring or directly create C-C bonds between their carbon atoms and those of the cell wall substance (Figure 2.5) (Fry, 1986; M. Li et al., 2020). These interactions enhance the structural rigidity of cell walls (Hamauzu & Suwannachot, 2019).

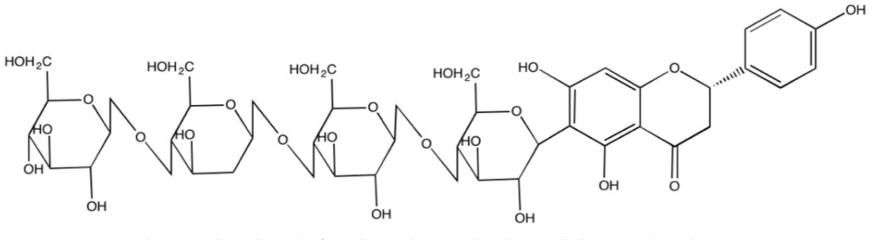




ester bond of 4-hydroxy benzoic acid



ether bond of catechin



carbon-carbon bond of naringenin attached to cellulose molecule

**Figure 2.5 :** Chemically covalent bonds and hydrogen bonds found in the insoluble-bound phenolics that are entrapped in food matrices (Shahidi & Yeo, 2016; B. Zhang et al., 2020).

Bound phenolics in plant material are covalently bound to cell wall structural components, including cellulose, hemicellulose, lignin, pectin, and rod-shaped structural proteins, as illustrated in Figure 2.6.

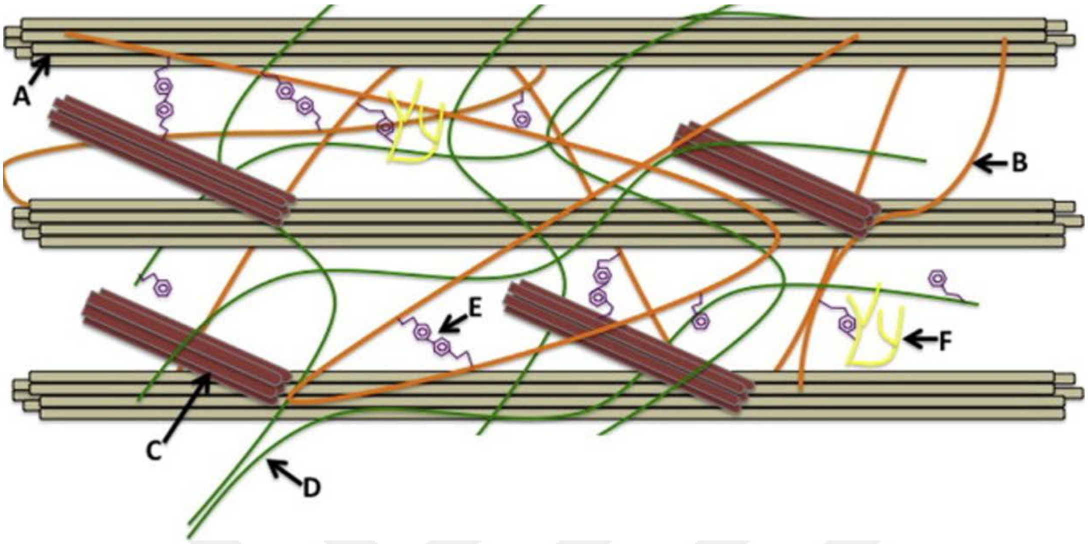


**Figure 2.6 :** Representations of primary cell wall structure of plant material and cross-linking between structural components and phenolic compounds. (A) Cellulose. (B) Hemicellulose. (C) Structural proteins. (D) Pectin. (E) Phenolic acids. (F) Lignin (Acosta-Estrada et al., 2014).

Mushrooms have cell walls that are significantly different from those of plants. Unlike plant cells, which typically have cell walls composed of chitin, chitosan, glucan, mannans, and glycoproteins in mushrooms (Figure 2.7) (Krüzselyi et al., 2020; Vetter, 2007). Chitin is the primary structural polymer found in mushroom cell walls and β-Glucans are intertwined with chitin. Phenolic compounds tend to avoid forming covalent bonds with chitin due to its acetylated amino group structure (Synytsya & Novák, 2013). However, non-covalent interactions like hydrogen bonding or hydrophobic interactions may occur between phenolics and chitin (Rinaudo, 2006). Chitosan can interact with phenolic acids more easily than chitin. These interactions may include ionic bonding between the amino groups of chitosan and the carboxyl groups of phenolic acids. Additionally, chitosan can form complexes with phenolic compounds, potentially through hydrophobic interactions and hydrogen bonding. Phenolics can also bind to beta-glucans, possibly through ester or ether linkages (Ojha, 2021; Wasser, 2002). However, such covalent bonds would typically require the involvement of mushroom metabolism.

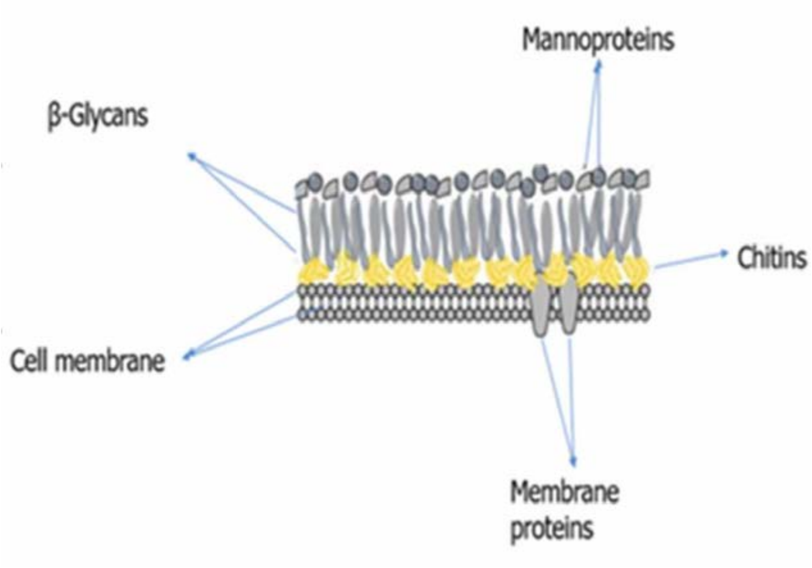


**Figure 2.7 :** Glycan on mushroom cell walls and fruiting bodies (Mwangi et al., 2022).

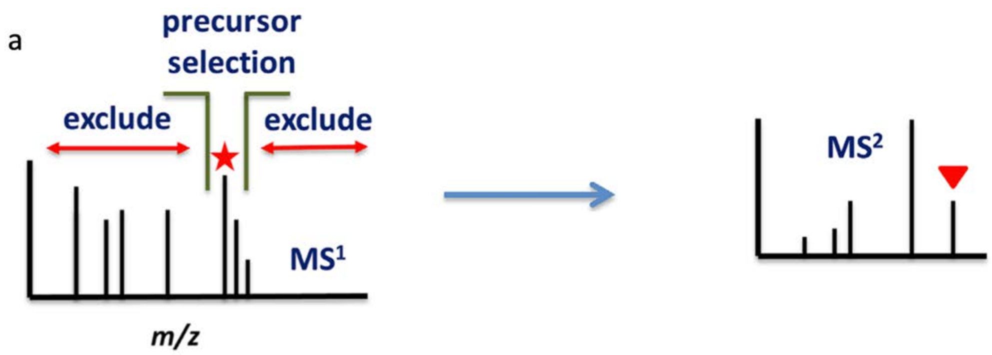
### 2.2.1 Identification of phenolics using tandem mass spectrometry

The extraction and identification of phenolic compounds found in mushrooms is an area of significant interest due to their beneficial properties and potential use in various industries (Abdelshafy et al., 2022). Current research is focused to improving extraction techniques and identifying these valuable compounds more effectively. Since the early 1980s, tandem mass spectrometers have enabled the development of spectral searching against reference databases for tandem mass spectral data (MS/MS) (Chu et al., 2023). This technique has become the quickest and most reliable method for identifying compounds in small molecule analysis (Stein, 2012).

To identify a compound using tandem mass spectrometry (MS/MS), the process entails a series of steps that rely on distinctive fragmentation patterns formed when molecules are ionized and disintegrated into smaller units. The following is a concise overview of the procedure (Kind et al., 2018):

1. Ionization: The compound of interest is first ionized using methods such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) to create charged molecules (ions).
2. Mass Analysis: The ions are then sorted and analyzed based on their mass-to-charge ratio (m/z) in the initial stage of mass spectrometry (MS 1). This step enables the selection of the precursor ion(s) of the compound for further fragmentation.

3. Fragmentation: The selected ions undergo controlled fragmentation using methods such as collision-induced dissociation (CID) or electron capture dissociation (ECD), resulting in a series of smaller fragment ions. This step generates a unique fragmentation pattern (spectrum) that serves as a molecular fingerprint.
4. Second Mass Analysis: The fragment ions derived from the precursor ion are analyzed in the second stage of mass spectrometry (MS2), providing a detailed spectrum that includes the mass-to-charge ratios of all the fragments.
5. Database Search and Matching: The resulting MS/MS spectrum is compared against theoretical or experimental spectra stored in databases such as ChemSpider, METLIN, or MassBank. The comparison employs algorithms and scoring systems to identify the best match based on the similarities between the experimental MS/MS spectrum and the database entries.
6. Compound Identification: The identification of a compound is achieved by matching its MS/MS spectrum to a database spectrum with the highest degree of similarity. This process takes factors like mass accuracy, the presence of specific fragment ions, and the intensity of peaks into account. Additional validation methods may involve matching retention times or orthogonal techniques like nuclear magnetic resonance (NMR) for confirmation.
7. Quantitative and Qualitative Analysis: MS/MS can provide both quantitative information on the concentration of the compound in the sample and qualitative data on its structure based on its fragmentation pattern. The schematic overview of these steps is present in Figure 2.8.



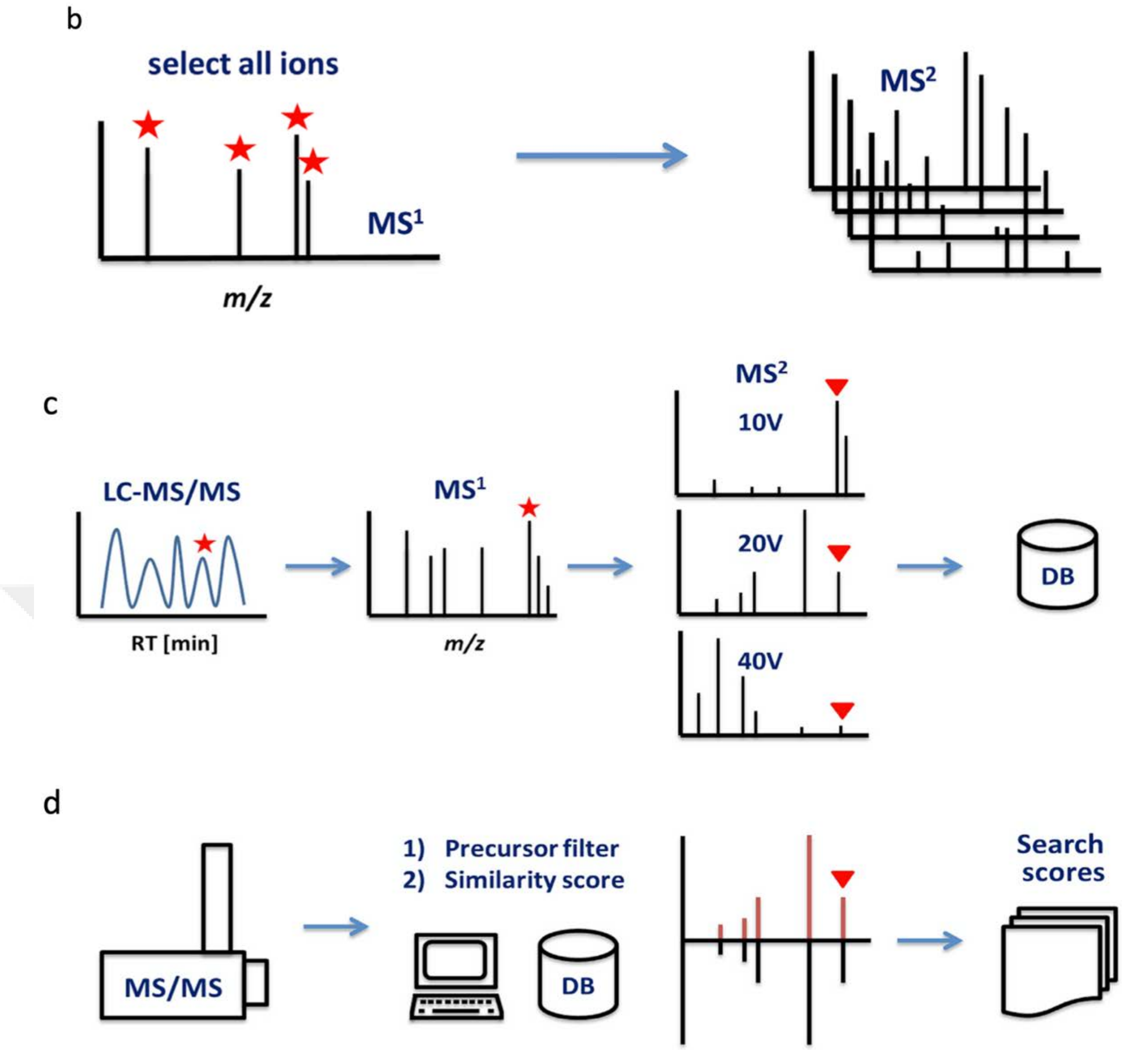


**Figure 2.8 :** The schematic overview of MS/MS approach identification (Adopted from Kind et al., 2018). (a) During data-dependent MS/MS spectral acquisition, the mass spectrometer selects a prominent MS1 peak and excludes all other peaks outside the specified precursor isolation range. The chosen ions produce an MS/MS spectrum that provides information about the selected precursor ion. (b) The all-ion fragmentation technique fragmentates all detected peaks, regardless of their intensity. This results in spectra that are informative but lack specific precursor ion details. (c) During LC-MS/MS analysis, the MS1 ion (precursor) undergoes fragmentation across different collision energies within the tandem mass spectrometer to generate a comprehensive database. This process captures various characteristic fragments (product ions) for inclusion. (d) In an MS/MS search, a precursor filter is employed to exclude candidates outside a specific mass accuracy window, ranging from 0.1 to 0.001 Da. Subsequently, a similarity algorithm assesses the remaining spectra by comparing them to the database spectra, generating a similarity score to rank the matches.

## 2.3 Fermentation

### 2.3.1 Lactic acid bacteria fermentation

Fermentation is a crucial biotechnological process that significantly contributes to food safety, nutritional value, sensory properties, and shelf-life improvement. This underscores the importance of both the fermentation process itself and the composition and dynamics of the microbiota in impacting the quality of the final product (Wu et al., 2020). Lactic acid bacteria (LAB) are capable of fermenting various food products and by-products, and LAB fermentation has become increasingly popular in the food industry as it improves nutritional quality. LAB fermentation enhances protein digestibility, mineral bioavailability, and the liberation of phenolics, peptides and amino acids. The efficiency of LAB in processing plant materials depends on the species and strains, substrate availability for fermentation, and inhibitory factors (De Montijo-Prieto et al., 2023a).

### 2.3.2 Effect of LAB fermentation on phenolic compounds

The metabolic pathways of phenolic compounds during fermentation are essential, given that the microbiota involved in the process plays a crucial role in the resulting biological activities of these compounds. Moreover, certain phenolic compounds within food are transformed by the fermentative microbiota. These transformations are necessary for their absorption and the subsequent modulation of their biological effects. Proposed LAB decarboxylases or reductases have been documented to biotransform hydroxycinnamic acids like caffeic, ferulic or p-coumaric acid, either cinnamic or benzoic acid derivatives (Figure 2.9) (Leonard et al., 2021). Prior research has highlighted that the metabolic alteration of phenolic compounds by specific fermentation enzymes including esterases, glucosidases, dehydroxylases, and decarboxylases significantly affects their bioavailability and biological functions (Leonard et al., 2021). The conversion of hydroxycinnamic and hydroxybenzoic acids is facilitated by distinct esterases, reductases, and decarboxylases showed in Figure 2.10 (Gaur & Gänzle, 2023). Moreover, the fermentation process can significantly increase the antioxidative potential of plant-based foods. This enhancement primarily arises from the increased liberation of phenolic compounds, contributing to an increased natural antioxidant profile. Additionally, the fermentation process may facilitate the structural alteration of plant cell walls, potentially enabling the liberation and/or synthesis of various bioactive components.

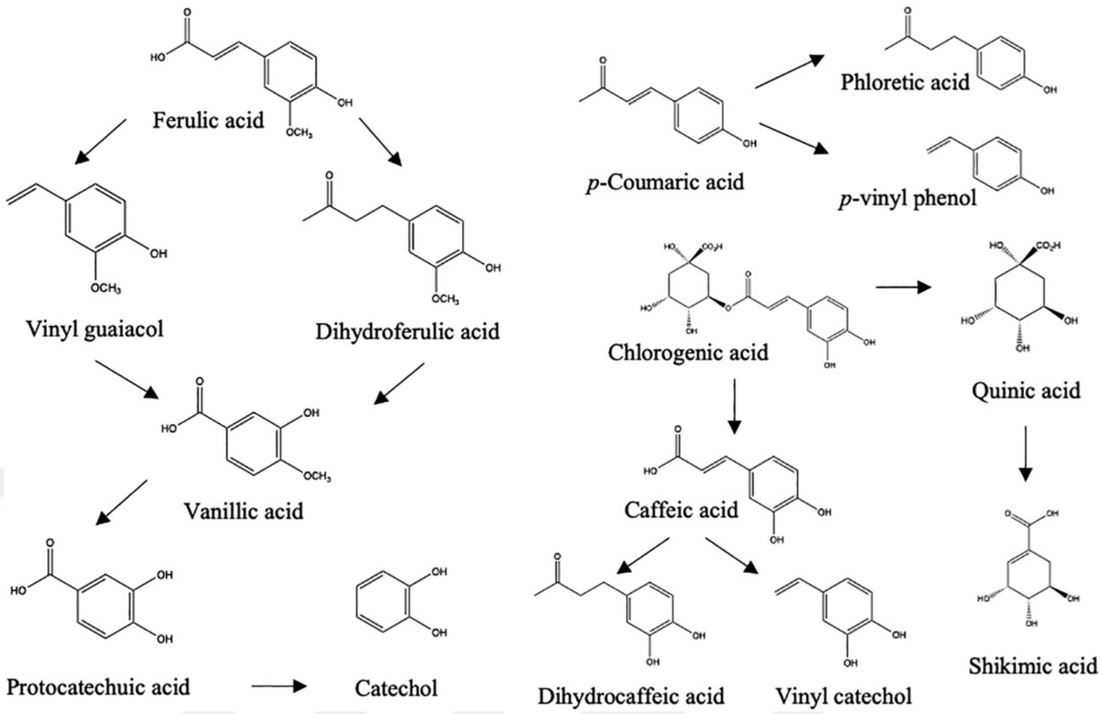


**Figure 2.9 :** Proposed biotransformation of selected phenolic acids during food fermentation (Leonard et al., 2021).

Additionally, the fermentation process may facilitate the structural alteration of plant cell walls, potentially enabling the liberation and/or synthesis of various bioactive components. Such phenomena are instrumental in the observed increase in total phenolic content and antioxidant activities post-fermentation (F. Yang et al., 2023; Yeo et al., 2021), underscoring the critical role of fermentation in augmenting the nutritional and functional properties of plant-derived foods. The scope of this improvement extends to a variety of foods, including beans (Yi et al., 2021), lentils (Yeo et al., 2021), soy flour (Teleky et al., 2020), oolog tea (Wang et al., 2012), quinoa (Melini & Melini, 2021), wheat bran (H. M. Zhao et al., 2017), rice (D. Zhang et al., 2022), and white cabbage (Lee et al., 2021), among others. The studies focusing effect of LAB fermentation on phenolic compounds are listed in Table 2.5.

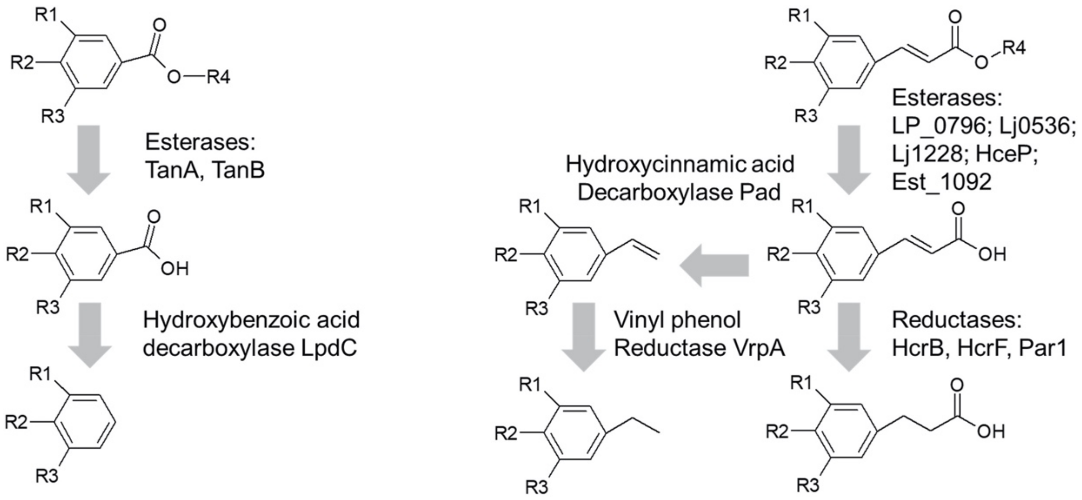


**Figure 2.10 :** Overview on enzymes of lactobacilli that convert hydroxycinnamic and hydroxybenzoic acids (Gaur & Gänzle, 2023).

The use of LAB in cereal fermentation has gained significant attention and is considered a significant development in food biotechnology. This interest is due to the enzymatic activities of both inherent grain enzymes and those introduced through bacteria, such as esterases, xylanases, and glycosidases, which induce profound alterations in cereal grains. Such enzymatic interactions do not modify the structure and composition of grains; instead, they significantly affect grain bioactivity (Adebo & Medina-Meza, 2020). Therefore, the introduction of lactic acid bacteria into cereal fermentation processes has been identified as a crucial step towards achieving significant nutritional improvements. These nutritional enhancements include increased folate availability, elevated levels of soluble dietary fibre, an augmented concentration of phenolic compounds, and notably, the improved digestibility of proteins (Hole et al., 2012).

Microbial enzymes like glucosidase, amylase, and lipase target glucosides, plant cell walls, and starch during fermentation. This enzymatic activity helps extract phenolics and flavonoids from plant materials, improving plant-derived foods' antioxidative capacity. The observed improvements in antioxidative activity are likely due to the structural modification of phytochemicals, which are significantly influenced by lactic acid bacteria. These bacteria are good at metabolising simple phenolic compounds and breaking down large phenolic molecules, increasing both total phenolic content and antioxidative activity.

**Table 2.5 :** The effect of fermentation on phenolic compounds in various fermented substrates.

| Fermented substrate | Fermentation strains | Main positive effect of fermentation | References |
|---|---|---|---|
| Cocoa seed | yeast and LAB | Decrease in TPC, ABTS and ferric reducing antioxidant power with fermentation time | (Albertini et al., 2015) |
| Liquorice root extract | *L. plantarum* | Effect on TPC was influenced by concentration of high fructose corn syrup. Greater DPPH inhibition activity | (Mousavi and Mousav, 2019) |
| Mungbean and soybean milk | *L. plantarum* | Increase in TPC. Increase and decrease in DPPH/ABTS, as affected by the fraction type and time. | (Gan, Shah, Wang, Lui, and Corke, 2016) |
| Mulberry juice | *L. plantarum,*<br>*L. acidophilus,*<br>*L. paracasei* | Increase in total antioxidant activity, anthocyanin and TPC. | (Kwaw et al., 2018) |
| Pomegranate juice | *L. plantarum* | Antimicrobial activity increased, resulting in higher content of volatile free fatty acids and better organoleptic properties. | (Valero-Cases et al., 2017) |
| Pomegranate juice | *L. plantarum,*<br>*L. paracasei* | Improved TPC, antioxidant activity and sensorial characteristics | (Mantzourani, Kazakos, et al., 2019)<br>(Mantzourani et al., 2020; Plessas, 2022) |
| Cornelian cherry | *L. paracasei* | Improved TPC and antioxidant activity. | (Mantzourani, Terpou, et al., 2019) |

**Table 2.5 (continued) :** The effect of fermentation on phenolic compounds in various fermented substrates.

| Fermented substrate | Fermentation strains | Main positive effect of fermentation | References |
|---|---|---|---|
| Apple juice | *L. plantarum* | Improved antioxidant capacity and enhanced bioaccasebility of polyphenols. | (Z. Li et al., 2019a) |
| Phyllanthus emblica fruit juice | *L. paracasei* | Improved TPC and antioxidant activity. | (Peerajan et al., 2016) |
| Extruded brown rice | *L. plantarum*, *L. fermentum* | Enhanced TPC, improved antioxidant activity and bioaccessibility | (Khan et al., 2020) |
| Sorghum silage | *L. farciminis*, *L. plantarum* | Increased glucosinolates and polyphenolic compounds | (Xie et al., 2021) |
| Mulberry juice | *L. strains* | Modified phenolic profile, enhanced antioxidant activities | (Kwaw et al., 2018) |
| Maize flour | *L. plantarum*, *L. acidophilus* | Modification in phenolic compounds kinetics, improved antioxidant activity | (De Beer et al., 2019) |
| Rooibos | *Various lactic acid bacteria* | Changes in phenolic composition, impact on antioxidant capacity | (De Beer et al., 2019) |
| Wheat bread doughs | *L. plantarum* | Increase in carotenoid and phenolic acid content, enhanced antioxidant capacity | (Antognoni et al., 2019) |
| Pomegranate juices | *L. plantarum*, *L. paracasei* | Formation of catechin and α-punicalagin derivatives, increased bioaccessibility of phenolic compounds | (Valero-Cases et al., 2017) |
| Tea extracts | *Various lactic acid bacteria* | Metabolism of tea phenolics, enhanced antioxidant activity and cellular uptake | (D. Zhao & Shah, 2016) |

**Table 2.5 (continued) :** The effect of fermentation on phenolic compounds in various fermented substrates.

| Fermented substrate | Fermentation strains | Main positive effect of fermentation | References |
|---|---|---|---|
| Rice bran | *Rhizopus oryzae* | Increase in TPC, enhancement of antioxidant activity | (Schmidt et al., 2014) |
| Black garlic | *L. plantarum*<br>*L. rhamnosus*<br>co-culture of them | Enhanced bioaccessibility and bioactivity of phenolic compounds | (L. Ma et al., 2021) |
| Barley and oat grains | *L. acidophilus*<br>*L. johnsonii*<br>*L. reuteri* | Increased levels of free phenolic acids, improved antioxidant activities | (Hole et al., 2012) |
| Cashew apple juice | *B. bifdum,*<br>*B. longum subsp. infantis,*<br>*L. plantarum,*<br>*L. acidophilus,*<br>*L. mesenteroides* | Modifying TPC, antioxidant activity and possible prebiotic action through prebiotic oligosaccharides. | (Vergara et al., 2010) |

Fermented soy foods, for example, have elevated aglycone content, which is produced from the conversion of glycosylated isoflavones during fermentation. This conversion is carried out by lactic acid bacteria, such as various species of *Lactobacillus* and *Bifidobacterium*, which can hydrolyse β-glucosides in soybeans and transform them into bioactive isoflavones like genistein and daidzein. Malvidin and delphinidin, which are valued for their antioxidant properties in food additives, are flavonoid β-glucosides that these bacterial strains can degrade. However, the fermentation process can also lead to the oxidative degradation of certain flavonols in green tea, illustrating the complex and varied effects of fermentation on the antioxidant profiles of plant-based foods (Adebo & Medina-Meza, 2020).

### 2.3.3 *Lactiplantibacillus plantarum*

*Lactiplantibacillus plantarum* is a highly adaptable and versatile bacterium that is widely used in food fermentation and the production of probiotic foods. It is classified as GRAS (Generally Recognized as Safe) and can thrive in highly acidic and ethanol-rich environments due to its large genome and metabolic flexibility (Plessas, 2022). *Lb. plantarum* can survive in diverse environments such as cereals, meats, dairy, vegetables, fruits, beverages, and human and mammal niches (Y. W. Liu et al., 2018). *Lb. plantarum* produces extracellular enzymes like tannase, β-glucosidase, α-glucosidase, and β-galactosidase that create value-added compounds, enhancing food aroma and antioxidant activity (Behera et al., 2018).

*Lb. plantarum* is a microbe that manages gastrointestinal disorders, lowers cholesterol and alleviates IBS symptoms. It also exhibits antimicrobial, antifungal, and antiviral activities, making it ideal for developing functional foods (Behera et al., 2018). It increases phenolic content in food and transforms phenolic compounds into potent antioxidants, making it crucial in food biotechnology for healthier food options (Ahire et al., 2021).

### 2.3.4 Fermentation of mushrooms

Fermentation is a traditional food processing technique that is widely used for mushrooms, particularly *L. edodes*. The purpose of fermenting mushrooms was to create longer-lasting and more nutritious foods that have improved nutritional profiles and organoleptic properties (Z. Chen, Fang, et al., 2021). During the fermentation process, lactic acid bacteria metabolize certain compounds, resulting in a unique flavour. It is important to note that the quality of the final result may be influenced by the specific bacterial strain utilized. Studies examining the fermentation of mushrooms are listed in Table 2.6. While some of the studies aimed at improving the taste of mushrooms, the majority were conducted through pickling and preservation methods.

Jabłońska-Ryś et al. (2016) explored acid fermentation using *Lb. plantarum, Lb. casei,* and *Lb. helveticus* of oyster and chanterelle mushrooms to study changes in their polyphenol content, antioxidant activities, and phenolic acid levels. Oyster mushrooms showed higher total phenolic content than chanterelles. Blanching reduced the polyphenols and antioxidant activities in both mushrooms, with no significant difference in total phenolic compounds among different LAB strains. *Lactobacillus plantarum* was identified as the most effective LAB for acid fermentation, it significantly lowered the pH of the fermented mushrooms and resulted in higher levels of specific phenolic acids, such as gallic, ferulic and homogentisic acids.

Bartkiene et al. (2023), explored the effects of 48-hour lactic acid fermentation on *Agaricus bisporus* (white and brown varieties) using strains of *Lacticaseibacillus casei, Lactiplantibacillus plantarum, Lacticaseibacillus paracasei*, and *Pediococcus acidilactici*. The study focused on certain compounds present in fermented mushrooms and evaluated their consumer acceptability. Fermented mushrooms had notable levels of spermidine and, in some instances, tyramine. Fatty acid analysis showed variations, with fermented mushrooms displaying higher monounsaturated and polyunsaturated fats, particularly in those fermented with *Lactiplantibacillus plantarum*. This research also marked the first analysis of volatile compounds in fermented mushrooms, identifying changes in specific compound. Consumer acceptability was high for fermented mushrooms, and emotional evaluations revealed significant impacts of LAB strain type and mushroom variety on emotional responses. The results highlight the effectiveness of the chosen LAB strains in the fermentation of mushrooms and provide valuable insights into fermented food products.

**Table 2.6 :** The effect of fermentation on bioactive compounds in fermented mushrooms.

| LAB Strains | Fermented Substrate | Main Positive Effect | References |
|---|---|---|---|
| *Lactobacillus pentosus* | Oyster mushrooms (*Pleurotus spp.*) | Enhanced organic acids content, controlled spoilage/pathogenic microorganisms, improved sensory characteristics. | (Y. Liu et al., 2016) |
| *Lacticaseibacillus casei LUHS210*,<br>*Liquorilactobacillus uvarum LUHS245* | Wild edible mushrooms (*Boletus edulis*, *Cantharellus cibarius*, *Rozites caperata*) | Inhibited fungal growth, improved fermentation safety, influenced color and pH, produced a variety of volatile compounds. | (Bartkiene et al., 2022) |
| *Lactiplantibacillus plantarum 299v*,<br>*L. plantarum EK3* | Button mushroom (*Agaricus bisporus*) | Altered sugar and organic acids content, enhanced LAB count, improved product stability. | (Jabłońska-Ryś et al., 2022) |
| *Lactobacillus plantarum* | King oyster mushrooms (*Pleurotus eryngii*) | Controlled spoilage/pathogenic microorganisms, reduced nitrite concentrations, safe and effective preservation. | (Zheng et al., 2018) |
| *Leuconostoc mesenteroides*,<br>*Lactiplantibacillus paraplantarum*,<br>*Lactiplantibacillus plantarum*,<br>*Lactococcus lactis* | White button mushrooms (*Agaricus bisporus*) | Identified LAB with potential functional and technological properties suitable for novel starter cultures. | (Skrzypczak et al., 2020) |
| *Lactobacillus plantarum* | *Agaricus bisporus* | Reduced polysaccharide content after processing, yet preserved antioxidant and antiproliferative activities | (Radzki et al., 2019) |
| *Lactiplantibacillus plantarum Ib*,<br>*L. casei Lby*,<br>*L. helveticus K1Lb* | *Pleurotus ostreatus* and *Cantharellus cibarius* | Modified phenolic profile, enhanced antioxidant activities. | Jabłońska-Ryś et al., (2016) |

**Table 2.6 (continued) :** The effect of fermentation on bioactive compounds in fermented mushrooms.

| LAB Strains | Fermented Substrate | Main Positive Effect | References |
|---|---|---|---|
| *Lactobacillus plantarum, Lactobacillus johnsonii* | Mushroom and vegetables | Improved product quality in microbial, chemical, and sensory aspects after storage | (Manowan et al., 2020) |
| *Lacticaseibacillus casei, Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, Pediococcus acidilactici* | *Agaricus bisporus* (white and brown varieties) | Evaluated changes in characteristics, including biogenic amine and volatile compound formation. | (Bartkiene et al., 2023) |
| *Saccharomyces cerevisiae, Aspergillus oryzae, Aspergillus niger, Lactobacillus plantarum* | *Lentinus edodes* | Enhanced umami flavor due to increased free amino acids and flavor nucleotides | (Z. Chen, Gao, et al., 2021) |
| *Lactobacillus plantarum KU5* | Spent mushroom substrate | Improved fermentability and aerobic stability of SMS silages | (J. S. Kim et al., 2016) |
| Human fecal microbiota | *Pleurotus eryngii* | Immunomodulating effects after in vitro fermentation by human fecal microbiota | (Vlassopoulou et al., 2022) |
| The fecal inocula anaerobic culturing | *Flammulina velutipes, Lentinus edodes, Auricularia auricular, Pleurotus osteratus, Agaricus bisporus and Pleurotus eryngii* | In vitro digestion and anaerobic culture effects on intestinal microbial community composition | (R. Zhao et al., 2018) |
| *Lactiplantibacillus plantarum GDM1.191* | *Lentinus edodes* | Improved contents of umami taste compounds | (Z. Chen, Fang, et al., 2021) |
| *Lactiplantibacillus plantarum* | *Pleurotus ostreatus, Cantharellus cibarius* | Maintained polyphenol content and antioxidant activities despite blanching | (Jabłońska-Ryś et al., 2016) |

Radzki et al. (2019) investigated how various processing methods affect the polysaccharides extracted from button mushrooms, including blanching, boiling in water, and blanching followed by lactic acid fermentation. The researchers analyzed water-soluble polysaccharides from the mushrooms and assessed their chemical composition, antioxidant capacity, and antiproliferative activity against cancer cell lines MCF-7 and T-47D. The results showed that processing methods led to a slight decrease in polysaccharide content, with boiling in water and blanching followed by fermentation showing minor reductions. The polysaccharides contained both α- and β-linkages, with molecular weights of 163.3 and 1.9 kDa. Processing also reduced the protein and phenolic content, yet the isolated polysaccharides still demonstrated antioxidant and antiproliferative activities, albeit slightly reduced by processing.

Bartkiene et al. (2022) examined the preservation of wild edible mushrooms by pickling, *Suillus luteus, Boletus edulis, Cantharellus cibarius*, and *Rozites caperata*. strains of *Lacticaseibacillus casei LUHS210* and *Liquorilactobacillus uvarum LUHS245* were used to ferment the mushrooms, which improved their quality and safety. The fermentation process inhibited fungal growth and produced various volatile compounds that affected the mushrooms' colour, pH, and overall taste. The research showed that using LAB fermentation is a promising method to enhance the appeal and safety of wild mushrooms for culinary purposes.

Manowan et al. (2020) investigated the quality characteristics of fermented mushroom and vegetable products using a mixed starter of *Lactobacillus plantarum* and *Lactobacillus johnsonii*. The study found that lactic acid fermentation can significantly improved product quality in microbial, chemical, and sensory aspects, ensuring consistent safety and enhancing the nutritional value of fermented foods. The fermented product had a favorable pH, titratable acidity, and phenolic content, while also maintaining high LAB counts even after 30 days of storage.

Liu et al. (2016) used *Lactobacillus pentosus* as a starter culture for the lactic acid fermentation of different types of oyster mushrooms (*Pleurotus spp*.). The results showed that the use of L. pentosus was effective in controlling spoilage and pathogenic microorganisms, with Enterobacteriaceae not being detected in the final products. The study also highlighted that *L. pentosus* was capable of producing fermented oyster mushrooms with higher concentrations of organic acids and acceptable sensory characteristics. Therefore, the study suggested that *L. pentosus* could be an effective starter culture for mushroom fermentation.

Bartkiene et al. (2022) investigated the effects of fermenting wild mushrooms (*Boletus edulis, Cantharellus cibarius,* and *Rozites caperata*) using strains of LAB, *Lacticaseibacillus casei LUHS210* and *Liquorilactobacillus uvarum LUHS245*. This research aimed to understand how LAB fermentation influences mushroom quality, safety, and consumer acceptance. It found that LAB fermentation, especially with the LUHS245 strain, improved mushroom preservation by inhibiting fungal growth and enhancing desirable color and flavor profiles. Pre-treatments like ultrasonication or thermal processing before fermentation were also evaluated, showing benefits in ensuring safer mushroom fermentation. Overall, the study underscores the importance of selecting suitable LAB strains for mushroom fermentation to maintain their quality and safety.

Jabłońska-Ryś et al. (2022) found that controlled lactic fermentation improves the quality of button mushroom fruiting bodies. The researchers used two strains of *Lactiplantibacillus plantarum (299v* and *EK3)*, as starter cultures and measured changes in sugars, organic acids, and lactic acid bacteria content. Lactic acid was the most dominant organic acid in the final product, indicating successful fermentation. The *L. plantarum EK3* strain was found to be superior, leading to a higher amount of produced lactic acid, lower pH, and higher LAB counts. The study recommends this strain as a suitable starter culture for mushroom fermentation.

Zheng et al. (2018) explored three traditional lactic acid fermentation processes (sauerkraut, pickling, and kimchi) to preserve king oyster mushrooms using *Lactobacillus plantarum* as a starter culture. LAB colonized the mushroom fruit bodies and controlled spoilage and pathogenic microorganisms. The fermentation resulted in high LAB populations and reduced nitrite concentrations below China's safety limits. The study suggests lactic acid fermentation as a viable alternative to heavy salting for long-term mushroom preservation, reducing environmental pollution associated with traditional preservation methods.

Skrzypczak et al. (2020) focused on identifying and examining lactic acid bacteria from spontaneously fermented *Agaricus bisporus* (white button mushrooms). The fermentation process was conducted at 28 °C for 5 days, leading to the isolation of

LAB which were then characterized through MALDI-TOF Biotyper and further analyzed for phenotypical, genotypical, and functional properties. The study discovered a predominance of *Leuconostoc mesenteroides* in the fermented mushrooms, alongside other LAB such as *Lactiplantibacillus paraplantarum, Lactiplantibacillus plantarum,* and *Lactococcus lactis*. Notable findings include the efficient acidification of the mushroom substrate by isolates EK55 and EK4 and the high cell hydrophobicity in *L. plantarum* isolates EK12, EK55, and EK5. The research highlighted the potential of certain LAB isolates, especially *L. plantarum EK55* and *L. paraplantarum EK4*, as promising starter cultures for developing novel fermented mushroom products due to their functional and technological capabilities.

Chen, Fang, et al. (2021), explored the impact of fermentation on the umami taste substances in *Lentinus edodes* by using different microorganisms, including *Saccharomyces cerevisiae, Aspergillus oryzae, Aspergillus niger,* and *Lactobacillus plantarum.* Through fermentation, the acidity and content of free amino acids and flavour nucleotides in the fermentation broth increased. Among the tested strains, *Lactobacillus plantarum* proved to be the most effective in providing a strong umami flavour, indicating enhanced taste profiles. This research demonstrates that fermentation can significantly improve the umami flavour of shiitake mushrooms, with *L. plantarum* being particularly effective.

J. S. Kim et al. (2016) focused on the fermentation of spent mushroom substrate using novel *Lactobacillus plantarum* strains, examining the fermentative quality and aerobic stability. The strain *L. plantarum KU5* was found to be the most effective in enhancing lactic acid production and lactic acid bacteria population. The researchers also discovered that adding molasses could improve the odour of the fermentation, lower the pH, and enhance the aerobic stability of SMS silages. The study concluded that *L. plantarum KU5* could be successfully used to ensile SMS, indicating a potential use as animal feed.

Vlassopoulou et al. (2022) investigated the immunomodulatory effects of *Pleurotus eryngii* mushrooms after in vitro fermentation by fecal microbiota of elderly volunteers. They focused on beta-glucans, which are known for their health benefits, and studied how they interacted with the fecal microbiota of elderly volunteers. The research found that the mushrooms had potential prebiotic activity and could modulate the immune function positively. The presence of *P. eryngii* led to modifications in immune response indicators across all volunteers, indicating the mushrooms' fermentation products' potential to have anticancer and immunomodulatory properties.

R. Zhao et al. (2018) assessed the impact of six different edible mushrooms on the intestinal microbial community composition and diversity during in vitro digestion and anaerobic culture. The research found that fermentation significantly altered short-chain fatty acid (SCFA) concentrations and pH values, suggesting mushrooms treated with edible mushrooms had a beneficial impact on the gut microbial environment. The study highlighted the potential of edible mushrooms to influence the gut microbiota favourably and improve gut health.

Chen, Fang, et al. (2021) investigated how the fermentation of shiitake mushrooms with *Lactiplantibacillus plantarum GDM1.191* affects their umami compounds. They found that the fermentation process enhanced all umami compounds, except for succinic and fumaric acids. The umami taste compounds significantly improved with increasing fermentation time. The study highlights the effectiveness of *L. plantarum GDM1.19*1 fermentation in enhancing the umami taste of shiitake mushrooms and provides insights into developing flavorful fermented mushroom products.

In conclusion, numerous studies have demonstrated that processing and fermentation can alter the structure, bioactivity, and nutritional profile of mushrooms and other foods. However, to our knowledge, there is a notable gap in research regarding the use of lactic acid bacteria fermentation specifically aimed at enhancing the bioaccessibility of bound phenolics in mushrooms. Additionally, the potential of using mushroom by-products for this purpose remains largely unexplored. This highlighted a significant opportunity for our investigation in this area. To summarise this literature review, mushroom by-products, including those that are misshapen and mushroom residues from the extraction of bioactive compounds, can be enhanced in terms of their phenolic compounds through lactic acid bacteria fermentation. The following chapters will explore the valorisation of mushroom by-products through LAB fermentation (*Chapter 3* and *Chapter 4*) and the enhancement of the biological activity and bioaccessibility of mushrooms through fermentation (*Chapter 4* and *Chapter 5*).

# 3. VALORIZATION OF MUSHROOM BY-PRODUCTS VIA LACTIC ACID FERMENTATION: FUNCTIONAL AND STRUCTURAL PROPERTIES

## 3.1 Abstract

Mushrooms are known to have health-promoting properties due to the presence of several bioactive compounds, including polysaccharides. The extraction of these polysaccharides generates a significant amount of mushroom residue. Our study aims to valorise this by-product by enhancing the functional and structural properties of both the mushroom and its residue. We investigated the effects of a 72 h fermentation with *Lactiplantibacillus plantarum LMG 17673* on protein characterisation, technofunctional properties, phenolic compounds, and antioxidant activity in *Lentinula edodes* and *L. edodes* residue. However, inconsistent changes were observed in mushroom fractions, indicating the complexity of bioactive compound interactions and the need for careful processing of these residues. The study observed fermentation metabolites, noting decreased free sugars and increased free organic acids, proven by decreased pH. The primary monosaccharides, disaccharides, and sugar alcohols identified in mushrooms were glucose, ribose, trehalose, and mannitol; however, only ribose remained after 72 h of fermentation. Protein degradation during fermentation led to an increase in free amino groups and the conversion of high molecular weight proteins into lower molecular weights, as evidenced by SDS-PAGE in *L. edodes*. This degradation also impacted the oil and water holding capacities. In contrast, LER exhibited an increase in free amino groups due to the breakdown of peptide bonds during fermentation, releasing smaller peptides and free amino acids. The antioxidant activity of both mushroom fractions were enhanced, particularly after 24 hours of fermentation. This study demonstrates for the first time the valorisation of mushroom by-product residue through lactic acid fermentation, which improved some functional and structural properties.

## 3.2 Introduction

*Lentinula edodes* is rich in a range of nutrients and bioactive compounds that offer numerous health benefitsbenefits (K. Kumar et al., 2021). This range includes a high carbohydrate content (comprising 68% to 78% of mono-, di-, tri-, and polysaccharides), essential amino acids such as arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan, as well as lipids (making up 5% to 8% of dry weight) ) (Roszczyk et al., 2022; Sheng et al., 2021). *L. edodes* is also a good source of vitamins (B1, B2, B12, C, D, E), minerals (Ca, K, Mg, Mn, P, Zn, Na), fibre, and various bioactive compounds with antibacterial, antiviral, and antifungal propertiesproperties (Koca & Gençcelep, 2011; Reis et al., 2012). Notably, numerous studies have been carried out to investigate the health benefits of polysaccharides obtained from *L. edodes* (Muñoz-Castiblanco et al., 2022; Roszczyk et al., 2022; Sheng et al., 2021; C. Yin et al., 2018).

Water-soluble polysaccharides such as beta-glucans and glycoproteins have immune-modulating effects and potential anticancer activities and can also help regulate cholesterol levels (Soua et al., 2020). Hot water extraction was the commonly used to extract water-soluble polysaccharides from mushrooms. However, water extraction at room temperature, called cold water extraction, may be preferable for some reasons (P. Chen et al., 2019). Cold water extraction can preserve the integrity and biological activities of thermally sensitive polysaccharides. This method is gentle and does not expose the compounds to high temperatures, making it ideal for sensitive compounds. Additionally, it uses less energy as no heating is required, making it more environmentally friendly and less costly in terms of energy use (Abreu et al., 2021a). Pérez-Bassart et al., (2023) developed a sequential fractionation of protocol for β-glucan in *Grifola frondosa, L. edodes* and *Pleurotus ostreatus*. They found that aqueous extracts at room temperature had the highest overall yields (56.3-82%) of proteins, sugars, and polyphenols. However, hot water extraction typically yields higher amounts of polysaccharides than cold water extraction because some polysaccharides are tightly bound within the cell walls and are not easily solubilised without applying heat (Leong et al., 2021). Therefore, these polysaccharides play an essential role in characterising extract residues.

Many studies have successfully isolated polysaccharides from mushrooms using sequential cold water extraction, effectively preserving their beneficial effects effect (Palacios et al., 2012; Castro-Alves & do Nascimento, 2016; Chen et al., 2019; Ji et al., 2019; Abreu et al., 2021). After extracting the soluble polysaccharides, the remaining mushroom residue may still contain biologically active compounds. Therefore, it is crucial to process these residues to maximise their value (Aguiló-Aguayo et al., 2017; K. Wang et al., 2021).

LAB fermentation is a biotechnological technique that has been used for a long time to process fruits, vegetables, cereals, legumes, and mushrooms. The technique helps improve safety, extend the shelf life, and enhance the substrates' nutritional quality and sensory characteristics. Fermentation is cost-effective and highly valuable and may increase the nutritional value of the food by reducing sugar levels and enhancing protein and peptide content (Emkani et al., 2021). Additionally, fermentation can occur in foods and/or their byproducts, either naturally or through the introduction of lactic acid bacteria starter cultures, intending to achieve specific outcomes such as improved digestibility and transformed structure (Razola-Díaz et al., 2023). For example, fermenting *L. edodes* with *Lactiplantibacillus plantarum* changed umami taste compounds in shiitake mushrooms, including organic acids, free amino acids, and 5′-nucleotides (Z. Chen et al., 2021). In addition, Jabłońska-Ryś et al. (2022) discovered that *Lb. plantarum* fermentation enhances button mushroom quality, altering sugars, organic acids, and lactic acid bacteria content.

This study aims to evaluate the effect of LAB fermentation on mushroom residue's functional and structural properties by evaluating its antioxidant activities and comparing it to *L. edodes*. In particular, the effects of *Lb. plantarum LMG 17673* fermentation on organic acids, sugar composition, phenolic compounds, antioxidant capacity, and protein characteristics were studied.

## 3.3 Materials and Methods

### 3.3.1 Media and chemicals

De Man-Rogosa-Sharpe (MRS) and plate count agar were purchased from Thermo-Fischer (Merelbeke, Belgium). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid

(ABTS), trolox, 2,4,6-trinitrobenzene sulfonic acid (TNBS), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), L-rhamnose monohydrate, DL-arabinose, D(+)-xylose, D(+)-mannose, D(−)-ribose, D(+)-glucose, D(+)-maltose monohydrate, D(−)-mannitol, trehalose, gallic acid, vanillic acid, salicylic acid, galacturonic acid, and glucuronic acid were purchased from Sigma–Aldrich Fine Chemicals (St.Louis, MO, USA). D(−)-Fructose was purchased from Acros Organics (Geel, Belgium). HPLC-grade water, methanol, citric acid, and lactic acid were purchased from VWR Chemicals (VWR International, France). Aluminium chloride, acetic acid, sodium nitrite, methanol, hydrochloric acid, sodium hydroxide, and sodium carbonate were purchased from ChemLab ( NV, Belgium).

### 3.3.2 Raw material and residue preparations

*L. edodes* fruiting bodies were sourced from a local market in Turkey. *L. edodes* fruiting bodies were cut into a thickness of 5 mm and frozen at -20°C. Following this, they were dried at -56°C for 72 h using a freeze dryer, resulting in the production *of L. edodes* mushroom powder (LE). *L. edodes* residue powder (LER) was generated by drying residue LE, which had its polysaccharides extracted using a sequential extraction technique, as outlined in Pérez-Bassart et al. (2023). Slight modifications were made to the LE extraction process; therefore, LE was added to 20 mL of 2.5% w/v NaCl solution at a ratio of 1:20. The mixture was stirred for 30 min and then centrifuged at 4°C for 20 min. The supernatant was separated as a cold water extract, and the pellets were collected and freeze-dried to create the LER.

### 3.3.3 Inoculum and fermentation

The fermentation process of mushrooms is shown in Figure 1. Briefly, for lactic acid fermentation, LER and LE (0.5 g) were mixed with 25 mL distilled water to obtain a 2% (w/v) mushroom solution and sterilised at 121 °C for 15 min. The Lactobacillus culture of *Lactiplantibacillus plantarum LMG 17673* was obtained from the BCCM/LMG Bacteria Collection, located within the Laboratory for Microbiology at Ghent University, Belgium. To activate the lactobacilli culture, 100 µL of the glycerol stock culture was aseptically transferred into 10 mL of sterile MRS broth and incubated at 37 °C for 24 h (He et al., 2021). *Lb. plantarum* was propagated in MRS broth and later used as a working culture to inoculate mushroom solutions. Flasks containing 25 mL of sterilised mushroom solution were inoculated with the working culture (1%, v/v) and then placed on a rotary shaker at 110 rpm and 30°C. Samples were collected at 0, 24, 48, and 72 h of fermentation for microbiological counting and pH measurement. The samples collected at certain times were then centrifuged at 4 °C for 20 min and filtered, and the pellet and supernatant were separately frozen and then freeze-dried. Thus, different fermented fractions were obtained, such as powdered soluble fractions of fermented *L. edodes* (S-FLE), *L. edodes* residue (S-FLER), powdered non-soluble fractions of fermented *L. edodes* (NS-FLE), and *L. edodes* residue (NS-FLER). The freeze-dried powder pellets and supernatants were stored in a freezer for further analysis.

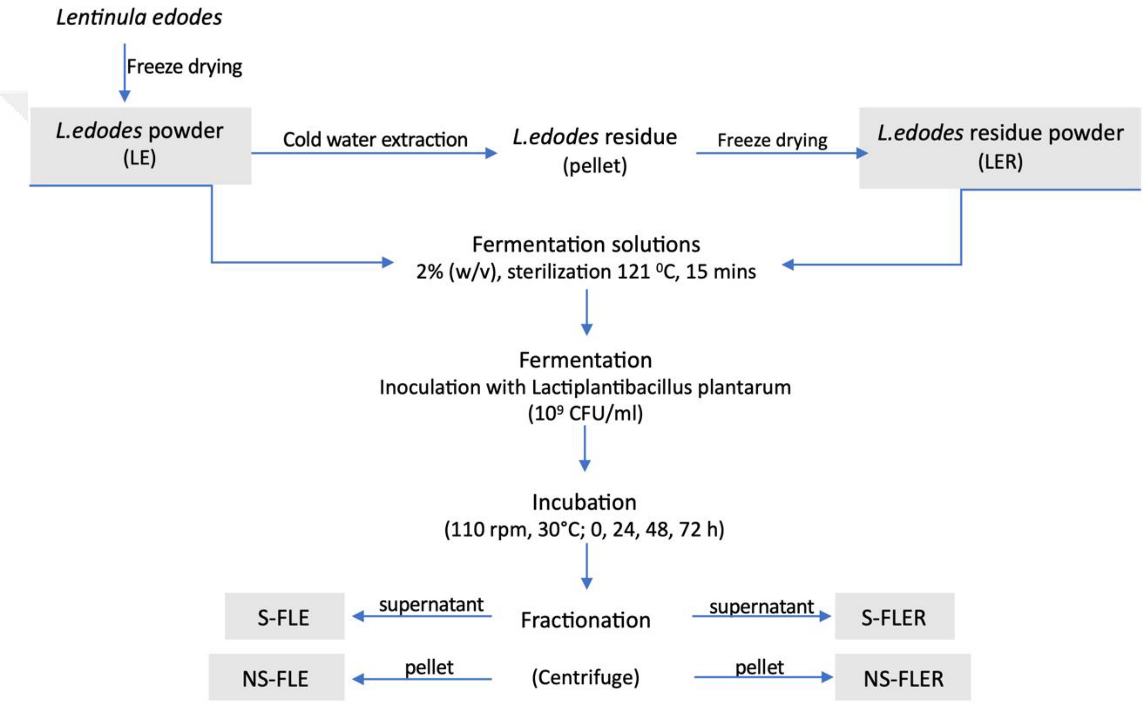


**Figure 3.1 :** Production of fermented LE and LER fractions. S-FLE: soluble fraction of fermented *L. edodes*, NS-FLE: non-soluble fraction of fermented *L. edodes*, S-FLER: soluble fraction of fermented *L. edodes* residue, NS-FLER: non-soluble fraction of fermented *L. edodes* residue.

### 3.3.4 Microbial analysis

Microbial composition during fermentation was evaluated by measuring the pH and counting the number of LAB. The standard plate count method analysed LAB counts in mushroom samples at specific time points (0, 24, 48, and 72 h). Serial dilutions were prepared using physiological water. Using the spread plate method, 100 µL diluted aliquots were plated onto MRS agar plates and then cultured at 30°C for 48 h. Finally, the microbial population was expressed as logarithmic colony-forming units per mL (log CFU/mL).

### 3.3.5 Fermentation metabolites

The fermentation metabolites of mushrooms were quantified across all solubility fractions at specific time points (0, 24, 48, and 72 h) using pre-column high-performance liquid chromatography (HPLC). The metabolite analysis employed an LC system (Agilent LC 1260 Infinity II, Gent, Belgium) equipped with a column (Agilent Hi-Plex 300 × 7.7 µm, 8 µm particle, Gent, Belgium). The column was maintained at 60◦C, the diode array detector was set to 210 nm, and the refractive index detector was set to 55 °C. A fixed flow rate of 0.7 mL/min was combined with a 5 mmol/L sulfuric acid mobile phase, and 20 µL injection volumes were used. Samples were prepared by mixing 1 g of fermented LE and LER samples with 5 mL of 5 mmol/L sulfuric acid using an Ultraturrax (IKA-T18, Staufen, Germany) for 1 min at 10.000 rpm at 21 °C. Following, the probe was washed with 5 mL of 5 mmol/L sulfuric acid and centrifuged (Hermle Z 366 K, Wehingen, Germany) at 4000 rpm for 10 min at 21°C. The resulting mixture was then filtered through a 0.45 µm disc filter, and the supernatant was collected and preserved at − 40 ◦C until analysis. The quantification of sugars and organic acids was based on calibration curves established for each compound by injecting known concentrations of external standards. Finally, the results were expressed as mg per g dry fermented mushrooms.

### 3.3.6 Metabolites analysis by UPLC-QTOF/MS

The pellet samples prepared in Section 2.5 were also analysed using an ultrahigh-performance liquid chromatography system (UPLC Infinity 1290, Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole time-of-flight mass spectrometer (UPLC-QTOF/MS) (Q-TOF 6546, Agilent Technologies). The column and mobile phase conditions were the same as those outlined in Section 2.5. The injection of 20 µL for each sample was performed. Mass spectrometry data were acquired in the full-scan mode with an *m/z* range of 100-1700. Peak identification was carried out in positive modes, while instrument control, data acquisition, peak alignment, and processing were performed using the MassHunter software (Qualitative Analysis, Agilent Technologies, Santa Clara, CA, USA).

### 3.3.7 Fourier transform infrared spectrum (FTIR) analysis

Fourier transform infrared spectrometer (Nicolet IS-20 FTIR, Thermo Scientific) coupled with a DTGS detector was employed to acquire sample spectra in the mid-infrared region, spanning from 4000 to 400 $cm^{-1}$, with a resolution set at 4 $cm^{-1}$ and 32 scans. The obtained spectra were processed using OMNIC 9 software (Thermo Scientific), facilitating smoothing and baseline correction. To generate the spectra, 2 mg (± <0.05 mg) of each homogenised sample from fractions of 0, 24, 48, and 72 h fermented NS-FLE and NS-FLER was placed on the diamond window of the FTIR spectrometer. Prior to acquiring the spectral data for each sample, a background air spectrum was collected.

### 3.3.8 Protein behaviour

#### 3.3.8.1 Soluble protein concentration

Protein concentration was assessed using the Lowry method, employing a bovine serum albumin (BSA) standard curve (Lowry et al., 1951). In detail, 5 mL of reagent 1 comprising 100 mL of 2.0% (w/v) sodium carbonate in 0.1N sodium hydroxide, 1 mL of 2.7% (w/v) sodium potassium tartrate, and 1 mL of 1.0% (w/v) copper sulfate was prepared and added to the fractions of 0, 24, 48, and 72 h fermented S-FLE and S-FLER. The mixture was incubated for 10 min. Subsequently, 0.5 mL of freshly prepared reagent 2 (diluted Folin-Ciocalteu at a 1:1 ratio) was added to each sample, followed by a 30-minute incubation at room temperature. The absorbance of the standards and samples was measured at 700 nm (UV-1800, Shimadzu). Protein concentrations in samples were expressed as mg BSA per gram of dry fermented mushrooms, resulting in a limit of detection (LOD) and a limit of quantification (LOQ) of 0.06 and 0.19 mg/mL, respectively.

#### 3.3.8.2 Determination of free amino group content

The free amino group ($NH_2$) content was determined according to the method reported by (Adler-Nissen (1979). Fractions of 0, 24, 48, and 72 h fermented S-FLE and S-FLER were dissolved in 1% SDS solution. Subsequently, 0.12 mL of the sample solutions were added to a test tube, followed by the addition of 1 mL sodium phosphate buffer (0.21 M, pH 8.2) and 1 mL of 1 mM TNBS. The mixture was shaken and incubated in the dark at 50°C for 1 h in a water bath. The reaction was stopped by acidifying with 2 mL of 0.1 N HCl and cooled before absorbance was measured at 340 nm (UV-1800 Shimadzu). Free amino groups were quantified against an L-leucine (0–3 mM) standard curve, and the results are expressed as millimolar free amino groups per gram of dry fermented mushrooms, resulting in LOD and LOQ of 0.09 and 0.29 mM, respectively.

#### 3.3.8.3 Protein profiling by gel electrophoresis of hydrolysates

To determine the protein/peptide profile of the fermented insoluble mushroom fractions, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted using a Criterion TGX Stain-Free Precast 12% gel (Bio-Rad Laboratories, Hercules, CA). Fermented NS-FLE and NS-FLER samples were mixed with Laemmli sample buffer (pH 6.8) containing 65.8 mM Tris-HCl, 2.1% SDS, 26.3% (w/v) glycerol, 0.01% bromophenol blue, and 2% (v/v) β-mercaptoethanol. After heating at 95°C for 5 min, the mixture was centrifuged at 4500 rpm for 10 min at room temperature. Electrophoresis was performed in a Bio-Rad Criterion cell (Bio-Rad Laboratories, Hercules, CA, USA) after loading 10 µg of supernatant per lane in the gel with SDS-PAGE running buffer 10×Tris/Tricine/SDS buffer (100 mM Tris, 100 mM Tricine, 0.1% SDS). The electrophoresis conditions included a constant voltage of 300 V and a starting current of 135 mA/gel for 25 min. Precision Plus Protein™ Unstained Protein Standards, ranging from 2-10 to 250 kDa, were run on the gel for reference. GelDoc Go Imaging system (Bio-Rad Laboratories, Hercules, CA) was used for gel image acquisition.

### 3.3.9 Phenolic content

The total phenolic content in the phenolic extracts of fermented soluble LE and LER fractions was assessed using the Folin-Ciocalteu method (Singleton et al., 1999). In this procedure, 0.1 g of freeze-dried S-FLE or S-FLER was dissolved in 5 ml of 90% methanol, followed by centrifugation and filtration before analysis. Then,1 mL of each extract was mixed with 0.5 mL–Folin-Ciocalteu reagent diluted tenfold, neutralised with 20% sodium carbonate (1.5 mL), and incubated for 2 h at room temperature. The absorbance at 760 nm was measured using a spectrophotometer (UV-1800, Shimadzu). TPC was calculated using gallic acid calibration curves ($R^2 > 0.99$) and the results were expressed as mg gallic acid equivalent (GAE) per g dry fermented mushrooms, resulting in LOD and LOQ of 0.18 and 0.56 mg/L, respectively.

### 3.3.10 Antioxidant activity

The method devised by Kumaran & Joel Karunakaran (2007) was used to evaluate antiradical activity against DPPH. Briefly, 100 µL extracts, obtained as mentioned in 3.3.2 section, were mixed with 0.1mM DPPH solution (2 mL), vortexed for 10 s, and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm (UV-1800, Shimadzu). DPPH free radical-scavenging capacity was calculated by measuring the extent of decolourization of the DPPH, with a blank (instead of sample methanol used) serving as a reference. Calculation of the DPPH was performed as follows,

$$DPPH\ (\%) = \left(Abs_{blank} - Abs_{sample}\right) / Abs_{blank} \times 100 \tag{3.1}$$

where $Abs_{sample}$ and $Abs_{blank}$ represent the absorbance at 517 nm of the tested mushroom samples and blank.

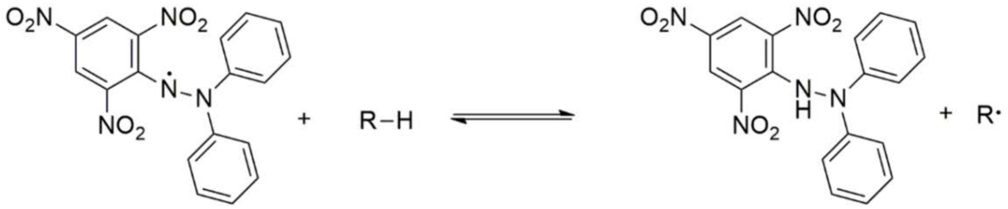


**Figure 3.2 :** Reaction between DPPH and a hydrogen donor (R-H) as an antioxidant (Bedlovičová et al., 2020).

The ABTS radical scavenging capacity was evaluated by using the method developed by Re et al., (1999). The ABTS compound was diluted with distilled water to obtain a stock solution with a concentration of 7 mM. Then, the ABTS radical cation was created by mixing the ABTS stock solution with 2.45 mM potassium persulfate and then left in the dark at room temperature for approximately 14 hours. Prior to use, the ABTS radical cation with an absorbance of 0.70 ± 0.02 at 734 nm was prepared by diluting it with 90% methanol. To conduct the assay, 2 mL of the fresh ABTS radical cation solution and 20 µL of sample extracts, obtained as mentioned in section 3.3.3., or Trolox standard were mixed and vortexed, followed by a 5-minute incubation period in the dark at 25°C, measured at 734 nm. ABTS scavenging capacity was calculated by measuring the extent of decolourization of the ABTS, with a blank (instead of sample methanol used) serving as a reference.

Calculation of the ABTS was performed as follows,

$$ABTS\ (\%) = \left(Abs_{blank} - Abs_{sample}\right) / Abs_{blank} \times 100 \tag{3.2}$$

where $Abs_{sample}$ and $Abs_{blank}$ represent the absorbance at 734 nm of the tested mushroom samples and blank.

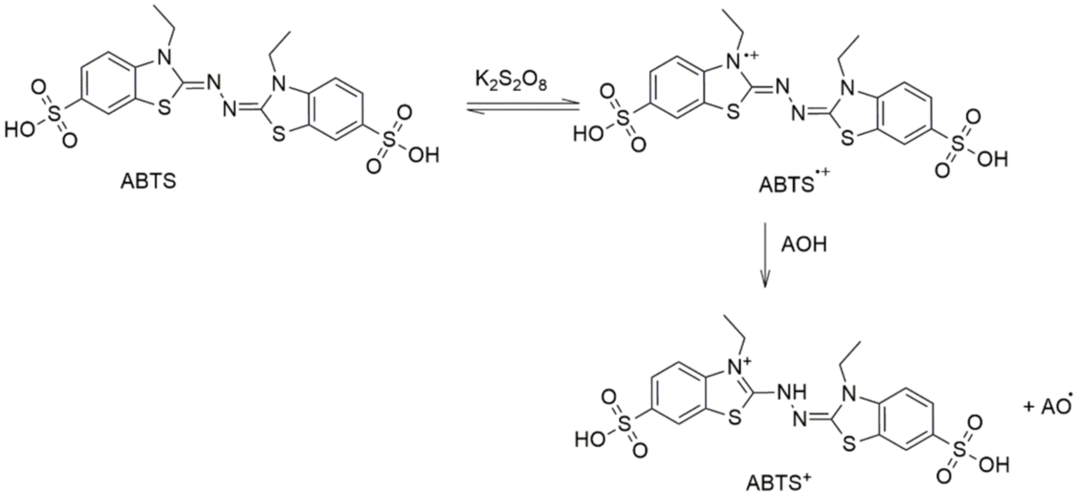


**Figure 3.3 :** Generating of ABTS working solution and observing its reaction with an antioxidant (AOH) (Bedlovičová et al., 2020).

### 3.3.11 Absorption capacities

#### 3.3.11.1 Water holding capacity

The determination of water-holding capacity (WHC) in fermented mushrooms followed the procedure outlined by Raghavendra et al. (2004). Initially, 250 mg of fermented LE and LER were mixed with 25 mL of distilled water and hydrated for 1 h at room temperature with orbital shaking at 300 rpm. Post-hydration, the mixture was centrifuged for 10 min at 5770 × g, and any remaining unabsorbed water was drained. The resulting residue was oven-dried at 103°C for 2 h. Finally, the WHC was calculated by dividing the absorbed water quantity by the weight of the fermented mushroom, as follows,

$$WHC = \left(M_{hydrated} - M_{dried}\right) / M_{hydrated} \tag{3.3}$$

#### 3.3.11.2 Oil holding capacity

The oil-holding capacity (OHC) of the fermented mushrooms was determined following the procedure outlined by Chau and Huang (2003). Initially, 250 mg of fermented LE and LER were precisely weighed and recorded as $M_{LE}$ and $M_{LER}$, respectively. Subsequently, a suspension was prepared by adding 25 mL of sunflower oil to the fermented LE and LER and shaking at room temperature with an orbital motion set at 300 rpm for 1 h. After the absorption period, the suspension was centrifuged for 10 min at 5770 × g to separate any residual unabsorbed oil. The unabsorbed oil residues were meticulously drained and the resulting residues were accurately weighed and recorded as $M_{oiled}$. Finally, OHC was calculated by dividing using the following formula,

$$OHC = M_{oiled} - M_{LE/LER} \tag{3.4}$$

### 3.3.12 Statistical analysis

Volcano plots were constructed using the Agilent Mass Profiler Professional (MPP, Agilent Technologies), employing the Benjamini-Hochberg method for statistical significance. Statistical analyses were performed using the SPSS version 28.0 program (IBM SPSS Statistics, SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was performed to investigate variations throughout mushroom fermentation. Subsequently, to compare the means and identify significant differences, a multiple range test, specifically Tukey's Honestly Significant Difference (HSD) test, was applied. A significance level of $p < 0.05$ was adopted for significant differences. Correlations between TPC and antioxidant activity were evaluated using Pearson's correlation coefficient test. The reported values are expressed as mean ± standard deviation (SD) based on three independent samples.

## 3.4 Results

### 3.4.1 The growth of *Lb. plantarum* LMG 17673 and pH changes

Changes in viable cell counts and pH values within the fermented LE and LER inoculated with *Lb. plantarum LMG 17673* during 72 h of fermentation are shown in Figure 3.4. *Lb. plantarum* was able to grow in the mushroom solutions of LE and LER (Figure 3.4a). The initial number of viable bacteria for *Lb. plantarum LMG 17673* in

LE and LER solutions was recorded as 7.62 ± 0.04 and 7.09 ± 0.07 log CFU/mL, respectively. After 72 h of fermentation, the viable cell counts in LE and LER were 5.15 ± 0.3 and 8.33 ± 0.19 CFU/mL, respectively. The high cell survivability of *Lb. plantarum LMG 17673* in LER fermentation indicates its adaptability to the LER environment. The viable bacteria counts in LE solutions were considerably low at the end of fermentation, potentially attributed to the low pH (3.88 ± 0.12). After 24 h of fermentation, the highest growth rate was observed for both mushroom samples; thereafter, it decreased in the fermented mushrooms. The populations of LAB are known to decrease due to the inhibitory effects of factors such as low pH, high concentrations of organic acids, and reduced availability of carbohydrates (Pau et al., 2022).

The pH of mushroom samples decreased during fermentation with *Lb. plantarum LMG 17673* (Figure 3.4b). LE and LER exhibited the most rapid decrease in pH during the initial 24-hour fermentation. The pH values of LE and LER dropped to 3.88 and 4.31 at the end of the fermentation, respectively. The pH is a critical factor in lactic acid fermentation, and severe acidic conditions, like those found in LE, can contribute to decreased viable bacteria counts (Pau et al., 2022). The pH range results were similar to the findings of pickled Agaricus bisporus mushrooms via Lb. plantarum LAB fermentation with a pH of 3.3 to 4.6 (Jabłońska-Ryś et al., 2022). The low pH can inhibit the growth of pathogenic bacteria in samples and extend the shelf life besides maintaining anaerobic conditions *Low* (Y. Liu et al., 2016). Furthermore, the observed decrease in pH during fermentation is linked to the accumulation of organic acids, especially lactic acid (Yang, Sun, Chen, et al., 2022). Moreover, the production of organic acids and the resulting pH levels varyexhibit variability depending on the specific composition of the fermentation substrates, fermentation conditions such as temperature, andor the additives used in the fermentation (X. Liu et al., 2023).

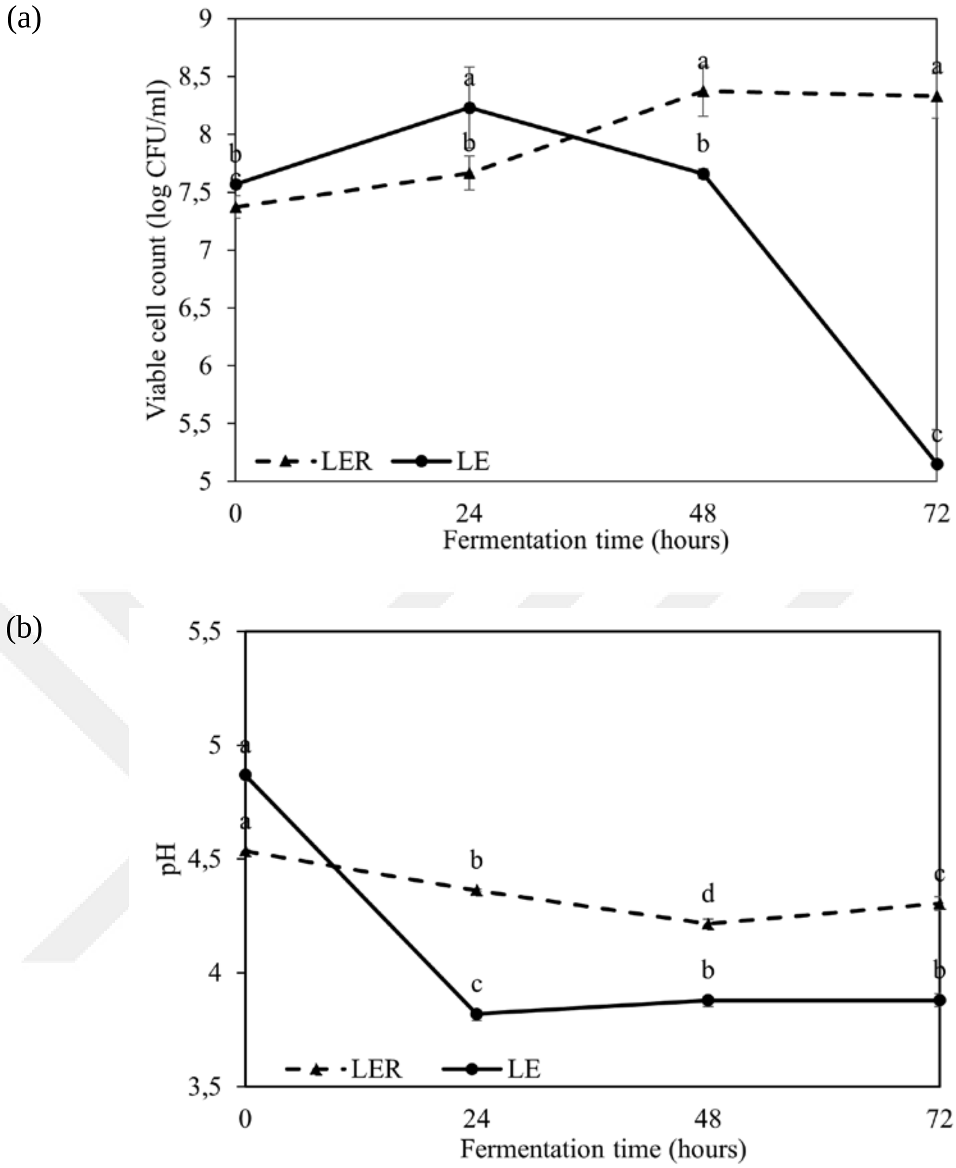


**Figure 3.4 :** Microbial activity of *Lb. plantarum* (a) and pH (b) throughout fermentation of LE and LER. CFU: colony forming unit.

### 3.4.2 Changes in the organic acid levels during *Lb. plantarum* fermentation

The changes in organic acids and sugars in LE and LER fermented with *Lb. plantarum* for 72 h are presented in Table 3.1. *Lb. plantarum* has the ability to process *L. edodes* (LE and LER) matrixes and generate organic acids and the accumulation of these organic acids can lower the pH of the fermented mushroom matrixes (Z. Chen, Fang, et al., 2021).

The content of formic acid, malic acid, lactic acid, citric acid, fumaric acid, and succinic acid in the samples was assessed. The level of organic acids was consistently higher in the supernatant than in the pellet throughout the fermentation process.  The total organic acid content showed a sharp increase followed by a period of slight increase or stability. The first 24 hours of fermentation marked a critical point for the alteration in organic acid content, which is consistent with our earlier determination of pH values in the fermentation matrixes. The LER samples had lower concentrations of organic acids compared to the LE samples. Lactic acid was the most abundant organic acid at the end of fermentation of LE and LER. During fermentation, there was a notable increase in the levels of lactic acid and formic acid across all fractions. On the other hand, organic acids can act as a carbon source, affectingimpacts the level of acidification (Perveen et al., 2023). Concurrently, a corresponding decrease was observed in the concentrations of malic acid, citric acid, fumaric acid, and succinic acid. This trend is consistent with findings reported by Yang, Sun, Gao, et al., (2022), who fermented apple juice using LAB strains. Malic acid can be converted to lactic acid with the help of the malolactic enzyme produced by LAB. The decomposition of citric acid may have caused the decreased citric acid content into various products such as diacetyl, lactic acid, and acetic acid. The trends observed in the fumaric and succinic acid contents in the fermented samples were similar to those of malic and citric acids.

### 3.4.3 Changes in the sugar levels during *Lb. plantarum* fermentation

The changes in free sugars in mushroom fractions fermented by *Lb. plantarum* for 72 h are presented in Table 3.2. The main mushroom monosaccharides, disaccharides, and sugar alcohols were glucose, ribose, trehalose, and mannitol, respectively, as has been reported for other edible mushrooms (Jabłońska-Ryś et al., 2019). During the fermentation of LE and LER, the free sugar concentration decreased. Previous studies have also reported decreased free sugar content in fermented foods such as cabbage during the initial fermentation stage (Lee et al., 2021).

**Table 3.1 :** The alterations in organic acids in LE and LER fermented with *Lb. plantarum* during 72 h.

| Compounds | Fractions | *Lentinula edodes* | | | | *L. edodes* residue | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fermentation Times (hours) | | | | | | | | LOD (mg/mL) | LOQ (mg/L) |
| | | 0 | 24 | 48 | 72 | 0 | 24 | 48 | 72 | | |
| Malic acid | pellet | 4.12±0.21 | ND | ND | ND | 2.01±0.15$^{a}$ | 0.54±0.00$^{b}$ | 0.45±0.02$^{b}$ | ND | 6.19 | 20.64 |
| | supernatant | 50.60±2.08$^{a}$ | 2.82±0.36$^{b}$ | 1.64±0.26$^{c}$ | ND | 35.46±0.32$^{a}$ | 12.66±0.18$^{b}$ | 13.31±0.24$^{b}$ | 12.88±0.17$^{b}$ | | |
| Succinic acid | pellet | 8.37±0.78$^{a}$ | 1.00±0.51$^{b}$ | 1.28±1.13$^{b}$ | 0.42±0.04$^{c}$ | 11.76±1.03$^{a}$ | 2.80±0.09$^{b}$ | 2.35±0.19$^{b}$ | 3.54±2.08$^{b}$ | 4.02 | 13.39 |
| | supernatant | 169.32±7.44$^{a}$ | 8.41±0.13$^{b}$ | 10.60±3.09$^{b}$ | 8.22±0.08$^{b}$ | 169.84±0.18$^{a}$ | 55.56±0.83$^{b}$ | 44.94±1.42$^{c}$ | 47.67±2.24$^{c}$ | | |
| Fumaric acid | pellet | 0.13±0.05 | ND | ND | ND | ND | ND | ND | ND | 2.00 | 6.66 |
| | supernatant | 0.98±0.10 | ND | ND | ND | 0.59±0.00$^{a}$ | 0.33±0.01a | D | D | | |
| Citric acid | pellet | 2.05±0.47$^{a}$ | 1.13±0.33$^{b}$ | 1.07±0.20$^{b}$ | 1.03±0.15b | 0.68±0.01$^{a}$ | 0.47±0.00$^{b}$ | 0.49±0.01$^{b}$ | D | 6.48 | 21.61 |
| | supernatant | 8.67±0.24$^{a}$ | 3.22±0.27$^{b}$ | 4.20±2.34$^{b}$ | 3.56±0.46$^{b}$ | ND | 7.66±0.24$^{b}$ | 9.62±0.20$^{a}$ | 7.63±0.16$^{b}$ | | |
| Lactic acid | pellet | 18.02±1.87$^{b}$ | 130.08±7.8$^{a}$ | 111.51±14.03$^{a}$ | 112.43±7.34$^{a}$ | ND | 10.31±0.38$^{a}$ | 6.07±0.58$^{b}$ | 4.80±1.36$^{c}$ | 6.88 | 22.92 |
| | supernatant | 88.27±3.88$^{d}$ | 695.37±0.55$^{a}$ | 463.24±5.20$^{c}$ | 585.61±1.01$^{b}$ | ND | 210.67±3.65$^{a}$ | 160.70±4.14$^{b}$ | 160.98±9.05$^{b}$ | | |
| Formic acid | pellet | 1.87±0.44$^{a}$ | 2.22±0.50$^{a}$ | 2.11±0.03$^{a}$ | 1.94±0.44$^{a}$ | 0.75±0.08$^{a}$ | ND | ND | 0.40±0.03$^{b}$ | 1.84 | 6.12 |
| | supernatant | 8.60±0.24$^{c}$ | 11.42±0.16$^{b}$ | 14.02±4.23$^{a}$ | 10.41±0.16$^{b}$ | 5.91±0.07$^{a}$ | 3.22±0.11$^{b}$ | 6.75±0.18$^{a}$ | 5.96±0.10$^{a}$ | | |
| Total | pellet | 34.57±0.64$^{c}$ | 134.42±4.87$^{a}$ | 115.98±2.56$^{b}$ | 115.83±1.33$^{b}$ | 15.20±0.21$^{a}$ | 14.12±0.08$^{a}$ | 9.36±0.14$^{b}$ | 8.74±0.57$^{b}$ | | |
| | supernatant | 326.44±2.33$^{d}$ | 721.25±0.24$^{a}$ | 493.69±10.0$^{c}$ | 607.80±0.29$^{b}$ | 211.80±0.09$^{c}$ | 290.10±0.84$^{a}$ | 235.32±1.01$^{b}$ | 235.12±1.96$^{b}$ | | |

Values are expressed as averages ± standard deviation ($n$ = 3). Different lowercase superscript letters within the same line indicate significant differences in the same mushroom fraction between means at $p < 0.05$. Acetic acid ND in all samples. LOD: Limit of detection; LOQ: Limit of qualification.

**Table 3.2 :** The alterations in sugars in LE and LER fermented with *Lb. plantarum* during 72 h.

| Compounds | Fractions | *Lentinula edodes* | | | | *L. edodes* residue | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fermentation Times (hours) | | | | | | | | LOD (mg/mL) | LOQ (mg/L) |
| | | 0 | 24 | 48 | 72 | 0 | 24 | 48 | 72 | | |
| D(+)glucose | pellet | 4.28±0.35 | D | D | ND | 5.89±0.40 | ND | ND | ND | 26.30 | 79.72 |
| | supernatant | 28.66±0.85 | D | ND | ND | 77.53±9.26 | ND | ND | ND | | |
| D(-)mannitol | pellet | 34.12±0.31$^a$ | 3.54±0.15$^b$ | ND | ND | 9.81±0.70$^a$ | 6.23±0.05$^b$ | 5.80±0.08$^b$ | 4.18±0.05$^b$ | 43.79 | 132.70 |
| | supernatant | 187.46±5.86$^a$ | 20.14±0.11$^b$ | 2.93±0.41$^c$ | ND | 131.20±0.04$^a$ | 117.44±0.09$^b$ | 119.01±0.38$^b$ | 113.52±0.48$^b$ | | |
| D(-)ribose | pellet | 7.24±0.01$^a$ | 5.54±0.20$^b$ | 6.18±0.21$^b$ | 5.80±0.38$^b$ | 1.97±0.13$^a$ | 1.44±0.04$^b$ | 1.60±0.13$^b$ | 1.03±0.02$^c$ | 29.57 | 74.72 |
| | supernatant | 43.99±0.66$^a$ | 29.96±0.13$^b$ | 42.58±8.63$^a$ | 31.44±0.11$^b$ | 27.01±0.10$^a$ | 28.21±0.13$^a$ | 29.73±0.09$^a$ | 27.70±0.08$^a$ | | |
| Trehalose | pellet | 1.72±0.28$^a$ | 0.86±0.15$^b$ | ND | ND | ND | ND | ND | ND | 29.58 | 89.63 |
| | supernatant | 8.57±0.34 | ND | ND | ND | 2.08±0.03 | D | D | D | | |
| Total | pellet | 47.36±0.24$^a$ | 9.94±0.12$^b$ | 6.18±0.05$^b$ | 5.80±0.1$^b$ | 17.66±0.31$^a$ | 7.66±0.02$^b$ | 7.39±0.05$^b$ | 5.22±0.02$^c$ | | |
| | supernatant | 268.68±1.93$^a$ | 50.10±0.06$^b$ | 45.51±2.26$^b$ | 31.44±0.03$^c$ | 237.82±2.3$^a$ | 145.65±0.06$^b$ | 148.74±0.12$^b$ | 141.22±0.14$^b$ | | |

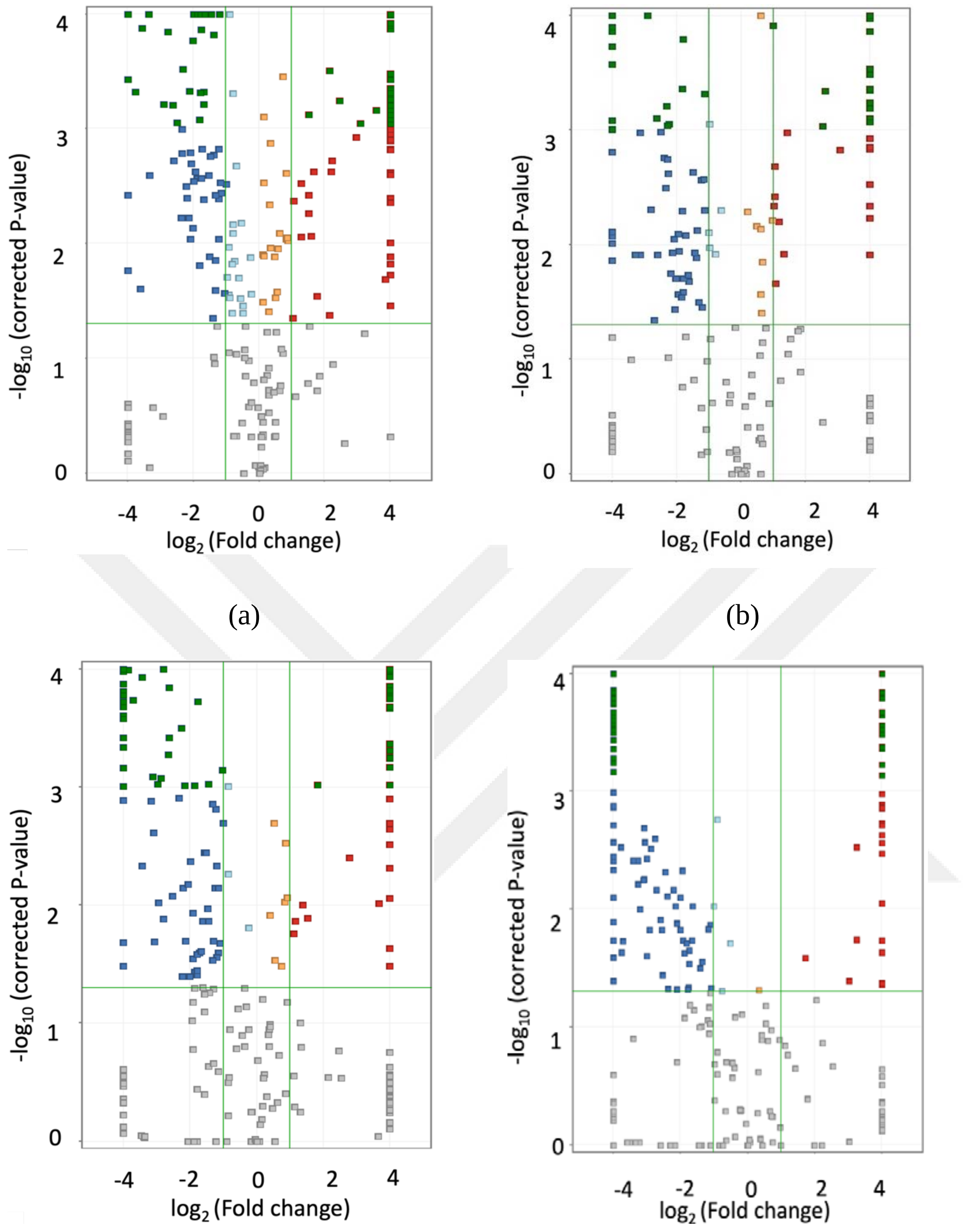
Values are expressed as averages ± standard deviation ($n = 3$). Different lowercase superscript letters within the same line indicate significant differences between means at $p < 0.05$. LOD: Limit of detection; LOQ: Limit of qualification.

In this study, the sugar level was higher in the supernatants of all fractions. Trehalose and glucose levels decreased rapidly at 24 h and were completely consumed at the end of the fermentation period, similar to that reported for button mushrooms (Jabłońska-Ryś et al., 2022). The decrease in free sugars, especially glucose, was primarily attributed to the consumption of sugar by LAB during LE and LER fermentation, rather than the mushrooms releasing a higher amount of free sugars (Lee et al., 2021). During the initial stages of fermentation, four different sugars were detected in both LE and LER fractions. However, after 72 h of fermentation, ribose was the only sugar found in both the supernatant and pellet fractions of LE and LER. In addition, during fermentation, the ribose content in fermented LE and LER showed certain fluctuations. The ribose content in both the supernatants and pellets decreased at 24 h, increased at 48 h, and then decreased again. A previous study also reported a similar finding (Jabłońska-Ryś et al., 2022), in which the ribose content in button mushroom fruiting bodies and brine fluctuated similarly during lactic acid fermentation.

Mannitol, a sugar alcohol, was found to be the most abundant sugar at the beginning of fermentation in the supernatant of fermented mushrooms. Although the consumption of mannitol was complete during LE fermentation, there was comparatively less consumption of mannitol during LER fermentation. Specifically, the mannitol level in the supernatant decreased rapidly in the first 24 h and remained stable until the end of the fermentation period. It is known that *Lb. plantarum* produces mannitol from glucose, the relatively stable mannitol levels in the LER during fermentation could be attributed to the production by *Lb. plantarum* (Martínez-Miranda et al., 2022).

### 3.4.4 Comparative analysis of differential metabolites

Untargeted metabolites were analyzed using UPLC-Q-TOF-MS datasets to investigate the effect of fermentation on the features of LE and LER. LE and LER were compared at certain fermentation time points and features that met the criteria of FC ≥ 2.0 and $p < 0.05$ or $p < 0.001$ were identified as differential features ( Figure 3.5). The horizontal green line represents the p-test limit, and the features positioned above this line were considered statistically significant ($p < 0.05$). The vertical green lines represent the fold-change limits. The volcano plot shows the passing features (coloured) and non-passing features (grey).



(a)

(b)

(c)

(d)

**Figure 3.5 :** V-plot of fermented LE and LER fractions; (a) Comparision of LE and LER features at 0 h; (b) at 24 h; (c) at 48 h; (d) at 72 h. LE: *Lentinula edodes*, LER: *Lentinula edodes* residue. The colours in the figure have the following meanings: grey squares are metabolites with no significant difference between samples at different fermentation times, pale blue or orange squares: are features that passed the significance test but did not meet the fold-change cut-off criteria, red squares are upregulated features, blue squares are down-regulated features ($p < 0.05$ and FC ≥ 2.0) and, green squares in the upper right and left corners are features ($p < 0.001$ and FC ≥ 2.0).

Each square, coloured pale blue or orange, corresponds to a feature that has successfully passed the significance test yet failed to meet the fold-change cut-off criteria. Differential features for each pairwise group of LE-LER comparisons were visualised and analysed using volcano plots, resulting in the selection of 252 significant differential features at 0 h, 222 at 24 h, 211 at 48 h, and 157 at 72 h. These findings suggest that there are significant molecular and metabolic differences between LE and LER through LAB fermentation, which can affect nutrient utilisation and metabolite production (J. J. Wang et al., 2023). This study also observed the impact of the LER-generating extraction process on the fermentation process.

### 3.4.5 Fourier transform infrared spectra

Fourier transform infrared (FT-IR) spectra of the fermented pellets of LE and LER are shown in Figure 3.6. The FT-IR spectrum displayed distinctive bands for carbohydrates, proteins, water, and aromatic compounds. Polysaccharides and polysaccharide-peptide complexes were the most abundant compounds in all fractions based on their characteristic bands. The FTIR spectrum indicated that samples with different fermentation times exhibited similar characteristic absorption peaks at ~3400, 2900, 1650, 1400, and 1000 cm$^{-1}$. The broad band at ~3400 cm$^{-1}$ can be attributed to the stretching vibrations of the $H_2O$, O–H, and N–H groups (C. Yin et al., 2018). Compared to LER, LE showed a decrease in the absorption peak at approximately 2900 cm$^{-1}$ which may due to the stretching vibrations of C–H groups (Baeva et al., 2019).

The Amide I and Amide II bands, located at ~1645 cm$^{-1}$ and 1530 cm$^{-1}$, respectively, indicated the presence of proteinaceous residues in the samples. The Amide I band arises from C=O stretching vibrations, while the Amide II band results from the bending vibrations of the N-H groups (Synytsya et al., 2009). The results of the analysis showed distinct absorptions in the range 1300-1800 cm$^{-1}$, which are typically associated with the presence of carbohydrates (Nascimento Santos et al., 2014). Additionally, the region between 500 and 1000 cm$^{-1}$ mainly corresponds to polysaccharides, including β-d-glucans and the pyranose form of glucose (Feher et al., 2021). Furthermore, the region between 950-1190 cm$^{-1}$ region exhibits strong absorption peaks attributed to the stretching vibrations of C-O-C, COH, and C-C.

The bands at ~930 cm$^{-1}$, 840-860 cm$^{-1}$, and ~760 cm$^{-1}$ can be associated with α-glycosidic bonds, indicating the presence of α-glucans. In addition, the spectra also revealed the presence of β-glycosidic bands between ~900 cm$^{-1}$, 1000, and 1100 cm$^{-1}$, specifically at 1045 cm$^{-1}$, indicating the existence of β-glucans due to O-substituted glucose residues. This is in agreement with previous studies, which found that polysaccharides from *L. edodes* contain both types of glycosidic bonds (Synytsya et al., 2009; Xue et al., 2019).

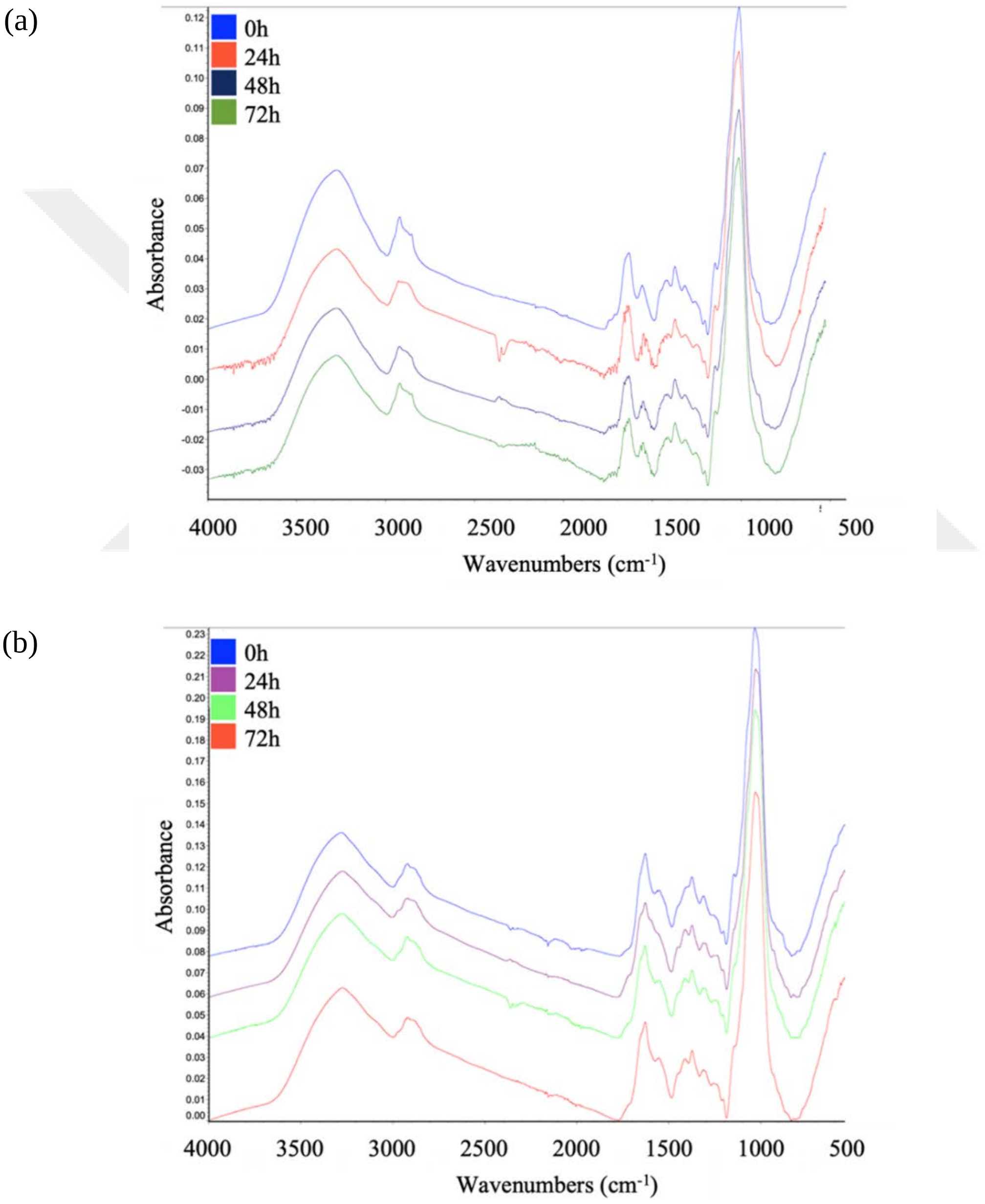


**Figure 3.6 :** Averaged and background subtracted FTIR spectra of different fractions during fermentation: (a) NS-FLE; (B) NS-FLER.

### 3.4.6 Protein behavior

The results of soluble protein content analysis were presented in Figure 3.7a. Initially, there was no significant difference ($p > 0.05$) in the protein content observed during the first 24 h for both S-FLE and S-FLER. However, at 48 hours, a notable increase in protein content was noted for both S-FLE and S-FLER, with values of 172.73 mg/g SF dm dm and 162.32 mg/g dm SF dm, respectively, which were significantly higher than earlier fermentation time points ($p < 0.05$).

The protein content is approximately 13–23% of the dry weight of shiitake mushrooms, which is significantly higher than that of ordinary vegetables (Morales et al., 2018). Notably, the highest protein content was obtained in LER, indicating a considerable amount of shiitake proteins to be water-insoluble, with most of the protein remaining in LER after water extraction. This is in accordance with the findings of Erjavec et al. (2012), who reported that approximately 90% of the protein content in *L. edodes* is water-insoluble. However, at the end of fermentation (72 h), soluble protein content tended to be the same as that at the beginning of fermentation ($p > 0.5$). The soluble protein concentration was also reported to remain steady in coix seeds inoculated with *Lb. plantarum* (H. Yin et al., 2020).

The results of the free amino group content analysis, aimed at determining any trends in smaller structures due to the absence of significant differences in soluble protein levels, are presented in Figure 3.7b. The highest amount of free amino group content ($–NH_2$) was observed at the beginning of LE fermentation. In addition, in the LER at 0 h, the quantity of the free amino groupsgroup was approximately half that of the amount of LE. This suggests that a significant number of *L. edodes* amino groups might be extracted with cold water, potentially due to the presence of water-soluble peptides, and amino acid derivatives in this fraction (Nie et al., 2020).

The amino group content in LE gradually decreased during fermentation. The noticeable decrease in free amino group content in LE may be due to the potential utilisation of these groups by microorganisms or their decomposition through deamidation during extended fermentation (Faithong et al., 2010).

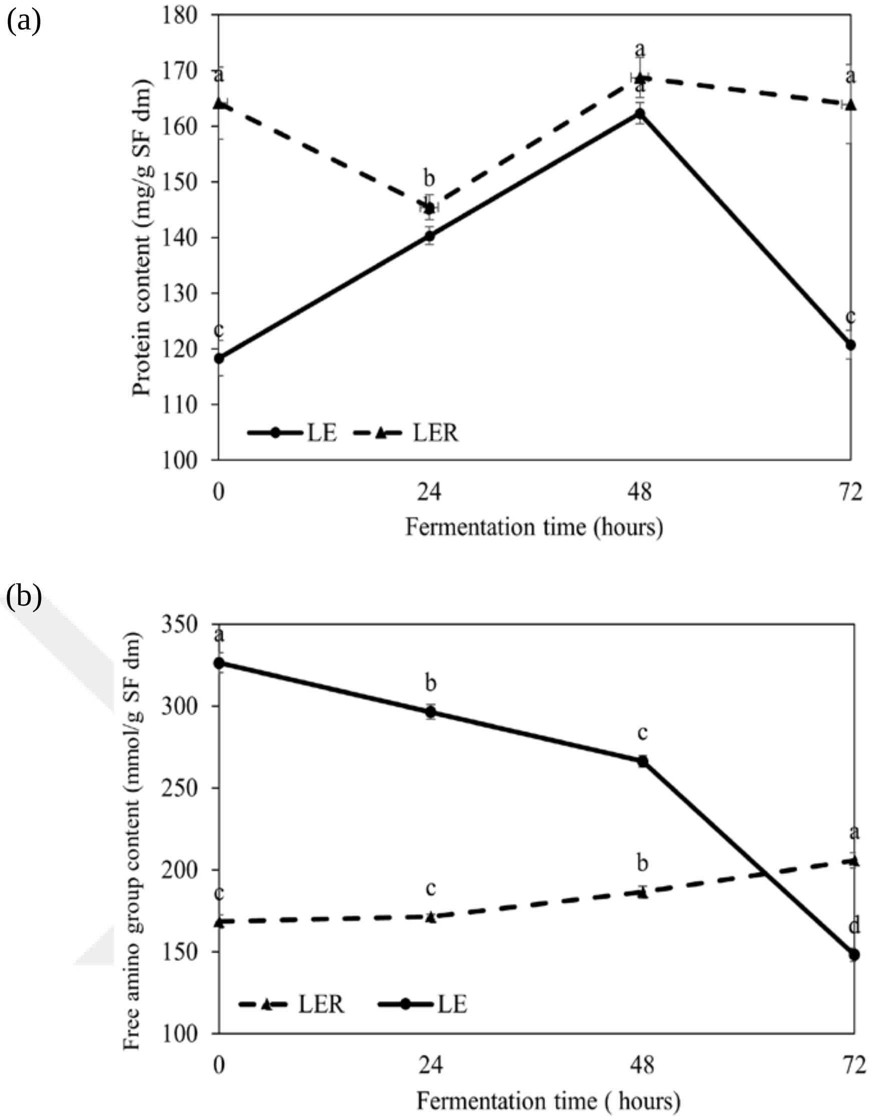


**Figure 3.7 :** (a) Soluble protein content of fermented LE and LER fractions; (b) Degree of hydrolysis of fermented LE and LER fractions. LE: *Lentinula edodes*, LER: *Lentinula edodes* residue; dm: dry matter. Different lowercase letters in the same mushroom fraction indicate significant differences between the various fermentation time points ($p < 0.05$). Values are presented as mean ± SD ($n = 3$).

However, the free amino group content in the LER increased during fermentation, which was attributed to peptide bond cleavage. This trend is in agreement with similar observations of protein degradation leading to elevated free amino groups that have been reported in various fermented products, including mealworms (Borremans et al., 2020), yoghurt (Tavakoli et al., 2019), suanyu, and fermented fish (W. Wang et al., 2017), and mao-tofu, fermented soybean (X. Zhao & Zheng, 2009). The increase in the free amino group content in fermented samples can be attributed to the proteolytic activity of bacteria during fermentation, which likely releases smaller polypeptides (<10 kDa), peptides, and free amino acids (Emkani et al., 2021). The elevated free amino group content suggests more extensive degradation in the fermented samples (Jia et al., 2021).

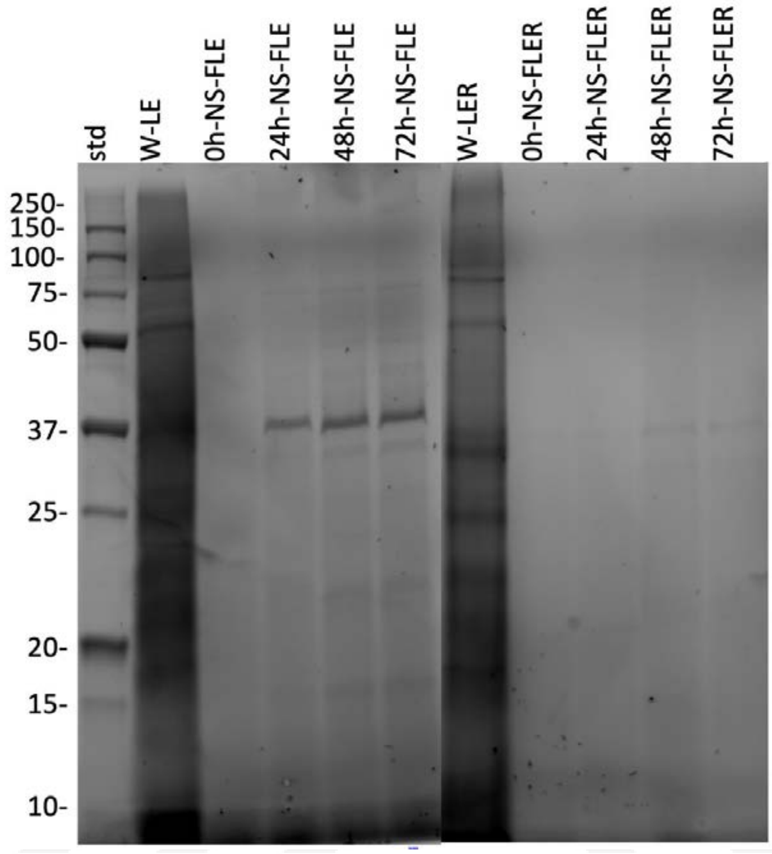


**Figure 3.8 :** SDS-PAGE of fermented and whole mushroom fractions. Lane std, molecular weight markers; Lane W-LE, unfermented whole *L. edodes*, Lane 0h-NS-FLE-Lane 72h-NS-FLE, different fermentation time points of fermented non-soluble LE fractions; Lane W-LER, unfermented whole *L. edodes* residue, Lane 0h-NS-FLER-Lane 72h-NS-FLER, different fermentation time points of fermented non-soluble LER fractions.

The molecular weight distribution of proteins in NS-FLE and NS-FLER fractions and unfermented whole *L. edodes* (W-LE) and *L. edodes* residue (W-LER) samples were examined using SDS-PAGE electrophoresis (Figure 3.8). To be able to see differences in smaller peptides, we also performed peptide SDS-PAGE analysis, revealing a diverse range of proteins with apparent molecular weights (Mw) ranging from 2 to 250 kDa in *L. edodes* fermented extracts (Figure 3.9). Nine differentially expressed proteins were identified in the fermented *L. edodes* extract, whereas 16 different proteins were observed in the mushroom extract. Consistent with previous findings by Hernández-Macedo et al. (2002), protein bands between 10 and 100 kDa were identified, including those at Mw= 10, 17, 21, 23, 29, 32, 57, 63, 68, 90, and 100.

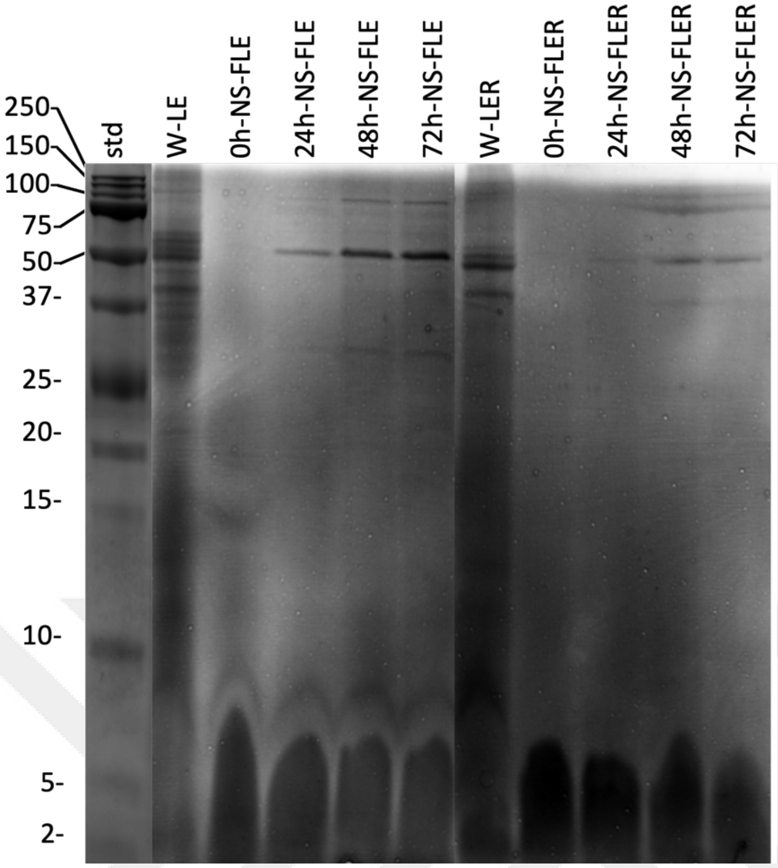


**Figure 3.9 :** Peptide SDS-PAGE of fermented and whole mushroom fractions. Lane std, molecular weight markers; Lane W-LE, unfermented whole *L. edodes*, Lane 0h-NS-FLE-Lane 72h-NS-FLE, different fermentation time points of fermented non-soluble LE fractions; Lane W-LER, unfermented whole *L. edodes* residue, Lane 0h-NS-FLER-Lane 72h-NS-FLER, different fermentation time points of fermented non-soluble LER fractions.

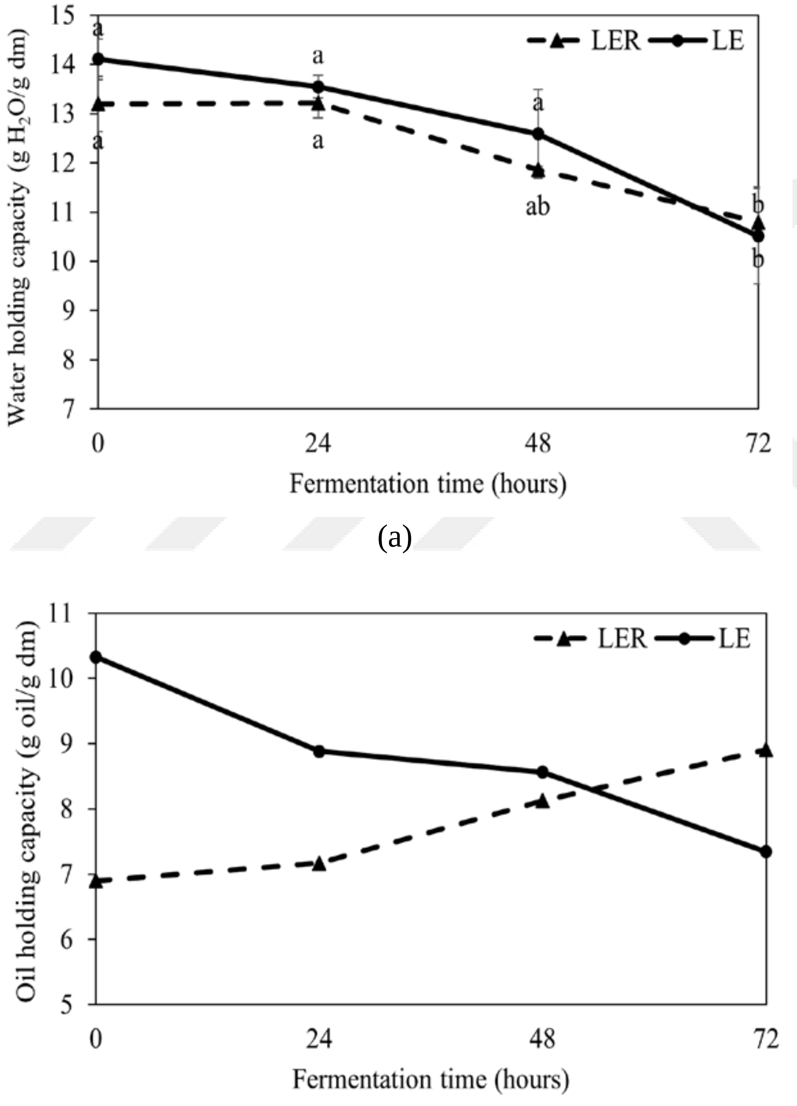
In Figure 3.9, bands corresponding to W-LE and W-LER can be observed at approximately 12, 18, 22, 27, 33, 43, 47, 92, 112, 151, and 245 kDa, with some of these bands also visible in the fermented LE fractions, but with slight shifting. However, further identification of these bands based on the available data remains challenging. During fermentation, some bands disappeared, particularly those with medium (25–70 kDa) and high (70–250 kDa) molecular weights, while others exhibited a decrease in molecular weight. For example, the high molecular weight band of W-LE (Mw=~112 kDa) gradually decreased to ~103 kDa (24h-NS-FLE), ~100 kDa (48-NS-FLE), and ~98 kDa (72-NS-FLE) during fermentation. The shift in molecular weight profiles of proteins indicates that higher molecular weight proteins are degrading into smaller polypeptides and peptides. This observation is consistent with the analysis of free amino group content in LE. The decrease in free amino groups is likely due to the proteolytic activity of *Lb. plantarum LMG 17673*, which also contributes to the degradation observed in SDS-PAGE. On the other hand, LER shows an increase in free amino groups because of the breakdown of peptide bonds during fermentation, which results in the release of smaller peptides and free amino acids. The SDS-PAGE analysis supports these findings by displaying that bands in W-LER tend to lower molecular weights with fermentation, displaying only faint bands mainly under . In contrast, LER fractions exhibited only faint bands with molecular weights smaller than 37 kDa during fermentation. The shift in molecular weight profiles of proteins indicates that higher molecular weight proteins are degrading into smaller polypeptides and peptides. This observation is consistent with the analysis of free amino group content in LE. The decrease in free amino groups is likely due to the proteolytic activity of

### 3.4.7 Water and oil holding capacity

The WHC and OHC of non-soluble fractions of LE and LER were displayed in Figure 3.10. The WHC and OHC of NS-FLER were lower than those of NS-FLE during 48 h fermentation. This discrepancy may be attributed to the lower soluble dietary fibre and proteins content in NS-FLER compared to NS-FLE. Soluble dietary fibre and proteins have a higher capacity to absorb water than insoluble dietary fibre (Shi et al., 2021; H. M. Zhao et al., 2017). The variations observed above in the soluble protein contents of LE and LER may account for the reduced protein levels in NS-LER. These changes likely contribute to the lower WHC and OHC observed in these fractions.The variations observed above in the soluble protein contents of LE and LER may account for the reduced protein levels in NS-LER. These changes likely contribute to the lower WHC and OHC observed in these fractions.

The WHC of all fermented samples was lower than that at the beginning of the fermentation (0 h). This decrease in WHC could be primarily attributed to the destruction of the cell walls and other supporting structures of *L. edodes* during fermentation (Su et al., 2020). Besides, despite NS-FLE showing higher values of WHC at the beginning of fermentation, the 72 h fermented LER and LE fractions were at the same level ($p>0.05$) (10.79 and 10.51 g $H_2O$/g dm, respectively) Additionally, the OHC of NS-FLE fractions was lower than at 0h, while the OHC of NS-FLER fractions become higher through fermentation. Compared with WHC, the OHC values of both fractions were much lower. The highest OHC values were found in NS-FLE at

0h of 10.32 g oil/g NS-FLE, while the NS-FLER had higher OHC values at 72 h fermentation of 8.9 g oil/g dm NS-FLER. The contrasting trends in WHC and OHC observed during fermentation may be attributed to the alteration of protein folding induced by fermentation, leading to heightened exposure of the hydrophobic regions (Shi et al., 2021). Consequently, LER fractions exhibited an improved affinity for oil than LE fractions at the end of fermentation ($p < 0.05$) (OHC of 8.9 and 7.34 g oil/g dm, respectively).



(a)

(b)

**Figure 3.10 :** Tecnofunctional properties of LE and LER fractions during fermentation (a) WHC; (b) OHC. LE: *Lentinula edodes*, LER: *Lentinula edodes* residue.

The changes in WHC and OHC observed during the fermentation of LE and LER can be attributed to various biochemical and structural modifications, including the breakdown of cell walls and other structural components, variations in soluble protein content, and alterations in protein structure. These changes impact the water and oil absorption capabilities of the samples, with NS-FLE exhibiting higher WHC likely due to its higher content of soluble dietary fibres and proteins, while NS-FLER shows an increase in OHC due to exposure to more hydrophobic regions in protein structure that enhance oil absorption. These findings suggest that fermentation influences the functional properties of LE and LER, which could have implications for their use in food products where moisture and fat retention are critical.The changes in WHC and OHC observed during the fermentation of LE and LER can be attributed to various biochemical and structural modifications, including the breakdown of cell walls and other structural components, variations in soluble protein content, and alterations in protein structure. These changes impact the water and oil absorption capabilities of the samples, with NS-FLE exhibiting higher WHC likely due to its higher content of soluble dietary fibres and proteins, while NS-FLER shows an increase in OHC due to exposure to more hydrophobic regions in protein structure that enhance oil absorption. These findings suggest that fermentation influences the functional properties of LE and LER, which could have implications for their use in food products where moisture and fat retention are critical.

### 3.4.8 Total phenolic content and antioxidant activity

The TPC and AA of soluble fractions of LE and LER were displayed in Figure 3.11. At the beginning of fermentation (0 h), the TPC of S-FLE was higher than that of S-FLER ($p < 0.05$), with values of 2.28 mg/g and 2.85 mg/g, respectively. Different studies have reported varying TPC values for *L. edodes*. Consistent with our findings, Kumar et al. (2022) reported that the TPC of acetone-extracted shiitake mushroom was 2.54 mg/g. Notably, both fractions showed an increase in TPC within 24 h of fermentation but did not change significantly afterwards ($p > 0.05$). The TPC trend of S-FLE and S-FLER exhibited a strong positive correlation, with a significant value of $p < 0.05$ and $R^2 = 0.95$. Additionally, by the end of fermentation, the TPC of S-FLE was higher than that of S-FLER.

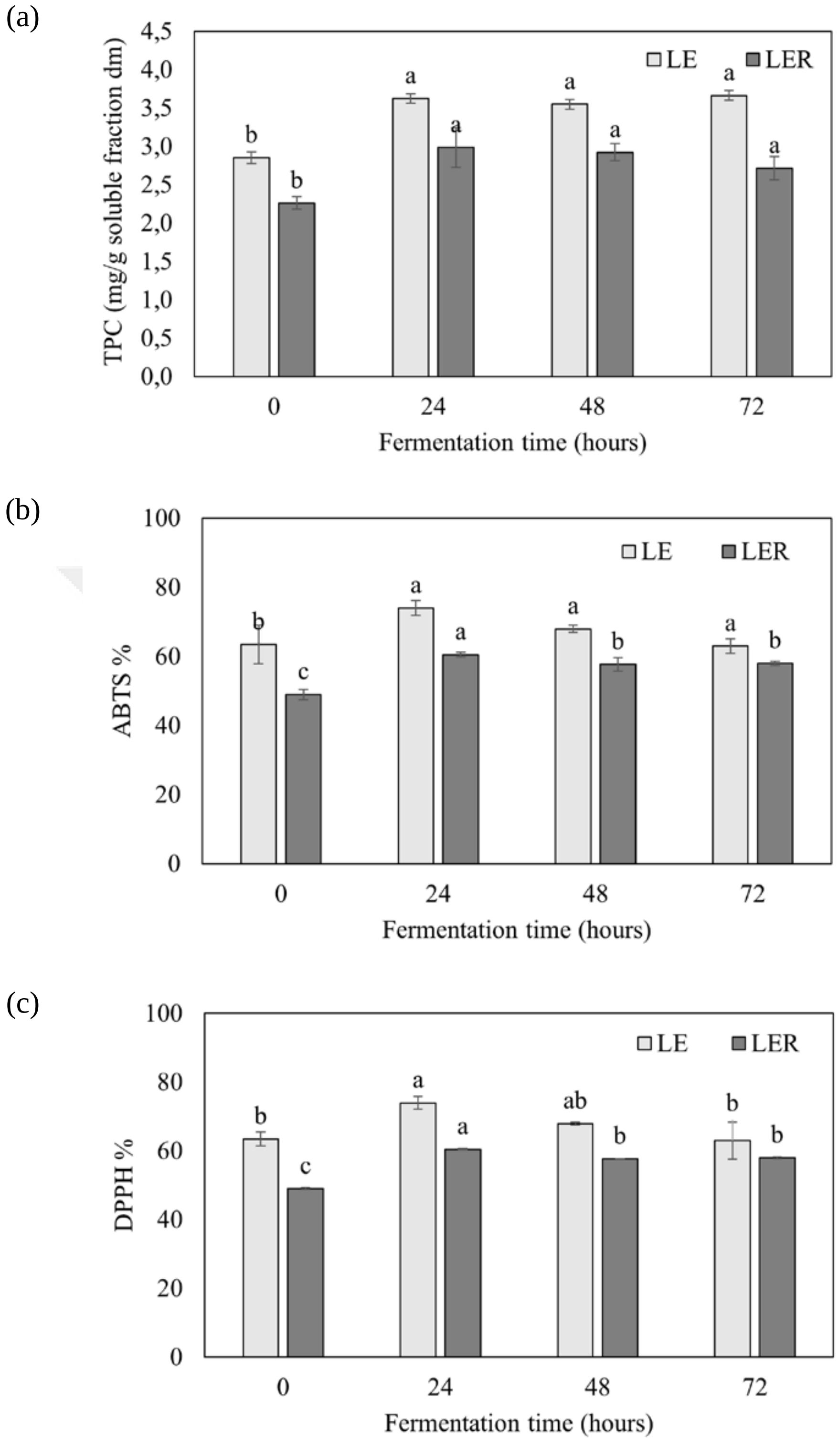


**Figure 3.11 :** Total phenolic content and antioxidant activity of LE and LER fractions during fermentation (a) TPC; (b) ABTS; (c) DPPH. LE: *Lentinula edodes*; LER: *Lentinula edodes* residue; TPC: total phenolic content; GAE: gallic acid equivalent antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′azino-bis(3-ethylbenzthiazoline-6- sulphonic acid) diammonium salt. Different lowercase letters in the same mushroom fraction indicate significant differences between the various fermentation time points ($p < 0.05$). Values are presented as mean ± SD ($n = 3$).

The increase in TPC could lead to an increase in the antioxidant activity of the fermented samples. In the process of fermenting mushroom fractions, improvement in antioxidant activity is closely linked to the transformation of phenolic substances (J. Yang, Sun, Chen, et al., 2022a). This is in accordance with our results which showed a strong correlation between TPC and antioxidant activity assays of S-FLE and S-FLER during fermentation ($p < 0.05$ and $R^2 = 0.95$).

The antioxidant activity of S-FLER was found to be maximal within 24 h of fermentation, after which it decreased but remained higher than that at 0 h. This was observed for both ABTS and DPPH by the strong positive correlation between them, with a significant value of $p < 0.05$ and $R^2 = 0.95$. During S-FLE fermentation, ABTS levels increased in the first 24 h and remained constant until the end of fermentation. However, the DPPH levels increased the most in the first 24 h and then decreased, ultimately reaching the same level as that at the beginning of the fermentation process. The AA of S-FLE was higher than that of S-FLER.

During the fermentation process, not only were phenolics transformed, but proteins also underwent breakdown into smaller peptides and free amino acids with antioxidant properties During the fermentation process, not only were phenolics transformed, but proteins also underwent breakdown into smaller peptides and free amino acids with antioxidant properties (Ayala-Ninõ et al., 2019; Sun et al., 2004). These molecules can chelate metals and inhibit enzymes generating free radicals. Increased levels of phenolics, peptides and amino acids have a synergistic effect on the antioxidant capacity of the fermented mushroom extracts. This synergism enhances the extracts' ability to counteract oxidative reactions, offering potential benefits for food preservation and health promotThese molecules can chelate metals and inhibit enzymes generating free radicals. Increased levels of phenolics, peptides and amino acids have a synergistic effect on the antioxidant capacity of the fermented mushroom extracts. This synergism enhances the extracts' ability to counteract oxidative reactions, offering potential benefits for food preservation and health promotion (Li et al., 2023).

## 3.5 Conclusions

The study demonstrates the successful use of lactic acid fermentation with *Lactiplantibacillus plantarum LMG 17673* to valorize mushroom residues. However, the changes observed in LE and LER were inconsistent, indicating the complexity of bioactive compound interactions and the need for careful processing of these residues. Organic acid content increased in both LE and LER, lowering the pH, while the soluble sugar content decreased due to its metabolism by the lactic acid bacteria during fermentation. The free amino group content decreased in LE, likely due to microbial utilization or degradation during fermentation, but it increased in LER, indicating protein degradation. This observation suggests that the presence of soluble polysaccharides may affect the behaviour of lactic acid bacteria. Changes in molecular weight detected in the samples confirmed this protein behaviour, which also affected the tecnofunctinal properties of LE and LER. Fermentation significantly enhanced total phenolic content and antioxidant activity, potentially due to peptides and amino acids released during protein breakdown. Overall, these findings confirm that lactic acid fermentation improves the nutritional and functional value of mushroom by-products and offers a promising strategy to enhance the sustainability and economic viability of mushroom processing industries.

# 4. IMPACT OF LACTIC ACID BACTERIA FERMENTATION BASED ON BIOTRANSFORMATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF MUSHROOMS

**Redrafted from:**

**Ayar-Sumer, E. N.,** Verheust, Y., Özçelik, B., & Raes, K. (2024). Impact of lactic acid bacteria fermentation based on biotransformation of phenolic compounds and antioxidant capacity of mushrooms. *FOODS*, *13*(11). https://doi.org/10.3390/ foods 13111616

## 4.1 Abstract

Mushrooms contain phenolic compounds that possess health-promoting properties, including antioxidant effects. However, the low solubility and form of phenolic compounds affect their bioactivity and bioaccessibility. To overcome this limitation, our study investigates the fermentation of mushrooms to increase their free phenolic content and enhance their bioactivity. Our research focused on the impact of fermentation on both free and bound phenolic fractions (FPs and BPs, respectively) in *Lentinula edodes* and *Lactarius deliciosus*, which were successively fermented with *Lactiplantibacillus plantarum LMG 17673* for 72 h. We examined the total phenolic content (TPC), phenolic profile, and antioxidant activity of both FPs and BPs. Our results showed that the TPC of BPs was higher than that of FPs in both mushrooms, with strong antioxidant capabilities. Fermentation significantly increased the TPC of FPs in both mushrooms, particularly after 24 h of fermentation. The TPC of BPs in mushrooms decreased during fermentation, indicating their release from the matrix. Additionally, we identified 30 bioactive compounds using UPLC-Q-TOF-MS/MS. Our study demonstrates for the first time that lactic acid bacteria fermentation of mushrooms with high phenolic content leads to the liberation of bound phenolics, enhancing their bioactivity and bioaccessibility.

## 4.2 Introduction

Consumption of edible mushrooms (cultivated and wild varieties) has been a global practice for centuries. *Lentinula edodes*, commonly referred to as shiitake mushrooms, is the second most cultivated edible mushroom worldwide (Lu et al., 2022). In Europe, the commercialisation of 268 distinct edible wild mushroom species has been officially sanctioned, and among these, *Lactarius deliciosus* is one of the top five best-selling varieties. Recognised as saffron milkcap, *La. deliciosus* is a popular and extensively consumed wild edible mushroom (Dogan et al., 2022; X. H. Wang et al., 2022).

The global mushroom cultivation market size is expected to reach approximately 24 million tons by 2028, which is 57% higher than that by 2021 (Dedousi et al., 2024). However, this growth poses environmental challenges for the market because of the short shelf life of the mushrooms. In addition, a significant amount of by-products are produced during mushroom production, leading to high environmental impact and management costs for the industry. These by-products consist of caps, stipes, mushrooms that do not meet commercial standards for size, shape, or calibration, and spent mushroom substrates. Approximately 5 kg of spent mushroom substrate by-products are generated per kilogram of fresh mushroom produced (Leong et al., 2022), whereas up to 20% of fresh mushroom production volume is attributed to misshaped mushroom by-products (Umaña et al., 2020). The misshapen mushrooms have basically the same chemical composition as that of the normal mushroom (Antunes et al., 2020). Therefore, applying food processing to mushrooms may not only serve as an effective means of valorisation to by-product mushrooms but also contribute to extending the shelf life of all mushrooms.

Consumers are becoming interested in the potential health benefits of adding mushrooms to their diet. Mushrooms are recognised for their nutritional value as plant-based protein sources containing essential vitamins, minerals, fibre, and antioxidants. Additionally, mushrooms contain bioactive compounds with anti-inflammatory, antioxidant, antitumour, antiviral, and antimicrobial properties that actively promote health and reduce the risk of diseases in the human body (Cateni et al., 2022). These positive effects on health are due to the presence of bioactive compounds, such as phenolic compounds, terpenoids, steroids, lectins, nucleotides, glycoproteins, and polysaccharides (Çayan et al., 2020), and much attention has been given to phenolic compounds as secondary metabolites in mushrooms (Zhou et al., 2023). Phenolic compounds are usually present in food matrices in both free and bound forms. As the bound phenolic compounds are linked to the cell wall matrices with covalent bonds, they cannot be absorbed in the small intestine, resulting in lower bioavailability compared to the free phenolic compounds. To increase the bioavailability of phenolic compounds, it is necessary to use additional processing techniques, such that bound phenolic compounds can be released and become available for absorption, similar to free phenolic compounds. This would also contribute to enhancing the health benefits of mushrooms (Yeo et al., 2021).

Fermentation is a traditional non-thermal food processing method that involves the metabolic activities of microorganisms such as bacteria and fungi. Lactic acid bacteria are gram-positive bacteria known for their ability to convert sugars to lactic acid during food fermentation. In addition to increasing the shelf life and improving the sensory qualities of food, LAB degrades macronutrients such as carbohydrates and proteins, resulting in alterations in the nutritional composition of the food product (Tao et al., 2022). This process results in food biotransformation by converting macronutrients, releasing antioxidative peptides, and altering phenolic compounds (Leonard et al., 2021). In particular, *Lactiplantibacillus plantarum LMG 17673*, a versatile and widely utilised LAB species, is particularly effective in fermenting fruits, vegetables, and dairy products, with beneficial effects on human health (Yi et al., 2021).

Many studies have investigated the antioxidant activity properties of cultivated and wild edible mushrooms. However, to our knowledge, no research has been conducted on the impact of LAB fermentation, as a possible processing technique for mushrooms, on the phenolic compounds in mushroom bodies. Since phenolic compounds are diverse and have complex structures, advanced analytical techniques are needed for which the ultra-performance liquid chromatography–electrospray ionisation quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS/MS) method is commonly used to tentatively identify extracts. Besides, in this study, for the first time, the impact of lactic acid fermentation on the possible changes in the structure of phenolic compounds in both free and bound fractions was investigated. This study is the first to use lactic acid bacteria and fermentation to enhance the bioactivity of mushrooms by liberating the bound phenolic compounds.

## 4.3 Materials and Methods

### 4.3.1 Media and chemicals

De Man-Rogosa-Sharpe (MRS) and plate count agar were purchased from Oxoid LTD (Basingstoke, Hampshire, England). Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2’-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)), trolox (6-hydroxyl-2,5,7,8- tetramethyl chroman-2-carboxylic acid), gallic acid, vanillic acid, and salicylic acid L-rhamnose monohydrate, DL-arabinose, D(+)-xylose, D(+)-mannose, D(−)-ribose, D(+)-glucose, D(+)-maltose monohydrate, D(−)-mannitol, trehalose, galacturonic acid, and glucuronic acid were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Louis, MO). D(−)-Fructose was purchased from Acros Organics (Geel, Belgium). HPLC-grade water and methanol were purchased from VWR Chemicals (VWR International S.A.S., Briare, France). Aluminium chloride, sodium nitrite, sodium hydroxide, sodium carbonate, hydrochloric acid, and methanol were purchased from Chem-Lab (ChemLab NV, Zedelgem, Belgium).

### 4.3.2 Sample preparation and lactic acid fermentation

In this study, although misshapen mushroom by-products were the preferred source for fermentation, our experimental outcomes remained unaffected by the use of either mushrooms or their misshapen by-products, as their nutritional compositions were identical. Due to practical considerations, fermentation was performed directly with mushrooms, which was more feasible than obtaining them from the mushroom by-product. Therefore, *L. edodes* and *La. deliciosus* were purchased from a local market in Turkey, known for their high standards and reliability to guarantee the correct identification of the mushrooms. Furthermore, the mushrooms bought were controlled for their identification based on the mushroom identification guides as described by Davis et al. (2012) and Stamets (2011) and thoroughly examined for their key morphological features (cap shape, colour, size, and spore print). The mushrooms were cleaned before being cut into small pieces measuring 1 × 1 cm, frozen at -20 °C and lyophilised (Christ Alphna 1-2 LDplus, Osterode am Harz, Germany). These lyophilised samples were then finely ground into a mushroom powder using a grinder (IKA-A11, Germany). The mushroom powder was mixed with physiological water to obtain a 2% (w/v) mushroom solution and sterilised.

The lactobacillus strain of *Lactiplantibacillus plantarum LMG 17673* was purchased from the BCCM/LMG Bacteria Collection, an integral component of the Belgian Co-ordinated Collections of Microorganisms situated within the Laboratory for Microbiology of the Faculty of Sciences of Ghent University, Belgium. The inoculum of the strain was prepared according to the method described by He et al. (2021) with slight modifications. The lactobacilli culture was aseptically activated by transferring 100 μL of glycerol stock culture into 10 mL of sterile MRS broth and then incubated at 37 °C for 24 h (He et al., 2021). *Lp. plantarum LMG 17673* was propagated in MRS broth at 37 °C for 24 h with shaking at 100 rpm before being used as a working culture to inoculate the mushroom solutions. Flasks containing 25 mL of sterilised mushroom solution were inoculated with 1% (v/v) working culture and then placed on a rotary shaker (110 rpm) at 30 °C. During fermentation, samples were collected at different time points (0, 24, 48, and 72 h), freeze-dried, and the powders were stored at -20 °C until further analysis.

### 4.3.3 Microbial analysis

Microbial composition during fermentation was evaluated by measuring the pH and counting the number of LAB. The standard plate count method analysed LAB counts in mushroom samples at specific time points (0, 24, 48, and 72 h). Serial dilutions were prepared using physiological water. Using the spread plate method, 100 μL diluted aliquots were plated onto MRS agar plates and then cultured at 30 °C for 48 h. Finally, the microbial population was expressed as logarithmic colony-forming units per mL (log CFU/mL).

### 4.3.4 Fermentation metabolites

The fermentation metabolites of mushrooms were quantified at specific time points (0, 24, 48, and 72 h) using pre-column high-performance liquid chromatography (HPLC) (Wen et al., 2005). The metabolite analysis employed an LC system (Agilent LC 1260 Infinity II, Gent, Belgium) equipped with a column (Agilent Hi-Plex 300 × 7.7 μm, 8 μm particle, Gent, Belgium). The column was maintained at 60 °C, the diode array detector was set to 210 nm, and the refractive index detector was set to 55 °C. A fixed flow rate of 0.7 mL/min was combined with a 5 mmol/L sulfuric acid mobile phase, and 20 μL injection volumes were used. Samples were prepared by mixing 1 g of fermented mushrooms with 5 mL of 5 mmol/L sulfuric acid using an Ultraturrax (IKA-

T18, Staufen, Germany) for 1 min at 10,000 rpm at 21 °C. Following, the probe was washed with 5 mL of 5 mmol/L sulfuric acid and centrifuged (Hermle Z 366 K, Wehingen, Germany) at 4000 rpm for 10 min at 21 °C. The resulting mixture was then filtered through a 0.45 µm disc filter, and the supernatant was collected and preserved at – 40 °C until analysis. The quantification of free sugars and organic acids was based on calibration curves established for each compound by injecting known concentrations of external standards. Finally, the results were expressed as mg per g dry fermented mushrooms. In this study, the total free sugar compound refers to the sum of glucose, ribose, trehalose, and mannitol, whereas the total organic acid refers to the sum of formic acid, malic acid, lactic acid, citric acid, fumaric acid, and succinic acid.

### 4.3.5 Extraction of free and bound phenolic fractions

The method by Gonzales, Smagghe, et al. (2014) was used to extract the free and bound phenolics. In summary, 2 g of fermented mushroom powder was blended with 15 mL of 100% methanol using Ultra-Turrax (IKA-T18D, Germany) at 3000 rpm for 45 s. The tubes were promptly cooled in an ice bath for 15 s before being centrifuged at 13000 × g for 10 minutes at 4 °C using a centrifuge (Z 300 K, Hermle Labortechnik GmbH, Germany). The residue was subjected to a second extraction with 10 mL of 80% methanol, using the same procedure as before. The resulting supernatant was filtered and the volume was adjusted to 25 mL using 80% methanol. The phenolic content of these extracts was referred to as the free phenolics (FPs). The residue was air-dried in a fume hood overnight to extract the bound phenolics. Briefly, 0.1 g of the air-dried residue was subjected to hydrolysis by adding 2 mL of 2M NaOH using ultrasound (UP 400S, Hielscher, GmbH, Germany) for 30 min at maximum amplitude (100%) and 60°C. The hydrolysed samples were neutralised with 2M HCl, followed by extraction with 4 mL of methanol containing 0.1% formic acid. After vortexing and centrifugation at 10,000 × g for 10 minutes at 4 °C, the residue underwent a second extraction with 4 mL of 0.1% formic acid in 100% methanol. Finally, the supernatants from both extractions were combined, and adjusted to 20 mL with 80% methanol. The phenolic content of these extracts is referred to as the bound phenolics (BPs). This procedure illusturated in Figure 4.1.

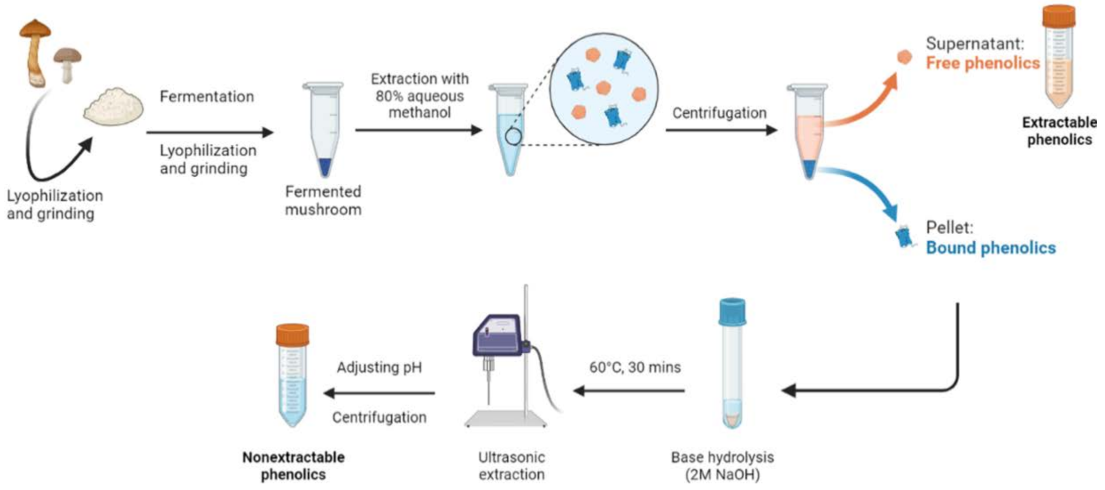


**Figure 4.1 :** Schemetic presentation of free and bound phenolc compounds extraction of fermenyed mushrooms.

### 4.3.6 Total phenolic content

The total phenolic content of the free and bound phenolic extracts was determined using the Folin-Ciocalteu method (Singleton et al., 1999). Each extract (1mL) was mixed with 0.5 mL of 10-fold diluted Folin-Ciocalteu reagent and then neutralised with 1.5 mL of 20% sodium carbonate solution. The mixture was then incubated for 2 hours at room temperature. After incubation, the blue colour that formed was measured using a 760 nm spectrophotometer (Model 4001/4, Thermo Spectronic, USA). The TPC was calculated using gallic acid calibration curves with $R^2$ values >0.99 and expressed as mg gallic acid equivalent (GAE) per gram of mushroom dry matter (dm). The limit of detection (LOD) and the limit of quantitation (LOQ) were determined to be 0.16 and 1.44 µg GAE/mL, respectively.

### 4.3.7 Antioxidant properties

#### 4.3.7.1 Determination of DPPH free radical scavenging activity

To evaluate the antiradical activity against DPPH radicals, the method devised by Kumaran & Joel Karunakaran, (2007) was applied. Briefly, a 0.1mM DPPH solution (2 mL) was mixed with 100 µL of the extracts by vortexing for 10 s. Then, the mixture was incubated in the dark for 30 min at room temperature and the absorbance of the solution was measured at 517 nm using a spectrophotometer. The DPPH free radical-scavenging capacity was calculated using the Trolox calibration curve with $R^2$ >0.99 and expressed as mg Trolox equivalent (TE)/100 g mushroom dm. LOD and LOQ were found to be 2.6 and 7.01 µg TE/mL, respectively.

#### 4.3.7.2 Determination of ABTS free radical scavenging activity

ABTS radical scavenging capacity was assessed according to the method described by Re et al. (1999). ABTS was dissolved in distilled water to create a stock solution with a concentration of 7 mM. Then, the reaction between the ABTS stock solution and potassium persulfate (2.45 mM) was initiated to generate ABTS radical cation, which was left in the dark at room temperature for 12-16 hours before utilisation. The ABTS radical cation was diluted with 90% methanol to prepare a working solution of fresh ABTS radical cation, achieving an absorbance of 0.70 ± 0.02 at 734 nm, and incubating at 30°C. Subsequently, 2 mL of fresh ABTS radical cation solution was mixed with 20 μL of sample extract and vortexed. Afterwards, the mixture was incubated for 5 min in the dark at 25°C and measured the absorbance of the solution at 734 nm. The results were calculated using the Trolox calibration curve with $R^2$ values >0.99. and expressed as mg Trolox equivalent (TE)/ g mushroom dm. LOD and LOQ were found to be 2.74 and 8.23 μg TE/mL, respectively.

### 4.3.8 Phenolic compound identification and characterisation

#### 4.3.8.1 UPLC-Q-TOF-MS/MS-based metabolite analysis

Fermentation metabolomic measurements were performed using an ultrahigh-performance liquid chromatography system (UPLC Infinity 1290, Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole time-of-flight mass spectrometer (Q-TOF 6546, Agilent Technologies) (Gonzales, Raes, et al., 2014). The system was equipped with an online degasser (Model 590, Alltech elite degassing system, Agilent Technologies, Santa Clara, CA, USA ), a sampler (G 7129B), a quaternary pump (G 7104A), Column18 5u (4.6 × 150 mm; GRACE, Deerfield, IL, USA), Column Oven (G 7130A), and photodiode array detector (DAD) (G 7117A, Agilent Technologies). Separation was carried out using a gradient elution method with two mobile phases: ultrapure water (0.1%, v/v, formic acid; eluent A) and LC-MS-graded methanol (0.1%, v/v, formic acid; eluent B). The gradient profile was as follows: 0-6 min, isocratic 20% B; 6-12 min, isocratic 20% B; 12–13 min, 20–30% B; 13-23 min, 30-50%; 23-30 min, 50-90%; 30-35 min, isocratic 90%; 35-40 min, 90-10%; 40-45 min, isocratic 10%. Each sample was run for a total of 45 minutes at a flow rate of 0.25 ml/min, and an injection volume of 20 μL. The eluent was directed into a Dual Agilent Jet Stream (AJS) electrospray ionisation (ESI) source within the mass spectrometer. The parameters for the MS were set as follows: capillary voltage, 3.5 kV; nozzle voltage, 4 V; gas temperature, 300 °C; vaporiser temperature, 350 °C; gas flow rate, 8 L min$^{-1}$; vaporiser flow, 11 L min$^{-1}$. A complete mass scan covering *m/z* 100–1700 was performed. For target analysis and fragmentation, MS/MS analysis was performed in the negative mode at different collision energies of 10, 20, and 40 V. Instrument control, data acquisition and processing were performed using Agilent MassHunter Workstation Qualitative Analysis software (version 10.0, Agilent Technologies, California, USA).

#### 4.3.8.2 Non-target screening on UPLC-Q-TOF-MS

All samples were analysed in triplicate in the negative mode. The acquisition procedure followed the described protocol, utilising Agilent MassHunter Workstation Profinder software (version 10.0) to extract non-target compounds via the Batch Recursive Feature Extraction workflow. Initially, the software identified molecular features in the first sample, where each feature represented a group of corresponding ions, including isotopes and adducts of the same compound. These groups formed a chromatographic peak at a specific retention time (RT). These detected features, accompanied by their precise monoisotopic mass and RT, were stored in a list, serving as a reference source for re-mining sample data using the Find-by-Ion method. Subsequently, the software used a binning algorithm to scan all the features across the samples. Detected features from all samples were compared, aligning exact masses and RT, and grouping corresponding features. This process generated a comprehensive list of features and their presence in respective samples. In a subsequent step, the average exact masses and RT of the consensus feature list were more sensitively scanned in each sample to detect any missed features in the initial round, facilitating recursive feature extraction. This approach enhances the feature identification accuracy by searching for low-level yet high-quality features that may have been overlooked initially, thus reducing false negatives and accelerating the binning process.

#### 4.3.8.3 Non-target metabolomics analysis

The acquired feature list was imported into Agilent Mass Profiler Professional (MPP, version 15.1, Agilent Technologies), which offers a complete array of advanced statistical tools and facilitates the powerful visualisation of the results. Due to the impracticality of identifying all features, statistical analyses were employed to focus on the relevant compounds. Initially, principal component analysis (PCA) assessed metabolic variations among replicate samples. Following this, Fold Change analysis was applied to features demonstrating abundance variations between different conditions. Paired analyses were conducted using the Benjamini-Hochberg method, and the findings were visually represented through volcano plots.

Qualitative analysis of phenolic compounds in fermented mushroom fractions was carried out using UPLC-Q-TOF-MS/MS in negative ionisation mode. The Agilent MassHunter Workstation Qualitative Analysis software (version 10.0) was used to process the data and extract additional details about the features of interest through a target MS/MS approach. The acquired MS/MS spectra are compared with the METLIN Agilent MassHunter Personal Compound Database and Library (PCDL) via the find-by-formula search feature. The software tentatively identifies target compounds by matching observed spectra to reference spectra. Only compounds with good matching (score >75/100) and mass errors within ±5 ppm of the metabolite results were tentatively identified. The software evaluates the similarity or match quality between the observed MS/MS spectra and reference spectra from the library, and assigns scores to these matches. Identification of compounds not found in the library was attempted again using MS/MS data from the ChemSpider database. The MS/MS data of the available authentic standards were also compared with the experimentally measured mass of the ions for verification.

### 4.3.9 Statistical analysis

Correlations between TPC and antioxidant activity were evaluated using Pearson's correlation coefficient test. Statistical analyses were performed using the SPSS version 28.0 program (IBM SPSS Statistics, SPSS Inc., Chicago, IL, USA). To examine variations through mushroom fermentation, a one-way analysis of variance (ANOVA) was used. Subsequently, a multiple range test, Tukey's Honestly Significant Difference (HSD) test, was used to compare the means and identify significant differences among them. A significance level of $p < 0.05$ was employed to determine the presence of statistically significant differences. The reported values are presented as the mean ± standard deviation (SD) based on three independent samples.

## 4.4 Results and Discussion

### 4.4.1 The bacterial growth and pH changes during mushroom fermentation

The changes in the viable cell count and pH values of the fermented mushrooms during the 72 hours fermentation period are shown in Figure 4.2. As shown in Figure 4.2a, *Lp. plantarum* LMG 17673 demonstrated robust growth in the mushroom samples, with no significant changes in viable cell count observed during the latter stages of fermentation (from 48 to 72 hours) ($p \geq 0.05$). Initially, the viable bacteria counts of *Lp. plantarum* LMG 17673 in *L. edodes* and *La. deliciosus* were 7.52 ± 0.07 and 7.67 0.06 log CFU/mL, respectively. After 24 hours of fermentation, both mushrooms exhibited a high growth rate, which remained stable throughout the fermentation process. In the later stages (from 48 to 72 h), the viable cell counts of *Lp. plantarum* LMG 17673 remained above 8.5 log CFU/mL, indicating sustained metabolic activity. These findings confirm that the selected mushrooms are suitable substrates for the fermentation of the particular species.

During fermentation, LAB strains can metabolise sugar and produce organic acids, leading to changes in the pH value of mushrooms. The pH changes of mushroom samples during fermentation with *Lp. plantarum LMG 17673* is shown in Figure 4.2b. The most rapid decrease in pH occurred during the initial 24 hours fermentation, dropping to 4.51 and 5.53 for *L. edodes* and *La. deliciosus*, respectively. These results are comparable to the pH of 4.61 observed in pickled *Agaricus bisporus* fermented with *Lb. plantarum* (Jabłońska-Ryś et al., 2022). A lower pH inhibits the growth of pathogenic bacteria, potentially extending shelf life and maintaining anaerobic conditions for preservation (Y. Liu et al., 2016). Additionally, the pH reduction during fermentation is attributed to the accumulation of organic acids, as noted in recent studies (J. Yang, Sun, Chen, et al., 2022a).

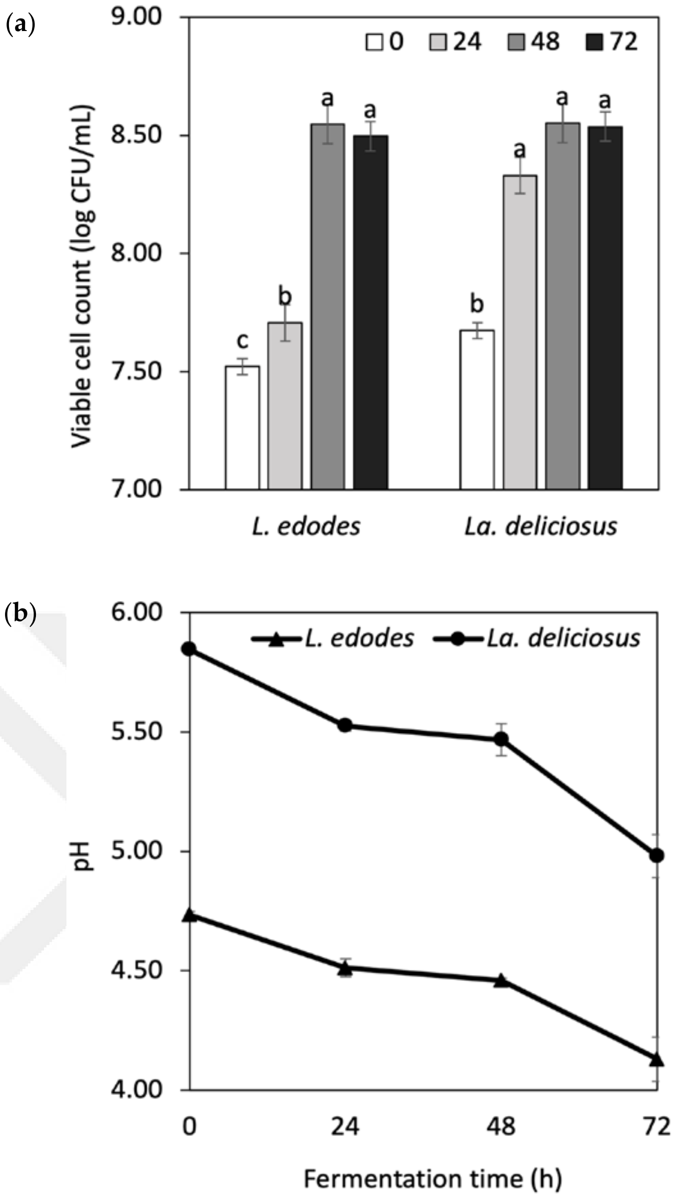


**Figure 4.2 :** Microbial activity of *Lp. plantarum LMG 17673* (a) viable cell count; (b) pH throughout the fermentation of *L. edodes* and *La. deliciosus*. CFU: colony forming unit. Different lowercase letters within the same mushroom species indicate significant differences during fermentation ($p < 0.05$). Values are presented as mean ± SD ($n = 3$).

### 4.4.2 Changes in the organic acid and sugar levels during mushroom fermentation

The changes in organic acids and free sugars in fermented mushrooms are presented in Table 4.1. During mushroom fermentation, *Lp. plantarum LMG 17673* can produce organic acids that lower the pH of the fermented mushroom matrixes. The organic acid contents of formic acid, malic acid, lactic acid, citric acid, fumaric acid, and succinic acid were assessed. During fermentation, the total organic acid content first increased, followed by a period of slight increase or stability (Figure 4.3a). The first 24 hours of fermentation marked a crucial point for the alteration in organic acid content. Throughout the fermentation, there was an increase in lactic acid and formic acid levels and a corresponding decrease in malic acid, citric acid, fumaric acid, and succinic acid concentrations. These trends are consistent with a study by Yang et al. (2022), who fermented apple juice using LAB strains. Malic acid can be transformed into lactic acid with the help of the malolactic enzyme produced by LAB, whereas the decomposition of citric acid may cause decreased citric acid content in various products. The fumaric and succinic acid contents in the fermented samples follow similar trends to those of malic and citric acids

The main mushroom monosaccharides, disaccharides, and sugar alcohols were glucose, ribose, trehalose, and mannitol, respectively, as has been reported for other edible mushrooms (Jabłońska-Ryś et al., 2019). During the 72 hours fermentation of mushrooms, the free sugar concentration in mushrooms decreased as shown in Figure 4.3b, primarily due to sugar consumption by LAB. Previous studies have also reported decreased free sugar content in fermented foods such as cabbage during the initial fermentation stage (Lee et al., 2021). Glucose and trehalose were rapidly consumed during the initial 24 hours, while ribose was the only sugar found in the mushrooms after 72 hours, similar to that reported for button mushrooms (Jabłońska-Ryś et al., 2022). At the beginning of the fermentation process, mannitol, a sugar alcohol, was the most abundant sugar in the fermented mushrooms. After 72 hours of fermentation, ribose was the highest level of sugar found in mushrooms.

**Table 4.1 :** Changes in free sugars and organic acids in fermented mushrooms during fermentation.

| Free sugars and organic acids (mg/g dm) | *L. edodes* | | | | *La. deliciosus* | | | | LOD (mg/L) | LOQ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fermentation time (hours) | | | | | | | | | |
| | 0 | 24 | 48 | 72 | 0 | 24 | 48 | 72 | | |
| D(+)glucose | 32.94±0.85 | D | ND | ND | 41.55±0.32$^{a}$ | 33.47±0.83$^{a}$ | ND | ND | 26.30 | 79.72 |
| D(-)mannitol | 221.46±6.16$^{a}$ | 23.90±0.15$^{b}$ | 2.93±0.41$^{c}$ | ND | 181.80±0.18$^{a}$ | 31.90±2.24$^{b}$ | 8.24±0.70$^{c}$ | 0.48±0.08$^{d}$ | 43.79 | 132.70 |
| D(-)ribose | 51.14±0.76$^{a}$ | 35.14±0.13$^{b}$ | 48.68±6.63$^{a}$ | 37.24±0.49$^{b}$ | 60.67±4.26$^{b}$ | 68.47±8.63$^{ab}$ | 76.40±2.26$^{a}$ | 78.48±5.26$^{a}$ | 29.57 | 74.72 |
| Trehalose | 10.29±0.64$^{a}$ | 0.86±0.15$^{b}$ | ND | ND | 25.43±0.11$^{a}$ | 0.79±0.03$^{b}$ | 0.89±0.02$^{b}$ | 0.82±0.02$^{b}$ | 29.58 | 89.63 |
| Malic acid | 54.72±2.28$^{a}$ | 2.82±0.86$^{b}$ | 1.64±0.26$^{b}$ | ND | 66.12±3.15 | ND | ND | ND | 6.19 | 20.64 |
| Succinic acid | 177.69±7.44$^{a}$ | 9.42±0.13$^{b}$ | 11.88±3.09$^{b}$ | 8.64±0.12$^{b}$ | 300.59±3.65$^{a}$ | 20.88±1.16$^{b}$ | 9.15±1.44$^{b}$ | 6.11±0.57$^{b}$ | 4.02 | 13.39 |
| Fumaric acid | 1.11±0.47 | ND | ND | ND | 1.20±0.01 | D | D | ND | 2.00 | 6.66 |
| Citric acid | 10.91±2.17$^{a}$ | 4.35±0.83$^{b}$ | 5.27±2.54$^{b}$ | 4.59±0.65$^{b}$ | 25.22±1.87$^{a}$ | 3.59±0.08$^{c}$ | 6.71±0.66$^{b}$ | 8.82±0.18$^{b}$ | 6.48 | 21.61 |
| Lactic acid | 106.33±3.88$^{c}$ | 525.76±8.1$^{b}$ | 574.00±19.03$^{b}$ | 697.60±9.34$^{a}$ | 136.07±5.44$^{b}$ | 1576.79±19.30$^{a}$ | 1480.47±15.23$^{a}$ | 1446.03±21.20$^{a}$ | 6.88 | 22.92 |
| Formic acid | 10.47±0.44$^{c}$ | 13.64±0.61$^{b}$ | 16.13±4.23$^{a}$ | 12.34±0.56$^{b}$ | 12.89±1.20$^{b}$ | 20.15±2.08$^{a}$ | 19.72±2.33$^{a}$ | 19.71±0.51$^{a}$ | 1.84 | 6.12 |

LOD: Limit of detection; LOQ: Limit of quantitation.

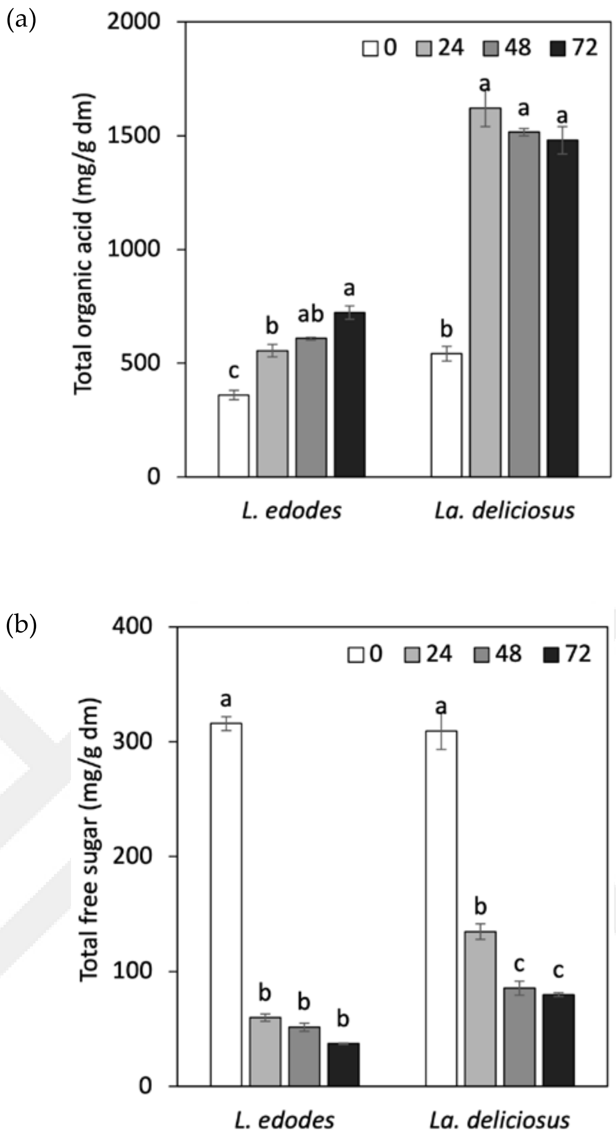


**Figure 4.3 :** Effect of *Lp. plantarum LMG 17673* fermentation on mushrooms (a) Total free sugar content; (b) Total organic acid content. Different lowercase letters within the same mushroom species indicate significant differences during fermentation ($p < 0.05$). Values are presented as mean ± SD ($n = 3$).

### 4.4.3 Changes in total phenolic content and antioxidant activity in free and bound phenolics during mushroom fermentation

The TPC and antioxidant activity of the mushroom fractions are listed in Table 4.2. The TPC of *L. edodes* free phenolic fraction (LEFPs) and *La. deliciosus* free phenolic fraction (LDFPs), at the beginning of fermentation, were 1.86 ± 0.38 mg GAE/g dm and 1.68 ± 0.14 mg GAE/g dm, respectively. A significant increase in FPs during fermentation occurred within 24 h, with the highest TPC increase of 29% and 27.3% observed for LEFPs and LDFPs, respectively, compared to the unfermented samples (2.40 ± 0.06 mg GAE/g dm and 2.14 ± 0.39 mg GAE/g dm, respectively). Similarly, De Montijo-Prieto et al. (2023) also showed that 24 h fermented samples had the highest level of TPC while investigating the impact of *Lp. plantarum* fermentation on the TPC of ethanolic avocado leaf extracts during 96 h of fermentation (De Montijo-Prieto et al., 2023b). In the present study, the TPC of LEFPs and LDFPs was steady between 24 h to 48 h fermentation ($p > 0.05$), whereas from 48 h to 72 h, the TPC of the FPs slightly decreased ($p < 0.05$). The decrease in TPC during fermentation has been associated with the degradation of phenolic compounds by *Lp. plantarum* (Carvalho & Guido, 2022). Comparatively, the bound fractions exhibited notably higher TPC levels than the free fractions did. After ultrasonic-alkaline hydrolysis, a greater amount of BPs was liberated from the cell wall components than FPs. Yeo et al. (Yeo et al., 2021) also found that phenolic compounds were more present in the bound form rather than the free form in lentils.

The highest TPC of *L. edodes* bound phenolic fraction (LEBPs) and *La. deliciosus* bound phenolic fraction (LDBPs) were measured at 0 h fermentation and amounted to 4.48 ± 0.26 mg GAE/g dm and 2.93 ± 0.53 mg GAE/g dm, respectively. Previous studies have indicated that the ratio between bound and free phenolic compounds can be altered depending on the processing technologies used, such as fermentation (B. Wang et al., 2021). In the present study, the TPC of BPs in both mushrooms decreased during fermentation. This finding may be due to the availability of endogenous hydrolysing enzymes throughout fermentation and/or the enzymes produced by fermenting microorganisms. The endogenous enzymes benefit from LAB fermentation, which lowers the pH and creates a more optimal environment for their function. The enzymes can break down bonds, and facilitate the mobilisation of phenolic compounds from their bound state to free form (Adebo & Medina-Meza, 2020; Salar et al., 2016). LAB can also transform phenolic metabolites and release phenolic compounds from the food matrices during fermentation. This process helps to offset the degradation of the original parent phenolic compounds during fermentation (Leonard et al., 2021).

**Table 4.2 :** Effect of fermentation on TPC and antioxidant activity of free and bound phenolic fractions of fermented mushrooms.

| Fermentation time | *L. edodes* | | *La. deliciosus* | |
|---|---|---|---|---|
| | Free | Bound | Free | Bound |
| | TPC (mg/g) | | | |
| 0 h | 1.86±0.04$^{c}$ | 4.48±0.08$^{a}$ | 1.68±0.01$^{c}$ | 2.93±0.05$^{a}$ |
| 24 h | 2.40±0.01$^{a}$ | 4.10±0.06$^{b}$ | 2.14±0.04$^{a}$ | 2.36±0.04$^{b}$ |
| 48 h | 2.33±0.04$^{a}$ | 3.79±0.02$^{c}$ | 2.09±0.07$^{a}$ | 2.35±0.02$^{b}$ |
| 72 h | 2.14±0.04$^{b}$ | 3.50±0.04$^{d}$ | 1.88±0.03$^{b}$ | 2.21±0.04$^{b}$ |
| | DPPH (mg TE/g) | | | |
| 0 h | 2.55±0.11$^{c}$ | 6.54±0.26$^{a}$ | 2.26±0.13$^{b}$ | 1.81±0.14$^{a}$ |
| 24 h | 3.77±0.10$^{a}$ | 5.55±0.16$^{b}$ | 2.88±0.36$^{a}$ | 1.66±0.15$^{ab}$ |
| 48 h | 3.36±0.13$^{b}$ | 5.21±0.11$^{b}$ | 2.64±0.03$^{ab}$ | 1.44±0.12$^{bc}$ |
| 72 h | 3.30±0.14$^{b}$ | 4.24±0.09$^{c}$ | 2.54±0.06$^{ab}$ | 1.31±0.11$^{c}$ |
| | ABTS (mg TE/g) | | | |
| 0 h | 6.29±0.21$^{b}$ | 23.68±1.51$^{a}$ | 5.69±0.14$^{a}$ | 26.71±1.43$^{b}$ |
| 24 h | 5.90±0.45$^{b}$ | 24.04±1.61$^{a}$ | 5.56±0.31$^{a}$ | 28.05±0.1$^{a}$ |
| 48 h | 5.61±0.71$^{b}$ | 23.40±1.5$^{a}$ | 5.67±1.23$^{a}$ | 25.45±0.23$^{c}$ |
| 72 h | 6.10±0.29$^{a}$ | 22.34±2.21$^{a}$ | 5.47±0.43$^{a}$ | 25.5±0.14$^{c}$ |

TPC: total phenolic content; GAE: gallic acid equivalent antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TE: trolox equivalent; ABTS: 2,2’azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt. Different lowercase letters in the same column indicate significant differences between the samples in the same column ($p < 0.05$). Values are presented as mean ± SD ($n$=3)

In the study of Tu et al. (2021), the free phenolic content of *L. edodes* was reported 7.16 mg GAE/g dry weight (dw), and the bond phenolic content of *L. edodes* was 7.47 ± 0.22 mg GAE/g dw, which corresponds with our results (Tu et al., 2021). Similarly, the TPC of the methanolic extract of *L. edodes* was found around 2.7 mg GAE/g dw, which closely corresponded with our FPs results (Xiaokang et al., 2020). On the other hand, the high TPC of *L. edodes* fractions was opposite to the results reported by Wang et al. (X. Wang et al., 2022a), who found the highest TPC of BPs of *L. edodes* as 26.67 mg GAE/100 g dm, and FPs content as 20.62 mg/GAE 100 dm g. Our study showed that TPC values were 10 times higher than these values. Several factors can account for the observed differences, such as differences in the environment, mushroom variety, location, extraction conditions, substrates, and maturity (Chu et al., 2023; Xiaokang et al., 2020). In a study conducted by Yao et al. (2023), the TPC of ethanolic extract of *L. edodes* was found to be 127 mg GAE/g dw (Yao et al., 2023), whereas, in the study by Pehlivan Karakas et al. (2023), the TPC of methanolic extract of *La. deliciosus* was measured as 193 ± 0.77 mg GAE/g dw (Pehlivan Karakas et al., 2023). The TPC values obtained in our study were lower than those reported in previous studies. It is clear that variations in the extraction and measurement methods exist across different studies. For instance, TPC in a previous study was measured using extracts obtained using 70% (v/v) ethanol and 80% (v/v) methanol. Despite these methodological differences, our study confirmed that *L. edodes* and *La. deliciosus* are rich in phenolic compounds.

Phenolic compounds can be used as reducing agents, free-radical scavengers, and singlet oxygen quenchers, and exhibit antioxidant activity primarily because of their ability to transfer hydrogen atoms or donate electrons to free radicals (De Montijo-Prieto et al., 2023b). As shown in Table 4.2, although LAB fermentation did not increase ABTS radical scavenging activity, it significantly increased DPPH radical scavenging activity in the free fractions of both mushrooms, indicating that LAB fermentation may have the potential to enhance the antioxidant activity of mushrooms. An increase in TPC could lead to an enhancement in the antioxidant activity of fermented samples (J. Yang, Sun, Chen, et al., 2022b). During the fermentation of both mushrooms, significant positive strong correlations ($p < 0.05$) were observed between DPPH and TPC for LEFPs and LDFPs ($R^2 = 0.95$, and $R^2 = 0.95$, respectively), and LEBPs and LDBPs ($R^2 = 0.98$, $R^2 = 0.95$, respectively). Thus, the higher DPPH activity observed in fermented mushrooms in this study may be attributed to the elevated bioaccessible free total phenolic content modulated by *Lp. plantarum LMG 17673* during lactic acid fermentation. In this study, *Lp. plantarum LMG 17673* fermentation increased the FPs by releasing BFs from the food matrix, potentially enhancing the bioavailability of mushroom phenolics. In addition, the TPC and DPPH radical scavenging activity of FPs increased, whereas the content in the BPs fractions decreased. Similarly, fermentation with selenium-enriched *Lp. plantarum* markedly improves the total phenolic content and antioxidant properties of *Pleurotus eryngii* (B. Wang et al., 2021). Interestingly, the ABTS activity of fermented mushrooms was not correlated with TPC and DPPH activities. The lack of correlation between ABTS levels and TPC and DPPH analysis is similar to the findings in pegaga, a medicinal herb (Tan et al., 2011), and galangal extracts (Ozdemir et al., 2024). The correlation between antioxidant capacities and antioxidant compounds is mostly complicated, as antioxidant capacity is determined by the number of antioxidants, as well as by environmental conditions, the interaction of antioxidants with one another and the matrix, and their physical location and structure (Tan et al., 2011). These factors can also be attributed to the ambiguous correlation between ABTS activity and TPC. Besides, the quantified TPC values may include other components that can exert antioxidant activity. Furthermore, assessing antioxidant activity using varying methods based on different mechanisms may yield different results; ABTS relies on the hydrogen atom transfer mechanism, whereas DPPH functions through an electron transfer mechanism (Adebo & Medina-Meza, 2020; Tao et al., 2022).

### 4.4.4 The non-targeted analysis of UPLC-Q-TOF-MS data by mushroom fermentation

An unsupervised PCA was applied to gain insights into the metabolic variations among different fermentation time points and phenolic fractions. Non-targeted metabolomics approach using UPLC-Q-TOF-MS datasets was employed for PCA, transforming the dataset into a series of values representing linearly uncorrelated variables. This widely utilised approach in metabolomic studies has allowed us to comprehensively overview metabolite variations among samples. Figure 4.4a shows the PCA score plot with PC1 and PC2, explaining 46.99% of the total variance (39.1% and 7.89%, respectively), which appeared to be between fermented *L. edodes* fractions. It could be observed that the BPs can be distinctly grouped from FPs. However, overlapping clusters at different fermentation time points in each fraction showed weakly explained variance.

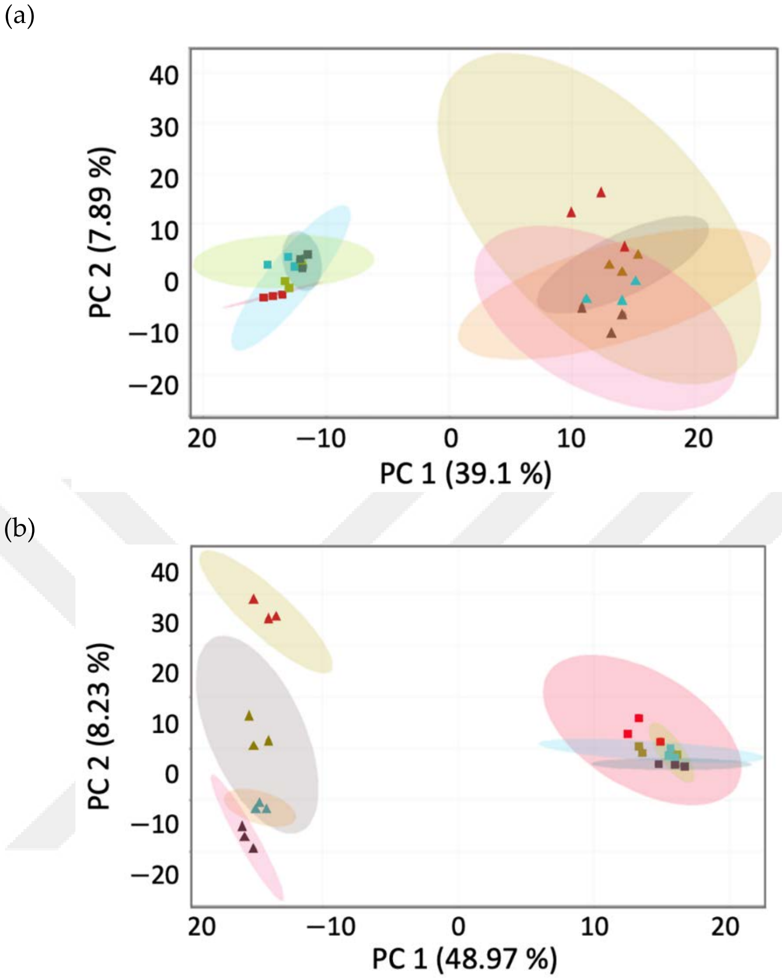
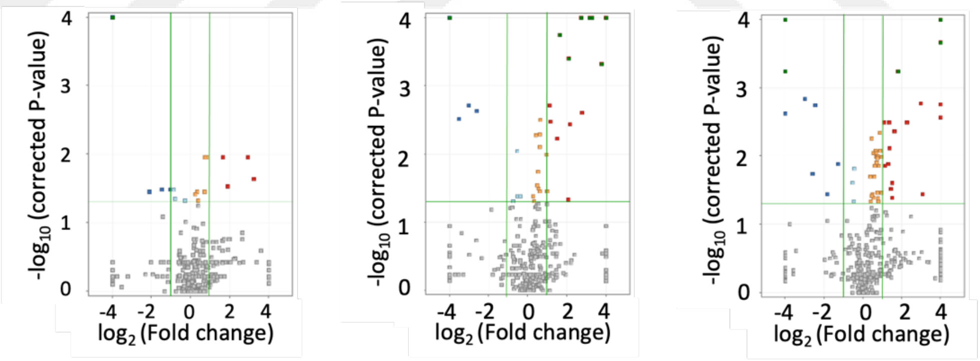


**Figure 4.4 :** Principal component analysis (PCA) plots with PC 1 and PC 2: (**a**) PCA plot of *L. edodes*; (**b**) PCA plot of *La. deliciosus*. The triangle represents bound phenolics, and the square represents free phenolics. Red colour represents 0 h fermented mushrooms, caramel colour represents 24 h fermented mushrooms, blue colour represents 48 h fermented mushrooms, and brown colour represents 72 h fermented mushrooms.

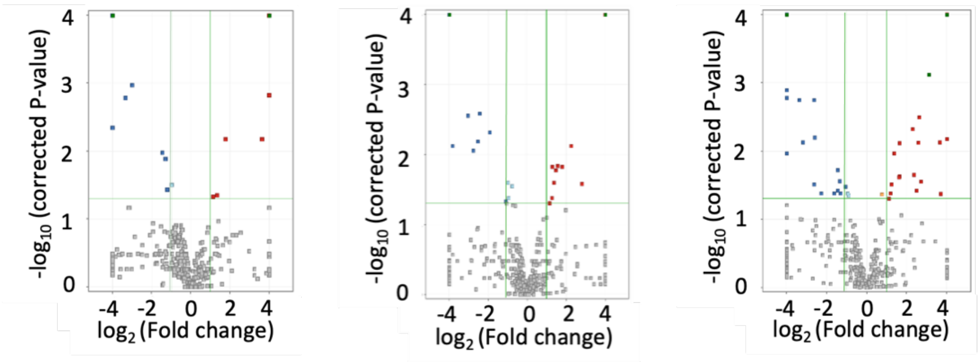
Figure 4.4b shows the PCA score plot with a higher explanation for *La. deliciosus*. PC1 and PC2 explained 57.2% of the total variance (48.97% and 8.23%, respectively), which also appeared to lie between those of fermented *La. deliciosus* fractions. The FPs of unfermented (0 h) *La. deliciosus* exhibited a clear difference from the fermented free fractions. PCA indicated that the form of phenolics was most affected by fermentation, leading to increased differentiation between the fermented and unfermented samples.

To further confirm the variance in metabolic profiles among fermented mushrooms, a volcano plot of fermented *L. edodes* (Figure 4.5a) and *La. deliciosus* (Figure 4.5b) showed the number of features which changed significantly during the fermentation. The horizontal green line represents the p-test limit, and the features positioned above this line were considered statistically significant ($p < 0.05$). The vertical green lines represent the fold-change limits. The volcano plot shows the passing features (coloured) and non-passing features (grey). Each square, coloured pale blue or orange, corresponds to a feature that has successfully passed the significance test yet failed to meet the fold-change cut-off criteria. During the fermentation of the mushrooms, pairwise group comparisons were conducted by comparing unfermented mushroom samples (0 h fermented) to samples at each further time point, including 24, 48, and 72 h of fermentation. Each red square represents upregulated features, each blue square represents downregulated features ($p < 0.05$ and FC $\geq 2.0$), the upper right and left corner green squares represent the features ($p < 0.001$ and FC $\geq 2.0$), and grey squares reveal metabolites that have no significant difference between different fermentation time points samples.

(**a**) 1) BPs 0h vs 24h 2) BPs 0h vs 48h 3) BPs 0h vs 72h



4) FPs 0h vs 24h 5) FPs 0h vs 48h 6) FPs 0h vs 72h



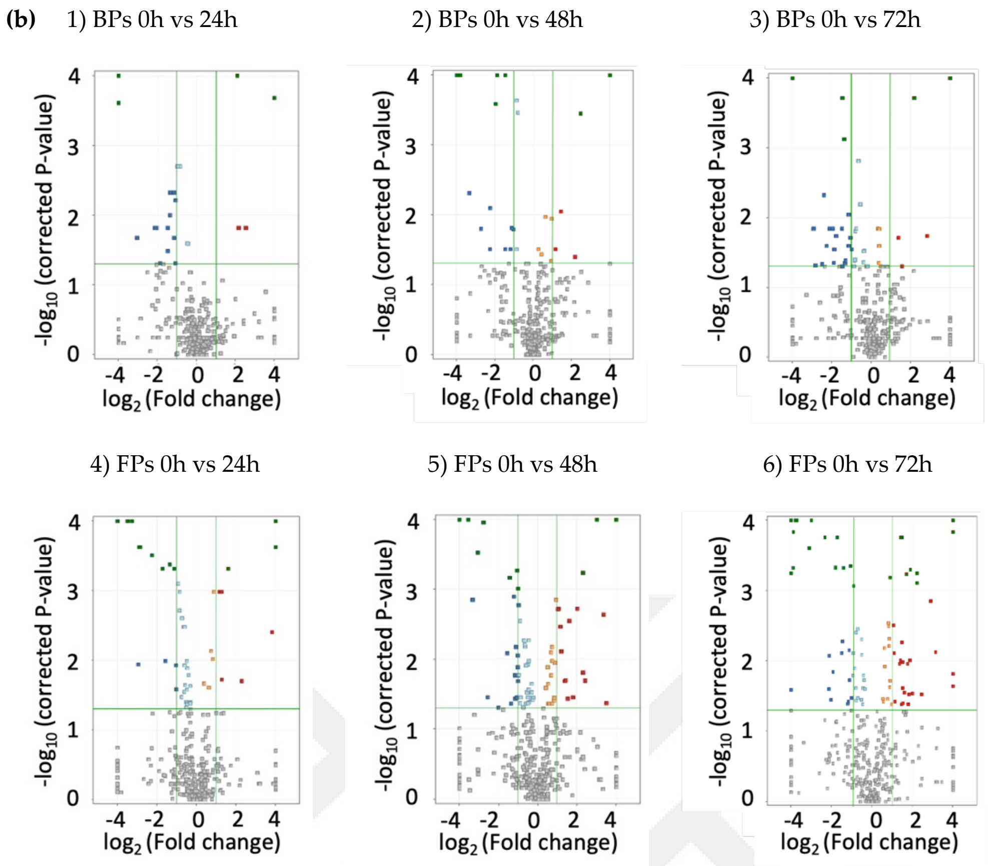
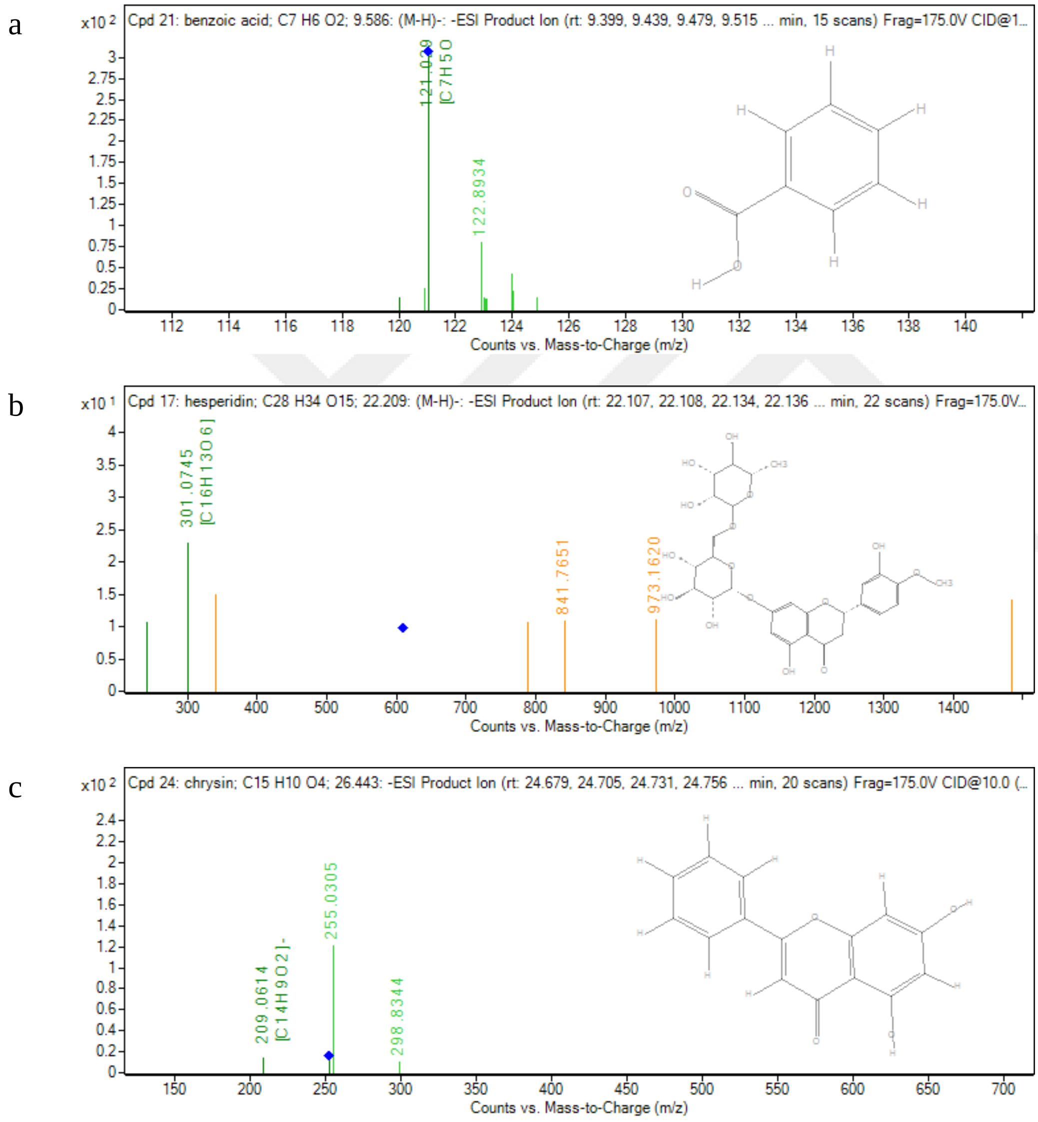


**Figure 4.5 :** Volcanic plots of differential metabolites in pairwise comparisons between unfermented (0h) and fermented: (**a**) *L. edodes*; (**b**) *La. deliciosus*. BPs; bound phenolics, FPs; free phenolics. The colours in the figure represent the following: grey squares reveal metabolites that show no significant difference between different fermentation time point samples; pale blue or orange squares indicate features that have successfully passed the significance test yet failed to meet the fold-change cut-off criteria. Red squares represent upregulated features, blue squares represent down-regulated features ($p < 0.05$ and FC ≥ 2.0), and green squares in the upper right and left corners represent features ($p < 0.001$ and FC ≥ 2.0).

A total of 468 features were evaluated in pairwise groups of fermented *L. edodes*. After the volcano plot analysis with $p < 0.05$ and FC ≥ 2.0, 216 out of 468 features were significantly different in *L. edodes* samples during fermentation. The volcano plots of fermented LEBPs showed that the number of features present at 0 h was significantly different from those present at 24, 48, and 72 h, with 17, 45, and 62 metabolites, respectively (as shown in Figures 4.5a 1, 2, 3) ($p < 0.05$). On the other hand, 0 h fermented LEFPs had 19 significantly different features compared to 24 h fermented LEFPs, which increased to 29 and 44 at 48 h and 72 h, respectively (Figures 4.5a 4, 5, 6) ($p < 0.05$). In addition, 332 of the 469 potential features with significantly different amounts were screened within the pairwise groups for *La. deliciosus* during fermentation ($p < 0.05$ and FC ≥ 2.0). The volcano plot of fermented LDBPs revealed that at 0h, the number of features was significantly different compared to 24 h, 48 h, and 72 h, with counts of 22, 32, and 49, respectively (Figures 4.5b 1, 2, 3) ($p < 0.05$). Similarly, LDFPs presenting the number of features at 0 h showed statistically significant differences compared to 24 h, 48 h, and 72 h were 50, 85, and 94, respectively (Figures 4.5b 4, 5, 6) ($p < 0.05$). As the fermentation duration increased, there was a corresponding increase in the number of features in the same fractions that exhibited statistically significant differences in their profiles. These findings suggest that LAB fermentation can alter both the composition and concentration of phenolic compounds, highlighting the fact that bound phenolics are predominantly affected by fermentation. This phenomenon may be linked to lower pH, which has the potential to influence the stability of phenolic compounds, leading to degradation or structural alterations (X. H. Wang et al., 2022). Moreover, fermentation contributes to the diversification of free phenolic compounds, which further enriches the nutritional profile. Z. Li et al. (2019b) also observed that the phenolic composition of apple juice could undergo variations during fermentation with *Lp. plantarum*. This alteration may result from the removal or hydrolysis of structural moieties in various phenolic compounds, leading to alterations in phenolic profiles.

### 4.4.5 Metabolite profiling and comparative analysis of mushrooms during different fermentation durations by UPLC-Q-TOF-MS/MS

To prevent overestimation of TPC using the spectrophotometric method, we examined variations in individual phenolic compounds that occur during the fermentation process. Secondary metabolites, such as phenolic acids and flavonoids, were detected using Agilent UPLC-ESI-QTOF-MS/MS Mass Hunter Qualitative Software by considering the MS/MS spectral data. Some of the examples of MS/MS spectras were presented in Figure 4.6. Speculative fragmentation pathway of ferulic acid in negative ionization mode was also present in Figure 4.7. A total of 30 metabolites were tentatively identified in the fermented mushroom fractions (Table 4.3). Protocatechuic acid, 3,4-dimethoxybenzoic acid, 5-feruloylquinic acid, ferulic acid glucoside, rosmarinic acid, caffeic acid, ursolic acid, formononetin, glycitein, gallocatechin, coumarin, riboflavin, niacinamide and, L-ascorbic acid were only found in the free fractions (LEFPs and LDFPs). With the exception of protocatechuic acid, 3,4-dimethoxybenzoic acid, and phenylacetic acid, all hydroxybenzoic acids were present in the free and bound forms in mushrooms. The cinnamic acid derivatives were found only in their free forms, except for quinic acid. Consistent with our results, *L. edodes* has been reported to contain hydroxybenzoic acid (Reis et al., 2012), hesperidin (X. Wang et al., 2022a), 3,4-dimethoxy benzoic acid, glycitein, l-ascorbic acid, riboflavin, niacinamide, salicylic acid, and shikimic acid (Nam et al., 2021b), whereas *La. deliciosus* has been reported to contain hydroxybenzoic acid, pyrogallol, protocatechuic acid (Çayan et al., 2020; Palacios et al., 2011), resveratrol, benzoic acid, kaempferol, chrysin, and ferulic acid (Kalogeropoulos et al., 2013b).



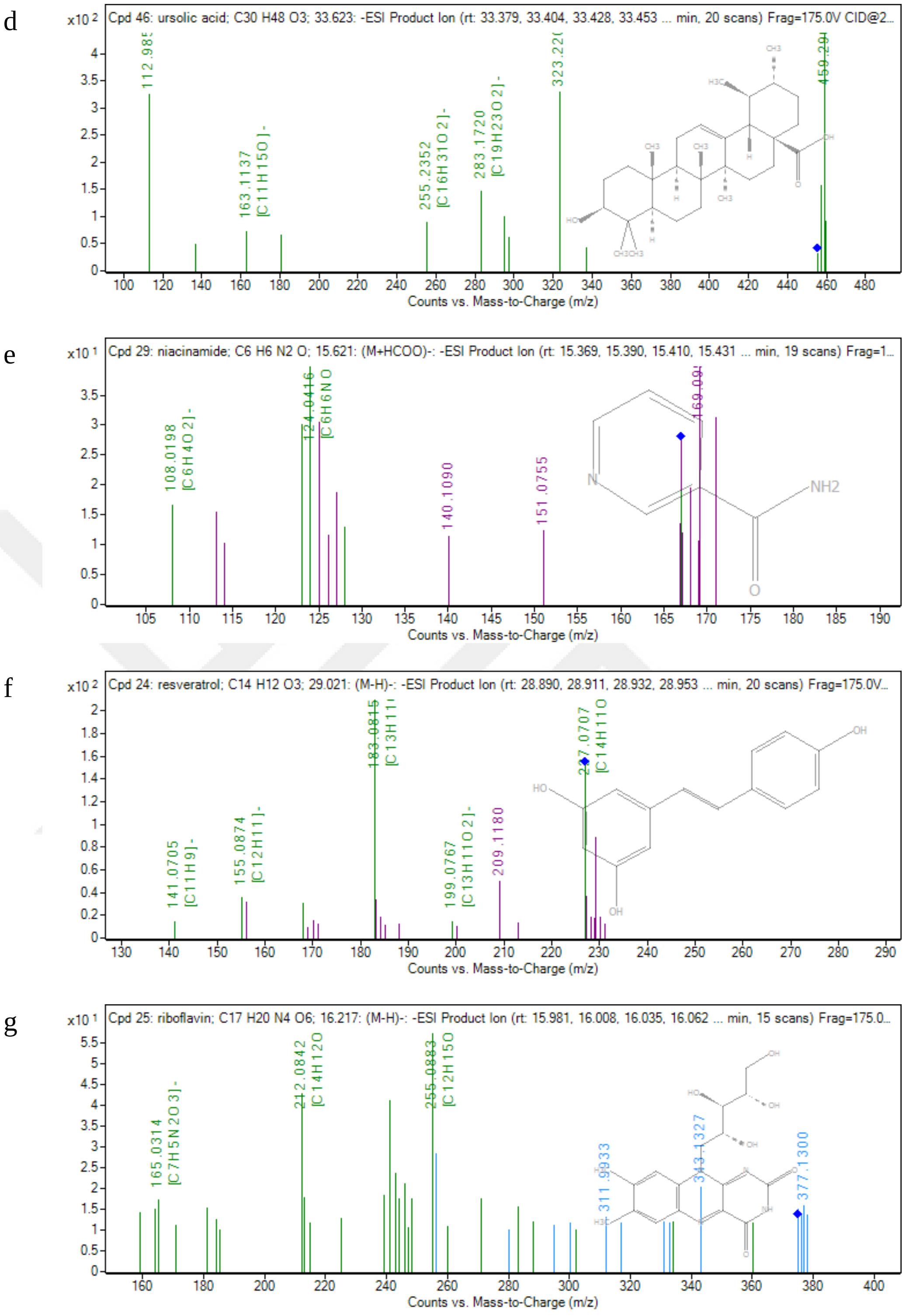


**Figure 4.6 :** Identification of some compounds in fermented mushrooms biosynthesis pathway including (a) benzoic acid; (b) hesperidin; (c) chrysin; (d) ursolic acid; (e)niacinamide; (f) resveratrol; (g) riboflavin by *m/z* matching using fragmentfor structure analysis.

**Table 4.3 :** Tentative identification of phenolic compounds, terpenic acids, and vitamins in the fermented *L. edodes* and *La. deliciosus* fractions.

| No | Proposed compound | Molecular formula | RT (min) | Adduct | Molecular weight | Theoretical (m/z) | Observed (m/z) | Mass error (ppm) | MS/MS Product Ions Collision Energies | | | Mushroom species | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 10V | 20V | 40V | *L. edodes* | *La. deliciosus* |
| | *Phenolic acids* | | | | | | | | | | | | |
| | *i.Hydroxybenzoic acids* | | | | | | | | | | | | |
| 1 | *p*-hydroxybenzoic acid glucoside | $C_{13}H_{16}O_8$ | 13.42 | (M–H)– | 300.085 | 299.0779 | 299.0772 | 2.1 | 299 | - | 106 | All-LEBPs<br>All-LEFPs | LDBPs-0h<br>LDBPs-24h<br>All-LDFPs |
| 2 | Protocatechuic acid | $C_7H_6O_4$ | 4.30 | (M–H)– | 154.0264 | 153.0191 | 153.0193 | -1.47 | - | 111 | - | All-LEFPs | All-LDFPs |
| 3 | 4-hydroxybenzoic acid* | $C_7H_6O_3$ | 7.91 | (M–H)– | 138.0318 | 137.0245 | 137.0244 | 0.76 | 109 | 111, 137 | - | All-LEBPs<br>All-LEFPs | All-LDBPs<br>All-LDFPs |
| 4 | Salicylic acid* | $C_7H_6O_3$ | 19.83 | (M–H)– | 138.0317 | 137.0238 | 137.0244 | -4,6 | - | 111 | - | All-LEBPs | All-LDBPs<br>LDFPs-24h<br>LDFPs-48h |
| 5 | Benzoic acid | $C_7H_6O_2$ | 9.6 | (M–H)– | 122.0368 | 121.0295 | 121.0295 | 0.35 | 120 | 121 | - | All-LEBPs<br>All-LEFPs | All-LDBPs<br>LDFPs-0h<br>LDFPs-24h<br>LDFPs-48h |
| 6 | 3,4-Dimethoxybenzoic acid | $C_9H_{10}O_4$ | 6.99 | (M–H)– | 182.0579 | 181.0506 | 181.0505 | -0.16 | 101, 141 | 120 | - | All-LEFPs | LDFPs-24h<br>LDFPs-48h<br>LDFPs-72h |
| 7 | Phenylacetic acid* | $C_8H_8O_2$ | 11.41 | (M–H)– | 136.0516 | 135.0452 | 135.0452 | 0.14 | 135 | 117, 109 | - | LEBPs-0h<br>LEBPs-24h | All-LDBPs |

**Table 4.3 (contunied) :** Tentative identification of phenolic compounds, terpenic acids, and vitamins in the fermented *L. edodes* and *La. deliciosus* fractions.

| No | Proposed compound | Molecular formula | RT (min) | Adduct | Molecular weight | Theoretical (m/z) | Observed (m/z) | Mass error (ppm) | MS/MS Product Ions Collision Energies | | | Mushroom species | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 10V | 20V | 40V | *L. edodes* | *La. deliciosus* |
| | *ii. Hydroxycinnamic acids* | | | | | | | | | | | | |
| 8 | Quinic acid | $C_7 H_{12} O_6$ | 7.25 | (M+HCOO)– | 192.0638 | 237.057 | 237.062 | 1.95 | 156,<br>126 | - | - | All-LEBPs<br>All-LEFPs | LDFPs-24h<br>LDFPs-48h<br>LDFPs-72h |
| 9 | 5-feruloylquinic acid | $C_{17} H_{20} O_9$ | 4.35 | (M+HCOO)– | 368.1093 | 413.1227 | 413.0143 | -3.8 | 312 | - | - | - | LDFPs-0h<br>LDFPs-24h<br>LDFPs-48h |
| 10 | Ferulic acid glucoside | $C_{16} H_{20} O_9$ | 1.77 | (M–H)– | 356.1109 | 355.1039 | 355.1035 | 0.36 | 193 | - | - | - | LDFPs-24h<br>LDFPs-48h |
| 11 | Rosmarinic acid | $C_{18} H_{16} O_8$ | 11.18 | (M+HCOO)– | 360.087 | 405.0809 | 405.0827 | -4.5 | 211 | 191,<br>227 | 139,<br>171 | All-LEFPs | LDFPs-0h |
| 12 | Caffeic acid | $C_9 H_8 O_4$ | 10.20 | (M–H)– | 180.0425 | 179.0352 | 179.0332 | 3.3 | 124 | 165 | - | - | LDFPs-0h<br>LDFPs-24h<br>LDFPs-48h |
| | *Phenols* | | | | | | | | | | | | |
| 13 | 2,6-dimethoxyphenol | $C_8 H_{10} O_3$ | 8.77 | (M+HCOO)– | 154.0628 | 199.06 | 199.061 | 0.32 | 123 | 128 | - | LEBPs-0h<br>All-LEFPs | LDBPs-0h<br>LDFPs-24h<br>LDFPs-48h<br>LDFPs-72h |
| 14 | Phenol | $C_6 H_6 O$ | 30.87 | (M+HCOO)– | 94.0416 | 139.039 | 139.0398 | -2.61 | 86 | - | - | All-LEBPs<br>All-LEFPs | All-LDBPs |
| | *Terpenic acids* | | | | | | | | | | | | |

**Table 4.3 (contunied) :** Tentative identification of phenolic compounds, terpenic acids, and vitamins in the fermented *L. edodes* and *La. deliciosus* fractions.

| No | Proposed compound | Molecular formula | RT (min) | Adduct | Molecular weight | Theoretical (m/z) | Observed (m/z) | Mass error (ppm) | MS/MS Product Ions Collision Energies | | | Mushroom species | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 10V | 20V | 40V | *L. edodes* | *La. deliciosus* |
| 15 | Ursolic acid* | $C_{30} H_{48} O_3$ | 35.81 | (M–H)–<br>(M+HCOO)– | 456.3603 | 455.3544<br>501.3584 | 455.3531<br>501.3585 | 2.82<br>-0.33 | 391<br>297, 337 | 255<br>152 | 163, 283<br>435 | All-LEBPs | LDFPs-0h<br>LDFPs-24h<br>LDFPs-48h |
| | *Flavonoids* | | | | | | | | | | | | |
| 16 | Genistein | $C_{15} H_{10} O_5$ | 28.13 | (M–H)– | 270.053 | 269.0457 | 269.0455 | 0.42 | 151, 107 | 119 | 187 | LEBPs-0h | All-LDBPs |
| 17 | Hesperidin* | $C_{28} H_{34} O_{15}$ | 22.21 | (M–H)– | 610.1888 | 609.1814 | 609.1825 | -1.75 | 609 | - | 301, 242 | LEBPs-72h | All-LDBPs |
| 18 | Formononetin | $C_{16} H_{12} O_4$ | 29.35 | (M–H)– | 268.0733 | 267.066 | 267.0663 | -1 | 251 | - | - | - | LDFPs-24h<br>LDFPs-48h<br>LDFPs-72h |
| 19 | Chrysin* | $C_{15} H_{10} O_4$ | 24.82 | (M–H)– | 254.0579 | 253.0506 | 253.0506 | -0.19 | 253 | 164, 208 | 154 | LEBPs-0h<br>LEBPs-24h | All-LDBPs |
| 20 | Glycitein | $C_{16} H_{12} O_5$ | 30.91 | (M+HCOO)– | 284.0745 | 329.0778 | 329.0774 | -0.92 | 285 | - | - | - | All-LDFPs |
| 21 | Gallocatechin | $C_{15} H_{14} O_7$ | 4.22 | (M–H)– | 306.0718 | 305.0702 | 305.0645 | -2.8 | 156 | - | - | LEFPs-0h<br>LEFPs-24h | LDFPs-0h<br>LDFPs-24h |
| 22 | Kaempferol-3-glucoside | $C_{21} H_{20} O_{11}$ | 22.99 | (M–H)– | 448.1001 | 447.0928 | 447.0933 | -1.07 | 224, 115 | - | - | - | All-LDBPs |
| | *Stilbenes* | | | | | | | | | | | | |
| 23 | Resveratrol | $C_{14} H_{12} O_3$ | 29.02 | (M–H)– | 228.0785 | 227.0713 | 227.0714 | -0.49 | 183, 199 | 155, 141 | 139, 167 | All-LEFPs | All-LDBPs<br>All-LDFPs |
| | *Other polyphenols* | | | | | | | | | | | | |

**Table 4.3 (contunied) :** Tentative identification of phenolic compounds, terpenic acids, and vitamins in the fermented *L. edodes* and *La. deliciosus* fractions.

| No | Proposed compound | Molecular formula | RT (min) | Adduct | Molecular weight | Theoretical (m/z) | Observed (m/z) | Mass error (ppm) | MS/MS Product Ions Collision Energies | | | Mushroom species | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 10V | 20V | 40V | *L. edodes* | *La. deliciosus* |
| 24 | Pyrogallol* | $C_6 H_6 O_3$ | 3.57 | (M–H)–<br>(M+HCOO)– | 126.0318 | 125.0244<br>171.03 | 125.0244<br>171.0299 | 0.21<br>0.59 | 124<br>103 | 108, 124<br>- | -<br>- | LEBPs-0h<br>All-LEFPs | LDBPs-0h<br>LDBPs-24h<br>LDBPs-48h |
| 25 | Catechol | $C_6 H_6 O_2$ | 5.17 | (M–H)– | 110.0366 | 109.032 | 109.0294 | 0.3 | 101 | - | - | All-LEFPs | All-LDBPs<br>All-LDFPs |
| 26 | Shikimic acid | $C_7 H_{10} O_5$ | 2.9 | (M–H)–<br>(M+HCOO)– | 174.0529 | 173.0457<br>219.0509 | 173.0455<br>219.051 | 0.82<br>-0.37 | 157, 109<br>113 | -<br>- | -<br>- | All-LEFPs | LDBPs-0h<br>All-LDFPs |
| 27 | Coumarin | $C_9 H_6 O_2$ | 15.5 | (M–H)– | 146.0368 | 145.0295 | 145.0245 | 0.12 | 142 | 108 | - | - | All-LDFPs |
| | *Vitamins* | | | | | | | | | | | | |
| 28 | Riboflavin | $C_{17} H_{20} N_4 O_6$ | 16.22 | (M–H)– | 376.1384 | 375.131 | 375,131 | -0.08 | 255,129 | 212,165 | 198,184 | - | All-LDFPs |
| 29 | Niacinamide | $C_6 H_6 N_2 O$ | 15.62 | (M+HCOO)– | 122.048 | 167.0462 | 167.0462 | -0.23 | 124,128 | 108 | - | All-LEFPs | - |
| 30 | L-ascorbic acid | $C_6 H_8 O_6$ | 1.66 | (M–H)– | 176.032 | 175.0247 | 175.0248 | -0.92 | 117,175 | - | - | LEFPs-0h | - |

MS/MS: Tandem mass spectrometry; LEBPs: *Lentinula edodes* bound phenolics; LEFPs: *Lentinula edodes* free phenolics; LDBPs: *Lactarius delicious* bound phenolics; LDFPs: *Lactarius delicious* free phenolics. “All” represents all fermentation time points in the mentioned fraction. 0h, 24h, 48h, and 72h represent fermentation time points. “-“ is not detected. “RT” stands for retention time. * The compounds were verified using authentic standards.

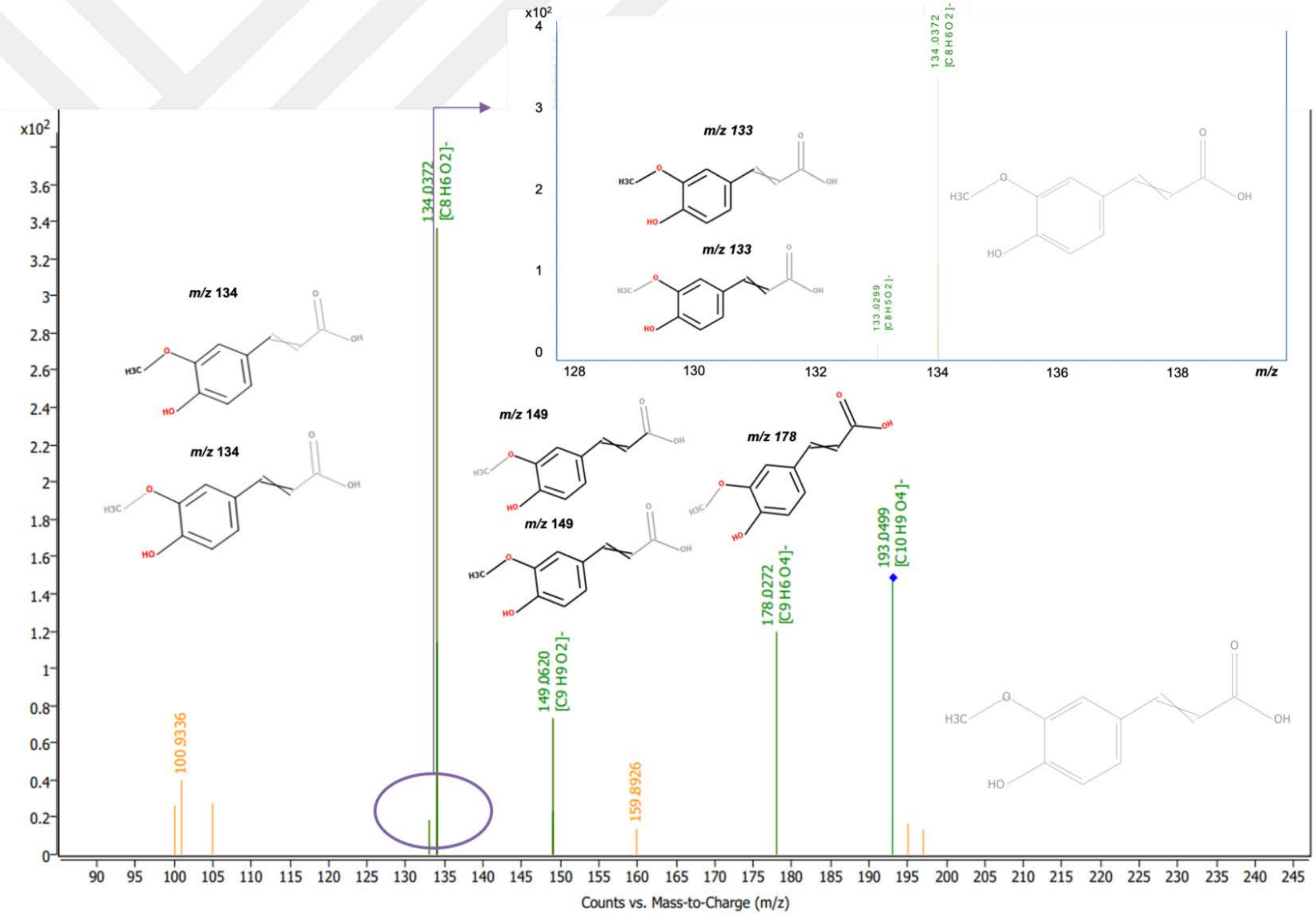


**Figure 4.7 :** Speculative fragmentation pathway of ferulic acid in negative ionisation mode (ESI−).

Heat map analysis was used to visualise the samples based on the concentration of bioactive compounds at distinct fermentation time points and phenolic fractions, where the colour scheme from red to blue shows the concentrations in decreasing order. Figures 4.8 and 4.9 show significant concentration differences ($p < 0.05$) in *L. edodes* and *La. deliciosus*, identifying 22 and 28 phenolic compounds, respectively, with three vitamins in each. The concentration differences during LAB fermentation can be explained by changes in the content and profile of bioactive compounds, modification of the parent phenolic compounds by changing their nutritional value and bioaccessibility, and structural breakdown of the mushroom cell walls (Rocchetti et al., 2022). In our study, certain compounds that became undetectable or reduced within FPs during fermentation could be associated with the metabolic activities of *Lp. plantarum LMG 17673*. For instance, there was a decrease in p-hydroxybenzoic acid glucoside in both LEFPs and LDFPs, which corresponded to a higher accumulation of its reduced metabolite, 4-hydroxybenzoic acid. As a result of this concentration difference, the concentration of quinic acid increased in both LEFPs and LDFPs. In contrast, that of 5-feruloylquinic acid, a derivative of quinic acid, decreased during LAB fermentation. This decrease might have contributed to the increase in quinic acid content, suggesting a potential conversion. Similarly, the concentration of quinic acid was significantly higher in avocado seeds fermented with *L. plantarum*, indicating possible hydrolysis of hydroxycinnamic acids (Razola-Díaz et al., 2023b). Additionally, hydroxycinnamic acids, such as caffeic acid, can undergo transformation into different compounds facilitated by reductase enzymes. This process may lead to the metabolism of dihydrocaffeic acids, which can further undergo decarboxylation to produce vinyl derivatives such as vinyl phenol and vinyl guaiacol (De Montijo-Prieto et al., 2023b; Degrain et al., 2020**.** Previous studies have reported that the *Lp. plantarum* may contribute to the degradation of phenolic compounds through processes such as depolymerisation, hydrolysis, decarboxylase-mediated metabolism, and reductase activity (Y. Wang et al., 2021).

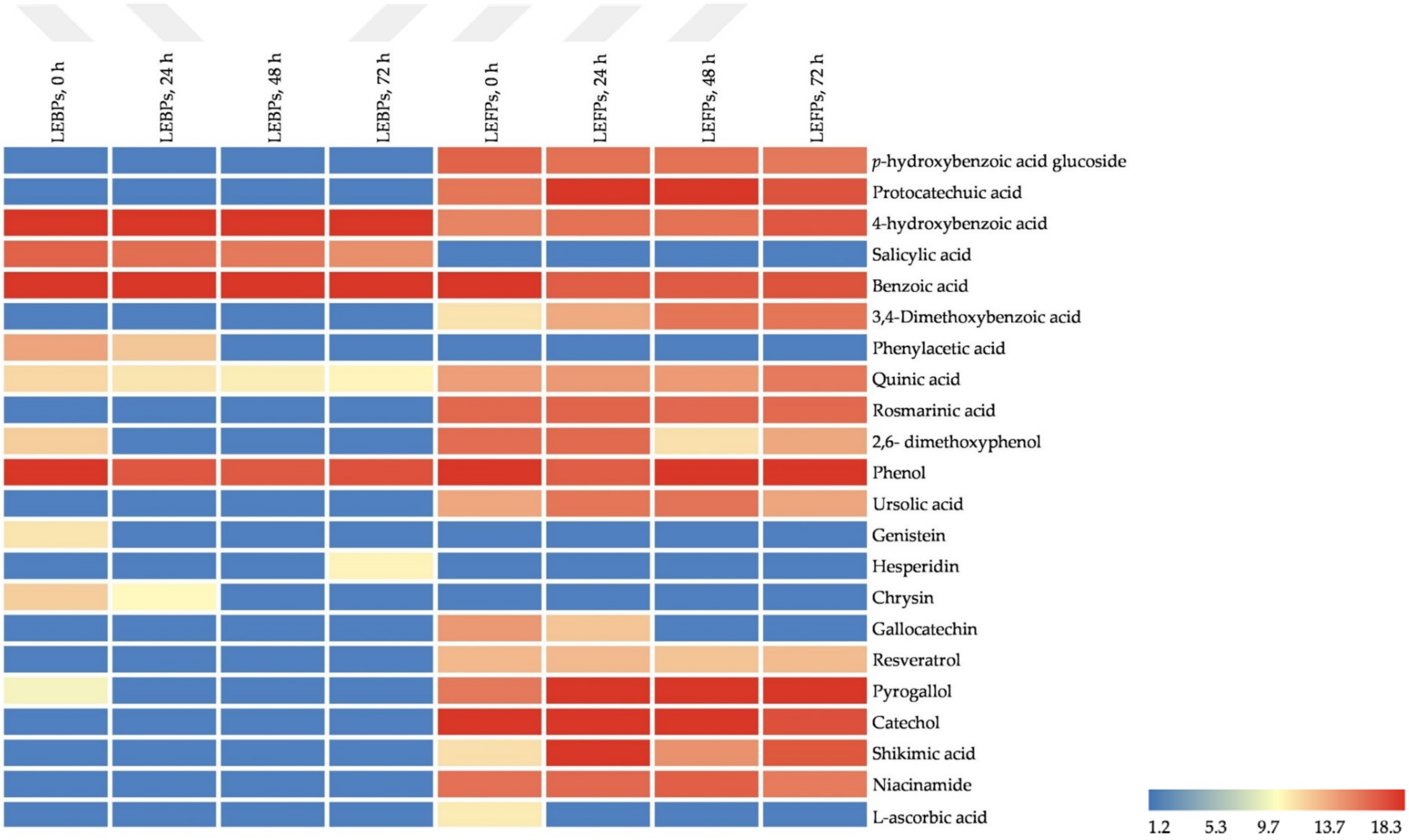


**Figure 4.8 :** Level of phenolic compounds and vitamins in *L. edodes* fractions during fermentation. LEBPs: *Lentinula edodes* bound phenolics; LEFPs: *Lentinula edodes* free phenolics. 0 h, 24 h, 48 h, and 72 h represent fermentation time points.

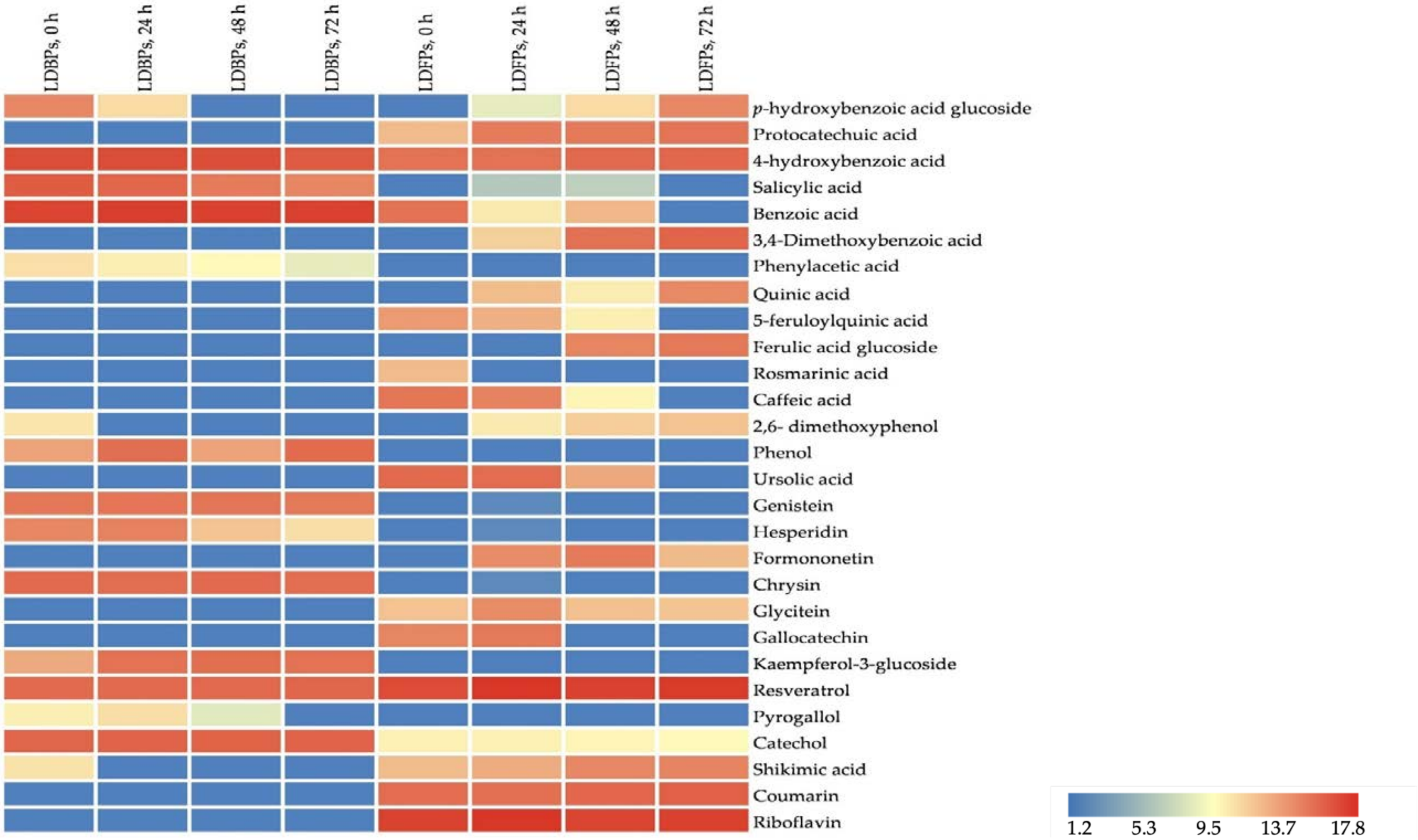


**Figure 4.9 :** Level of phenolic compounds and vitamins in *La. deliciosus* fractions during fermentation. LDBPs: *Lactarius delicious* bound phenolics; LDFPs: *Lactarius delicious* free phenolics. 0 h, 24 h, 48 h, and 72 h represent fermentation time points.

Fermentation increased the levels of certain components, such as protocatechuic acid, 4-hydroxybenzoic acid, 3,4-dimethoxybenzoic acid, quinic acid, phenol, pyrogallol, and shikimic acid in *L. edodes* FPs. Likewise, in the free fraction of *La. deliciosus*, the concentrations of protocatechuic acid, 4-hydroxybenzoic acid, salicylic acid, 3,4-dimethoxybenzoic acid, quinic acid, ferulic acid glucoside, 2,6-dimethoxyphenol, formononetin, glycitein, resveratrol, shikimic acid, and coumarin were significantly higher than those in the initial stages of fermentation. Furthermore, the biotransformation of ferulic acid to vanillin has been well-studied in various bacterial species, including LAB (Lubbers et al., 2019). However, we found increased ferulic acid glucoside levels in LDFPs without identifying vanillin, possibly because of its low concentration below the threshold (score < 75). Moreover, during *L. edodes* fermentation, quinic acid, 2,6-dimethoxyphenol, phenol, and pyrogallol concentrations decreased in the bound fraction but increased in the free fraction. Similarly, during *La. deliciosus* fermentation, the levels of 4-hydroxybenzoic acid, salicylic acid, 2,6-dimethoxyphenol, and shikimic acid decreased in the bound fraction and increased in the free fraction. This phenomenon can be explained by the ability of LAB to break down large molecules in favour of phenolic compounds in mushrooms, which may include breaking down cell wall polysaccharides and proteins, wherein bound phenolics are linked (D. Zhang et al., 2022). Thus, LAB fermentation can potentially enhance biological activity by converting bound phenolics to a free state. Additionally, LAB fermentation can biotransform complex phenolic compounds and flavonoids into simple bioactive compounds, such as phenolic acids, thereby increasing their bioavailability (J. Yang, Gao, et al., 2022).

## 4.5 Conclusions

This study demonstrated the efficiency of LAB fermentation in the biotransformation of phenolic compounds. *Lp. plantarum LMG 17673* was successfully fermented for 72 hours on the substrate of *L. edodes* and *La. deliciosus*, resulting in a high viable cell count. *L. edodes and La. deliciosus* were rich in bound and free phenolics, with the former fraction being notably more abundant. LAB fermentation enhanced the releasing of bound phenolics and modified their parent phenolic compounds and structure. The phenolic compound composition and concentration changes were assessed using UPLC-MS-TOF-MS/MS. This study detected thirty phenolic compounds in the mushroom fractions during LAB fermentation. Notably, DPPH scanning activity analysis indicated that the release of bound phenolics significantly contributed to antioxidant activity through fermentation. Hence, this study demonstrated the effect of bound phenolics on the bioaccessibility of mushrooms. Subsequent investigations concerning the conversion mechanism of phenolic compounds within mushrooms through LAB fermentation should be pursued in future studies.

# 5. THE IMPACT OF LACTIC ACID BACTERIA FERMENTATION ON PHENOLIC BIOACCESSIBILITY

## 5.1 Abstract

Consuming mushrooms may have health benefits due to their bioactive compounds, such as phenolic compounds. However, most of the phenolic compounds in mushrooms are in bound form. Thus, phenolic compounds have low bioavailability and bioaccessibility. Lactic acid bacteria can alter the structure of polyphenols in foods, consequently influencing their bioavailability and antioxidant capacity. This study used *Lactiplantibacillus plantarum LMG 17673* to investigate the effects of lactic acid bacteria fermentation and in vitro digestion of bioactive compounds in *Lentinula edodes* and *Lactarius deliciosus.* The changes in the antioxidant capacity of mushrooms have also been studied. Our results demonstrated that *L. edodes* and *La. deliciosus* are suitable matrixes for *Lb. plantarum*. Lactic acid fermentation increased the total phenolic content and improved the bioaccessibility of phenolic compounds during in vitro digestion. Fermentation had no remarkable influence on ABTS but increased DPPH. Additionally, fermentation improved the peptide profile before and after in vitro digestion, mainly because of protein metabolism. This study provides valuable information regarding the development of *L. edodes* and *La. deliciosus* as functional products with improved phenolic bioavailability.

## 5.2 Introduction

Polyphenols need to be bioaccessible to provide health benefits. Their bioactivity depends on their bioaccessibility and bioavailability. Bioaccessibility refers to the amount of a compound released from its matrix during digestion (Valero-Cases et al., 2017). Phenolic bioaccessibility is influenced by their chemical complexity and interactions with various compounds during digestion. In vitro digestion procedures and fermentation by lactic acid bacteria play a crucial role in phenolic bioaccessibility assessment and absorption (Mashitoa et al., 2021).

Mushrooms have been found to have numerous health benefits due to their rich composition of polyphenols, terpenes, steroids, and polysaccharides. These benefits include antimicrobial, antioxidant, antitumor, antihypertensive, and anti-ageing properties. Phenolic compounds, a type of polyphenol, are the main contributors to mushrooms' antioxidant capabilities (X. Wang et al., 2022b). Moreover, as an essential secondary metabolite, phenolic compounds can be found in both free and bound forms in both plant and mushroom matrices, as highlighted by various studies (Çayan et al., 2020; Chu et al., 2023; Reis et al., 2012; X. Wang et al., 2022b). Free phenolics (FPs) do not interact with other cellular molecules and are therefore easily extractable using aqueous or aqueous-organic solvents, making them extractable phenolics (M. Li et al., 2020). However, bound phenolics (BPs) are linked to cell wall components such as lignin and polysaccharides through ester, ether or glycosidic bonds, making them non-extractable without breaking these linkages through acid, base, or enzymatic hydrolysis (Alves et al., 2021; Rocchetti et al., 2022). There has been extensive research on the free phenolics in shiitake mushrooms, highlighting their potential benefits (Reis et al., 2012). However, to the best of our knowladge there is only one study focused on the extraction methods of bound phenolic compounds to identify and quantify phenolic compounds in *L. edodes* (X. Wang et al., 2022b).

The bioavailability and bioaccessibility of bound phenolics are generally lower compared to free phenolics due to their complex-bound form, which makes them less accessible for absorption and requires their release before absorption can occur (Alves et al., 2021). The fermentation process can biotransform and metabolise phenolic compounds into smaller, potentially more bioactive forms that are easier for the body to absorb and utilise. In addition, fermentation can degrade complex molecules and release bound phenolics (Myo et al., 2021; Wu et al., 2020). Thus, fermentation enhances the release of a plant's bioactive compounds, increasing their bioaccessibility and bioavailability. This enhancement significantly improves the nutritional and functional properties of the food, leading to beneficial health effects (Cuvas-Limon et al., 2022). Hole et al. (2012) found that probiotic LAB strain fermentation can significantly increase the levels of free phenolic acids in cereals, thereby improving the bioavailability of dietary phenolics Valero-Cases et al. (2017) discovered that fermentation can lead to the biotransformation of phenolic compounds in pomegranate juices and the formation of new phenolic derivatives, thereby increasing phenolic compounds' antioxidant capacity and bioaccessibility. Khan et al. (2020) demonstrated that co-culture fermentation by LAB and yeast enhances the bioaccessibility of phenolics and flavonoids in extruded brown rice.

Ucar & Karadag (2019) reported that different drying methods altered the structure of substances and affected the digestibility of bioactive compounds. Vacuum drying preserved more phenolic content and made it more accessible during digestion while freeze-drying impacted the structural integrity of phenolics differently and reduced bioaccessibility. Ng & Rosman (2019) explored the effects of different cooking methods on the antioxidant and enzyme-inhibitory activities of mushrooms. In vitro digestion improved antioxidant and anti-α-glucosidase activities but decreased anti-α-amylase activity, showing a selective effect depending on the type of enzyme. However, to our knowledge, there have been no reported studies on the effect of LAB fermentation on the biotransformation, bioaccessibility, and biological activity of phenolic compounds in fermented mushrooms. This study aims to investigate the behaviour of bioactive compounds, antioxidant activity, and bioaccessibility of *Lentinula edodes* and *Lactarius deliciosus* that have been fermented with *Lactiplantibacillus plantarum* during simulated in vitro gastrointestinal digestion. This research may provide valuable insights to encourage the development of functional mushroom formulations with added benefits, using natural resources that can enhance human health.

## 5.3 Material and Methods

### 5.3.1 Media and chemicals

De Man-Rogosa-Sharpe (MRS) and plate count agar were purchased from Thermo-Fischer (Merelbeke, Belgium). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Trolox, gallic acid, α-amylase (Type VI-B, > 10 units/mg solid, from porcine pancreas), pepsin (3200-4500 units/mg protein, from porcine gastric mucosa), pancreatin (8xUSP, P7545, from porcine pancreas), and bile salts (P1001879903, from porcine bile extract) were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA).  Louis, MO, USA). Aluminium chloride, acetic acid, sodium nitrite, methanol, hydrochloric acid, sodium hydroxide, and sodium carbonate were purchased from ChemLab (NV, Belgium). Technical-grade $CH_3OH$, $FeCl_3$, NaOH, HCl, KCl, NaCl, $KH_2PO_4$, $NaHCO_3$, $CaCl_2(H_2O)_2$, $MgCl_2(H_2O)_6$, $NH_4Cl$, and $HNO_3$ were obtained from VWR Chemicals (VWR International, Leuven, Belgium).

### 5.3.2 Mushroom fermentation

*L. edodes* and *La. deliciosus* were acquired from a local market in Turkey, and the mushrooms were cleaned, cut into small pieces, frozen at -20°C, and lyophilised using a Christ Alphna 1-2 LDplus device (Osterode am Harz, Germany). The lyophilised samples were ground into a mushroom powder (IKA-T10, Germany). For fermentation, the mushroom powder of 2 g was mixed with 100 mL of physiological water, creating the mushroom solution and sterilised.

The lactobacillus strain of *Lactiplantibacillus plantarum LMG 17673* was purchased from the BCCM/LMG Bacteria Collection, an integral component of the Belgian Co-ordinated Collections of Microorganisms situated within the Laboratory for Microbiology of the Faculty of Sciences of Ghent University, Belgium. The inoculum of lactobacilli was prepared according to the method described by He et al. (2021) with slight modifications. The lactobacilli culture was aseptically activated by transferring 100 μL of glycerol stock culture into 10 mL of sterile MRS broth and then incubated at 37 °C for 24 h. *Lp. plantarum* was propagated in MRS broth before being used as a working culture to inoculate the mushroom solutions. Flasks containing 25 mL of sterilised mushroom solution were inoculated with 1% (v/v) working culture and then placed on a rotary shaker (110 rpm) at 30°C. During fermentation, samples were collected at different time points (0 and 24), freeze-dried, and the powders were stored at -20°C until further analysis.

### 5.3.3 Static in vitro digestion

The INFOGEST standardised consensus model developed by Brodkorb et al. (2019) was used to perform static in vitro digestion. This model consists of three phases, as explained below. Freeze-dried samples were used for in vitro digestion analysis. It is important to note that mushrooms contain approximately 90% water; therefore, before starting the in vitro digestion analysis, a specific amount of mushroom powder was mixed with distilled water to reconstitute them as recommended in the protocol.

During the oral phase, 5 g fresh mushroom solution was mixed with an equal volume of simulated gastric juice. The mushroom solution was first mixed with 4 mL of a simulated salivary fluid (SSF) electrolyte stock solution. The SSF is an electrolyte with a pH of 7 and is made up of a mixture of KCl, NaCl, $KH_2PO_4$, $NaHCO_3$, $MgCl_2(H_2O)_6$, and $(NH_4)_2CO_3$ at varying concentrations, prepared in bi-distilled water. Then, the mixture was supplemented with 0.75 mL of α-amylase solution (150 units/mL) in SSF solution, 25 μL of 0.3 M $CaCl_2$ solution, and 975 μL of double-distilled water. After incubation for 2 min at 37°C in a shaking water bath, an oral bolus was prepared.

During the gastric phase, an oral bolus was mixed with an equal volume of simulated gastric fluid (SGF) electrolyte stock solution. The SGF electrolyte solution had a pH level of 3 and was composed of a mixture of KCl, NaCl, $KH_2PO_4$, $NaHCO_3$, $MgCl_2(H_2O)_6$, and $(NH_4)_2CO_3$ at different concentrations, prepared in distilled water. Afterwards, 0.75 mL of pepsin (4000 U/mL) in SGF and 5 μL of 0.3 M $CaCl_2$ solution were added to the mixture. The pH of the mixture was adjusted to 3 using 6 M HCl and 1 M NaOH. The volume of SGF was adjusted to 10 ml by adding sufficient water to the mixture.

Finally, the mixture was incubated at 37°C in a shaker water bath. For the intestinal phase, an equal volume of the simulated intestinal fluid (SIF) electrolyte stock solution was added to the mixture. SIF is a mixture of KCl, NaCl, $KH_2PO_4$, $NaHCO_3$, and $MgCl_2(H_2O)_6$ which were prepared in bi-distilled water at varying concentrations. The mixture contains 5.0 mL of pancreatin solution (400 U/mL) in SIF, 3 mL of fresh bile (20 mM), 40 μL of 0.3 M $CaCl_2$ solution, and 1.31 mL of water. The pH of the mixture was adjusted to 7 and then incubated for 2 h at 37°C in a shaking water bath.

### 5.3.4 Antioxidant activity and total phenolic content

After undergoing gastric and intestinal digestion, the samples were centrifuged, and the supernatant was freeze-dried and used for analysis. The phenolic compound extracted based on the method by Gonzales, Smagghe, et al. (2014). In summary, 2 g of fermented mushroom powder was blended with 15 mL of 100% methanol using Ultra-Turrax (IKA-T18D, Germany) at 3000 rpm for 45 s. Afterwards, the tubes were immediately cooled in an ice bath for 15 s before being centrifuged at 13000 × g for 10 minutes at 4°C using a centrifuge (Z 300 K, Hermle Labortechnik GmbH, Germany). The residue was resubmitted to extraction with 10 mL of 80% methanol, using the same method as before. The resulting clear liquid was filtered, and its volume was adjusted to 25 mL using 80% methanol. TPC and AA analyses of these methanolic extracts of both digested and undigested *L. edodes* and *La. deliciosus*, as well as the 0 h and 24 h fermented samples, were performed according to the method described in *Chapter 4*.

### 5.3.5 Bioaccessibility index

The bioaccessibility index assesses the amount of phenolic compounds released during gastrointestinal digestion, which can be absorbed into systemic circulation. The bioaccessibility index of phenolic compounds was calculated as follows:

$$\text{Bioaccessibility (\%)}=\frac{TPC_{digested}}{TPC_{undigested}}\times 100 \tag{5.1}$$

where $\mathrm{TPC}_{\mathrm{digested}}$ refers to the TPC of after intestinal digestion of fermented and unfermented *L.edodes* and *La.deliciosus*; $\mathrm{TPC}_{\mathrm{undigested}}$ refers TPC of fermented and unfermented *L.edodes* and *La.deliciosus*, which calculates according to the method described in *Chapter 3.*

### 5.3.6 SDS-PAGE gel electrophoresis

Protein/peptide profile of fermented and digested *L. edodes* and *La. deliciosus* were examined using SDS-PAGE following the method described in *Chapter 3*. Shortly, the analysis was performed on 12.5% resolving Tris-HCl acrylamide gels containing 375 mM Tris-HCl, pH 8.6, 0.001% SDS, 0.0005% TEMED, and 0.0005% APS. To prepare for electrophoresis, 25 µl of each protein sample was mixed with 12.5 µl of β-mercaptoethanol and 237 µl of Laemmli buffer consisting of 62.5 mM Tris-HCl pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue, and 5% β-mercaptoethanol. The samples were then heated for 5 minutes at boiling temperature. For electrophoresis, 10 µl of each sample was loaded per lane and run at a steady 20 V for 60 minutes using a 10× Tris/Tricine/SDS running buffer containing 25 mM Tris, pH 8.3, 192 mM glycine, and 0.1% SDS. After electrophoresis, the gels were rinsed briefly three times in distilled water, stained with Coomassie Brilliant Blue R-250, and then destained using distilled water. The protein molecular weights were estimated using BioRad Precision Plus Protein Standards, which range from 10 to 250 kDa.

### 5.3.7 UPLC-Q-TOF-MS/MS-based phenolic compound characterisation

In this study, since fermentation is examined as a treatment method, the impact of the steps applied during the fermentation process was investigated by comparing the phenolic composition of mushrooms that have not undergone treatment with those that have been treated but not fermented (0 h fermented) mushrooms. For this purpose, the total phenolic extracts of the mushrooms and the amounts of free and bound phenolics were compared. The free and bound phenolic extraction is performed as explained in *Chapter 4.* The extracts were analysed using an ultrahigh-performance liquid chromatography system (UPLC Infinity 1290, Agilent Technologies, Santa Clara, CA, USA) linked to a quadrupole time-of-flight mass spectrometer (Q-TOF 6546, Agilent Technologies). This setup included a quaternary pump (G 7104A), a Column18 5u (4.6 × 150 mm; GRACE, Deerfield, USA), a Column Oven (G 7130A), and a photodiode array detector (DAD) (G 711l7A, Agilent Technologies). The system also featured an online degasser (Model 590, Alltech elite degassing system, USA), a sampler (G 7129B), and the same specifications for the column and detector. Chromatographic separation was achieved using a gradient elution with two mobile phases: ultrapure water with 0.1% formic acid (eluent A) and LC-MS-grade methanol with 0.1% formic acid (eluent B). The gradient protocol was detailed as follows: 0-6 minutes at 20% B isocratically, 6-12 minutes maintained at 20% B, a gradient increase from 20% to 30% B over 12-13 minutes, followed by 30-50% B from 13-23 minutes, a sharp rise to 50-90% B from 23-30 minutes, held at 90% B from 30-35 minutes, then reduced to 10% B from 35-40 minutes, and finally maintained at 10% B from 40-45 minutes. Each sample underwent a 45-minute run at a flow rate of 0.25 ml/min and an injection volume of 20 µL. The eluate was introduced into a Dual Agilent Jet Stream (AJS) electrospray ionization (ESI) source of the mass spectrometer. Mass spectrometry settings included a capillary voltage of 3.5 kV, nozzle voltage of 4 V, gas temperature of 300 °C, vaporiser temperature of 350 °C, gas flow rate of 8 L/min, and vaporizer flow of 11 L/min. A comprehensive mass scan from m/z 100 to 1700 was conducted.

### 5.3.8 Non-target screening and metabolomics analysis UPLC-Q-TOF-MS

The mushrooms that were both treated and untreated were analyzed using Agilent MassHunter Workstation Profinder software (version 10.0) in triplicate and negative mode. The software employed the Batch Recursive Feature Extraction workflow to identify non-target compounds. At first, the software identified molecular features in the first sample, where each feature represented a cluster of related ions, including isotopes and adducts of the same compound, forming a chromatographic peak at a specific retention time (RT). These features, along with their precise monoisotopic mass and RT, were cataloged in a list used as a reference for revisiting sample data via the Find-by-Ion method. Then, the software applied a binning algorithm to examine all features across the samples, aligning and grouping features by exact mass and RT. This comparison generated an extensive list of features, noting their occurrence in various samples. In the next phase, the software conducted a more sensitive scan of the average exact masses and RTs in the compiled feature list across each sample to uncover any initially missed features. This recursive extraction method improved the precision of feature identification, focusing on low-abundance but high-quality features, thus minimizing false negatives and enhancing the speed of the binning process.

The obtained feature list was uploaded into Agilent Mass Profiler Professional (MPP, version 15.1, Agilent Technologies). MPP is a software that is equipped with advanced statistical tools that improve the visualization of results. Since it was impractical to identify every feature, statistical analyses were used to focus on the relevant compounds. Metabolic variations among replicate samples were initially assessed using principal component analysis (PCA). Afterward, Fold Change analysis was conducted on the features that showed variations in abundance under different conditions. Paired analyses were carried out using the Benjamini-Hochberg method. Finally, the results were visually displayed using volcano plots.

### 5.3.9 Statistical analysis

Statistical analyses were performed using SPSS version 28.0 program (IBM SPSS Statistics, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed to investigate the variations in mushroom fermentation and digestion. To compare the means and identify significant differences, the honestly significant difference (HSD) test was applied ($p < 0.05$). The fermentation process was carried out in triplicate using three independent inocula. The reported values are expressed as mean ± SD based on three independent samples.

## 5.4 Results

### 5.4.1 The changes in phenolic content during fermentation and in vitro digestion

The TPC and AA during fermentation and digestion are presented in Table 5.1. Following fermentation with *Lb. plantarum* difference between mushrooms with and without fermentation ($p < 0.05$). During simulated gastric digestion (SGD), the TPC of fermented *L. edodes* was significantly higher ($p < 0.05$) than that of unfermented *L. edodes,* whereas the TPC of fermented *La. deliciosus* was slightly higher ($p > 0.05$) than that of unfermented *La. deliciosus*. However, all TPC values during SGD were higher than those of the unfermented samples, likely due to the hydrolysis of bonds between phenols and proteins in the acidic environment (Blanco Canalis et al., 2020). Conversely, during SID, the TPC either decreased or remained constant as phenolic compounds became unstable and degraded in an alkaline environment (Yan et al., 2019). The bioaccessibility index of the mushroom samples also indicated this trend (Figure 5.1). Nevertheless, fermented mushrooms exhibited higher TPC after digestion than unfermented mushrooms, indicating that lactic acid fermentation may enhance phenolic bioaccessibility (Dong, 2023). This suggests that the biotransformation of phenolic compounds, including the conversion from complex to simpler forms, and the release of bound phenols from the plant cell wall, may contribute to the enrichment of phenolic compounds. Additionally, a decrease in pH during fermentation may enhance the dissolution and stability of phenolic compounds (Dong, 2023). *Chapter 4* indicates that the conversion of phenolics can influence their bioactivity, potentially altering the composition of bioactive compounds in mushrooms during lactic acid fermentation.

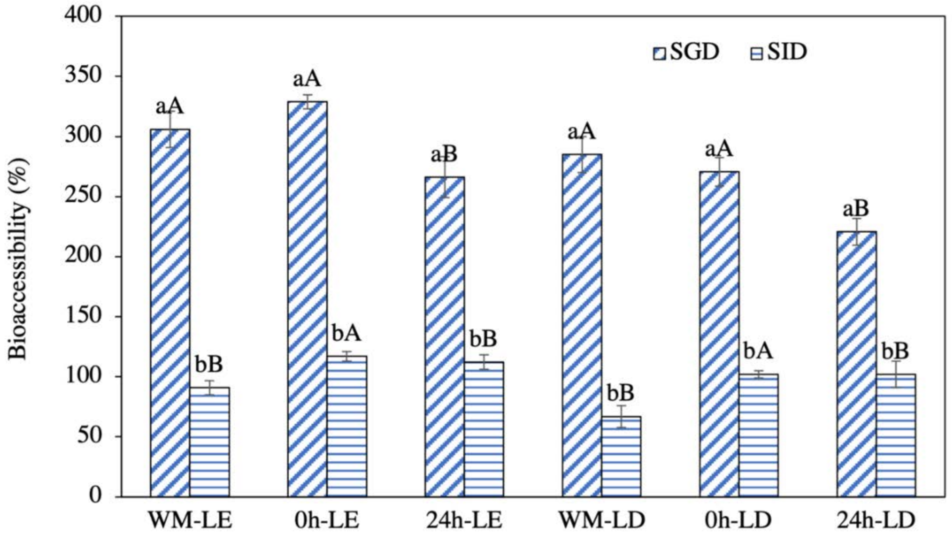


**Figure 5.1 :** Bioaccessibility of fermented and unfermented mushrooms. Different small letters represent statistically differences in the same sample in different *in vitro* stages, different capital letters represent statistically differences between same *in vitro* stages in different samples ($p < 0.05$). Values are presented as mean ± SD ($n$=3).

Since there is a lack of studies on fermented mushrooms and their in vitro digestion concerning phenolic compound bioaccessibility, we compared our findings with those of similar research on fermented vegetables and fruits. For example, Mashitoa et al. (2021) investigated the effects of fermenting papaya puree with various LAB strains on the bioaccessibility of phenolic compounds during in vitro digestion. They observed that the TPC, which was 395.4 mg/kg before *Lb. plantarum* fermentation, increased to 502.4 mg/kg and 372 mg/kg after in vitro digestion (gastric and intestinal phases, respectively). Furthermore, they found that the antioxidant activity of fermented papaya puree subjected to simulated gastric digestion was higher up to 3-fold than that of unfermented papaya puree (from 1.2 µmol TEAC/100 g FW to 3.7 µmol TEAC/100 g FW).

Santana Andrade et al. (2022) investigated the influence of in vitro gastrointestinal digestion and *Lactobacillus* probiotic fermentation on the bioaccessibility of phenolic compounds in Brazilian fruit residues. They reported that the aqueous and ethanolic extracts of soursop seeds showed bioaccessibility of the total phenolic content exceeding 100% after the gastrointestinal digestion stage in vitro, representing 283.63 ± 0.31% (aqueous extract) and 445.26 ± 8.34% (ethanolic extract).

Attri et al. (2017) reported that pancreatin influenced the binding of phenolic compounds to the food matrix, leading to the release of phenolics in the intestinal phase. Our study revealed that mushrooms released varying amounts of phenolics in each digestion phase, which may be attributed to differences in chemical composition, dietary fibre, hydrophobic interactions, hydrogen bonding, and covalent bonds. In conclusion, *L. edodes* exhibited a higher phenolic content released in the gastric and intestinal phases than *La. deliciosus* in this study. Regarding TPC, no statistical difference was observed before and after SID of fermented mushrooms, but SID significantly decreased the TPC of simulated gastric digested unfermented mushrooms (WM-LE and WM-LD). These findings suggest that lactic acid fermentation could increase the amount of phenolic compounds that are not extracted or absorbed in the small intestine reaching the colon, potentially enhancing the functional activity of phenolic compounds in *L. edodes* and *La. deliciosus*.

Overall, following the simulation of gastrointestinal digestion in vitro, there was a notable increase in the TPC concentration and bioaccessibility index for both mushrooms. Furthermore, during the fermentation stage, TPC was higher than that during the unfermented digestion stage. This suggests that the compounds contribute to the heightened bioaccessibility of phenolic compounds upon metabolism by the intestinal microbiota. The observed increase in phenolic content following fermentation and digestion may be attributed to the breakdown of bound phenolic compounds, facilitated by enzymatic action and low pH. However, variations in phenol content during gastric digestion could stem from differences in LAB strain survival and activity, affecting phenolic compound metabolism. However, the quantification of LAB populations during gastrointestinal transit was not performed in this study. The LAB survival rate was determined for the fermented samples, as discussed in *Chapter 4*. The decline in phenolic content during intestinal digestion may be linked to a shift in pH from acidic to alkaline conditions.

### 5.4.2 The changes in antioxidant activity during the fermentation and in vitro digestion

Two methods were used to assess the antioxidant capacity of mushrooms. As shown in Table 5.1, fermented mushrooms exhibited a significant increase in DPPH radical scavenging activity and ABTS activity compared to unfermented mushrooms during gastrointestinal digestion, indicating that lactic acid fermentation enhanced the antioxidant capacities of both *L. edodes* and *La. deliciosus*. In general, both mushrooms and all periods showed an increase in antioxidant activity during digestion. Polyphenols, which are structurally diverse and their mechanisms are pH-dependent, are easily deprotonated below the pH range of 7-9, which supports the mechanisms of the antioxidant action of these compounds (Q. Zhao et al., 2023a). Consequently, the inhibitory activity after intestinal digestion was generally higher than that after gastric digestion. Additionally, the increase in the release of phenolic compounds, the rise in the release of reactive compounds, and the formation of new compounds may be associated with the increase in antioxidant activity (Y. Ma et al., 2020).

Andrade et al. (2022) assessed the impact of in vitro gastrointestinal digestion of fermented fruit residues with antioxidant activity assessed using ORAC, ABTS, and DPPH assays. All assays indicated a notable increase in the bioaccessibility of the antioxidant activity, surpassing 100% (Andrade et al., 2022). Particularly, the ORAC method displayed superior efficacy compared to other antioxidant assays, revealing a significant rise in AA in vitro digestion (ranging from 71.02 ± 8.66 to 122.16 ± 4.49 μmol Trolox/g of sample) and fermentation (ranging from 77.20 ± 3.12 to 94.46 ± 6.25 μmol Trolox/g of sample) of both aqueous and ethanolic extracts, in contrast to undigested samples (ranging from 15.02 ± 0.64 to 60.17 ± 2.18 μmol Trolox/g of sample). Bioaccessibility indices ranged from 131.40 ± 1.92 to 813.32 ± 21.13%, with a notable emphasis on soursop seed extracts. These findings align with our results, which showed that the antioxidant capacity of fermented samples, as determined by ABTS and DPPH assays in SGD and SID, was significantly higher than that of unfermented mushrooms (WM-LE and WM-LD).

**Table 5.1 :** TPC, DPPH and ABTS values of fermented and unfermented mushrooms before digestion and after simulated gastric digestion (SGD) and simulated intestinal digestion (SID).

|  | Sample | WM-LE | 0h-LE | 24h-LE | WM-LD | 0h-LD | 24h-LD |
|---|---|---|---|---|---|---|---|
| TPC (mg GAE/100 g dm) | UD | 136.64±2.05$^{bC}$ | 186.64±2.05$^{bB}$ | 246.53±1.18$^{cA}$ | 151.52±1.22$^{cB}$ | 168.72±3.27$^{bAB}$ | 214.58±2.87$^{bA}$ |
|  | SGD | 418.03±5.23$^{aC}$ | 613.75±12.37$^{aB}$ | 655.92±19.27$^{bA}$ | 431.89±5.71$^{aC}$ | 456.51±3.78$^{aB}$ | 473.41±11.62$^{aA}$ |
|  | SID | 379.62±8.27$^{aC}$ | 718.02±19.64$^{aB}$ | 735.63±21.34$^{aA}$ | 288.68±2.06$^{bC}$ | 465.98±6.22$^{aB}$ | 482.32±3.08$^{aA}$ |
| DPPH (mg TE/g dm) | UD | 2.03±0.14$^{bC}$ | 2.55±0.11$^{cB}$ | 3.77±0.10$^{bA}$ | 1.87±0.14$^{bB}$ | 2.26±0.13$^{cA}$ | 2.88±0.36$^{bA}$ |
|  | SGD | 5.09±0.71$^{aB}$ | 8.27±0.12$^{bA}$ | 10.44±0.69$^{aA}$ | 2.24±0.32$^{aC}$ | 3.70±0.12$^{bB}$ | 5.48±0.16$^{aA}$ |
|  | SID | 5.29±0.67$^{aC}$ | 9.92±1.06$^{aB}$ | 11.37±0.78$^{aA}$ | 2.42±1.09$^{aC}$ | 4.29±2.06$^{aB}$ | 5.93±0.21$^{aA}$ |
| ABTS (mg TE/g dm) | UD | 5.65±1.87$^{bB}$ | 6.29±0.21$^{aA}$ | 5.90±0.45$^{aA}$ | 5.73±1.77$^{bB}$ | 5.69±0.1$^{bA}$ | 5.56±0.31$^{aA}$ |
|  | SGD | 21.39±4.12$^{aB}$ | 33.95±4.21$^{bA}$ | 36.02±6.23$^{bA}$ | 26.42±5.32$^{aB}$ | 36.43±6.85$^{aA}$ | 38.97±8.42$^{bA}$ |
|  | SID | 21.90±5.88$^{aB}$ | 39.75±6.52$^{cA}$ | 40.00±7.22$^{cA}$ | 25.32±3.83$^{aC}$ | 37.59±8.7$^{aB}$ | 43.12±2.38$^{cA}$ |

Values are means of triplicate samples and expressed as mean ± SD. Capital letters compare the same mushroom species: Unfermented whole *L. edodes* mushroom (WM-LE); 0 h fermented *L. edodes* (0h-LE); 24 h fermented *L. edodes* (24h-LE); unfermented whole *La. deliciosus* (WM-LD); 0 h fermented *La. deliciosus* (0h-LD); and 24 h fermented *La. deliciosus* (24h-LD). Lowercase letters are the comparison among the evolution of period: Undigested mushroom samples (UD), Simulated Gastric Digestion (SGD), and Simulated Intestinal Digestion (SID). Different letters indicate significant differences ($p$ <0.05).

As demonstrated in *Chapter 4*, mushrooms contain a higher amount of bound phenolics than free phenolics, where bound phenolics are in glycoside conjugates or linked to cellular wall components, which can limit the availability of free hydroxyl groups in the phenolic structure (Wang et al., 2018). However, an increase in free phenolic hydroxyl groups owing to the microbial enzymatic release of aglycones from sugar moieties and the liberation of bound phenolics from the cell wall matrix can explain the greater antioxidant activities exhibited by fermented substrates (X. Wang et al., 2022b). Moreover, *Chapter 3* showed a positive correlation between the free phenolic content and the antioxidant activity of mushrooms. Therefore, we hypothesised that the observed increase in DPPH and ABTS in fermented mushrooms compared to unfermented ones during gastrointestinal digestion might be associated with the disruption of cell walls and sub-cellular compartments facilitated by LAB fermentation. This process is likely to enhance the release or biotransformation of certain antioxidant compounds. Solid-fermented mulberry leaves exhibited a similar trend, with their TPC decreasing during in vitro digestion, while their antioxidant activity increased throughout the digestion process (Q. Zhao et al., 2023b).

Although the fermented mushroom samples displayed reduced or stable TPC after SID compared to SGD, the highest antioxidant activity was observed in samples following SID. This disparity could be attributed to the ongoing degradation and regeneration of certain functionally bioactive compounds facilitated by LAB fermentation. In summary, our findings suggest that fermentation enhances antioxidant activity during in vitro digestion.

### 5.4.3 Electrophoresis of fractions

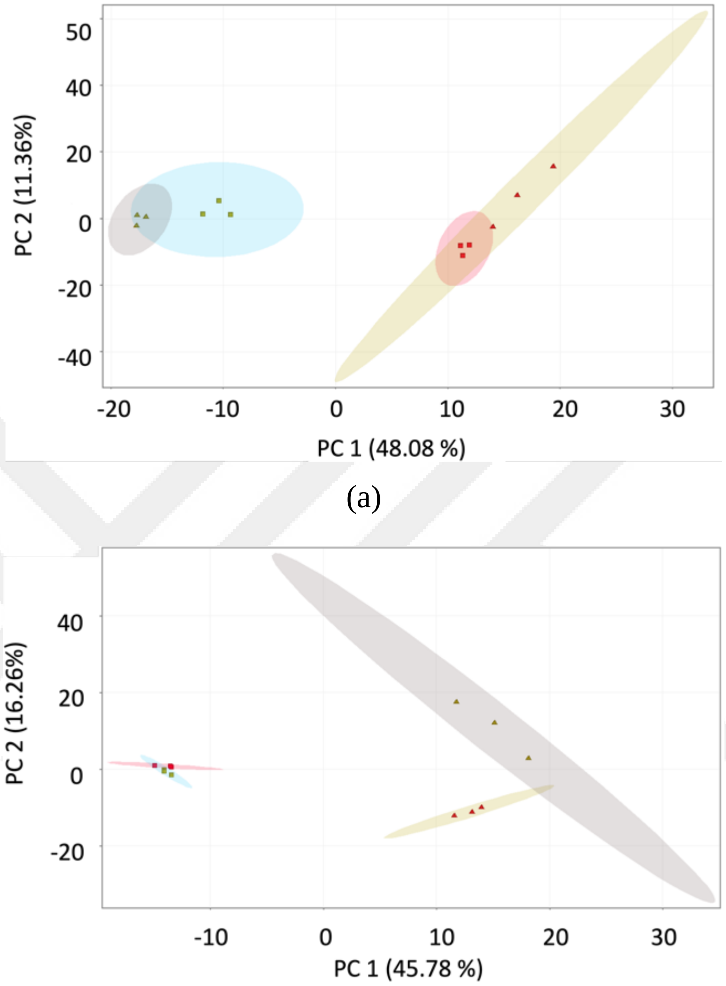
The increasing trend in antioxidant activity may be associated with the TPC, as discussed in section 5.2. Furthermore, protein hydrolysis resulting from fermentation and in vitro digestion may also contribute to antioxidant activity by generating antioxidant peptides (Gu et al., 2024; Verni et al., 2019). Therefore, the protein hydrolysis of *L. edodes* and *La. deliciosus* after gastric and intestinal digestion was examined using SDS-PAGE (Figure 5.2). SDS-PAGE analysis revealed a diverse range of proteins with apparent molecular weights ranging from 2 kDa to 250 kDa in mushrooms. SDS-PAGE patterns revealed strong bands in the simulated intestinal-digested samples, whereas the bands appeared weak in the simulated gastric-digested samples. The alkali pH was in favour of protein solubility during in vitro intestinal digestion. Notably, bands corresponding to pancreatic alpha-amylase were observed at approximately 50 kDa, whereas those corresponding to pancreatin ranged from 50 to 20 kDa (Sousa, 2020). The high molecular weight proteins (around 150-250 kDa) became less visible in the 24 h fermented *L. edodes* samples compared to unfermented and 0-hour fermented mushroom samples. Concurrently, there was a notable increase in the density of low-MW peptides/proteins (<10 kDa) in the 24-hour fermented mushroom samples. This suggests that fermentation and digestion lead to an increase in protein hydrolysis, potentially resulting in the generation of antioxidant peptides.

**Figure 5.2 :** SDS-PAGE of simulated digested fermented mushrooms: Lane 1, molecular weight markers in kDa, Lane 2, simulated gastric phase (SGP) unfermented *L.edodes*; Lane 3, simulated intestine phase (SIP) unfermented *L.edodes*; Lane 4, SGP of 0h fermented *L.edodes*; Lane 5, SIF of 0h fermented *L.edodes*; Lane 6, SGP of 24h fermented *L.edodes*; Lane 7, SIP of 24h fermented *L.edodes*; Lane 8, simulated gastric phase (SGP) unfermented *La.deliciosus*; Lane 9, simulated intestine phase (SIP) unfermented *La.deliciosus*; Lane 10 SGP of 0h fermented *La.deliciosus*; Lane 11, SIF of 0h fermented *La.deliciosus*; Lane 12, SGP of 24h fermented *La.deliciosus*; Lane 13, SIP of 24h fermented *La.deliciosus*; Lane 14, molecular weight markers in kDa.

### 5.4.4 The effect of fermentation process on phenolic compound characterisation

The observing different trends of phenolic content in untreated mushroom and 0 h fermented (treated) mushroom during in vitro digestion needed to be explained deeper. For this reason, pair comporasion between untreated and treated mushrooms performed. An unsupervised PCA was applied to gain insights into the metabolic variations untreated and treated mushrooms. Non-targeted metabolomics approach using UPLC-Q-TOF-MS datasets was employed for PCA, transforming the dataset into a series of values representing linearly uncorrelated variables. This widely utilised approach in metabolomic studies has allowed us to comprehensively overview metabolite variations among samples. Figure 5.3a shows the PCA score plot with PC1 and PC2, explaining 59.44% of the total variance (48.08% and 11.36%, respectively), which appeared to be treated and untreated *L. edodes* fractions. It could be observed that the FPs can be distinctly grouped from BPs. However, overlapping clusters at different fermentation time points in each fraction showed weakly explained variance. Figure 5.3b shows the PCA score plot with a higher explanation for *La. deliciosus*. PC1 and PC2 explained 61.99% of the total variance (45.78% and 16.21%, respectively), which also appeared to lie between those of treated and untreated *La. deliciosus* fractions. The FPs of untreated *La. deliciosus* exhibited a clear difference from the treated fractions. PCA indicated that the form of phenolics was most affected by fermentation, leading to increased differentiation between the treated and untreated samples.

To further confirm the variance in metabolic profiles among mushrooms, a volcano plot of fermented *L. edodes* (Figure 5.4a) and *La. deliciosus* (Figure 5.4b) showed the number of features which changed significantly during the treatment. The horizontal green line represents the p-test limit, and the features positioned above this line were considered statistically significant ($p < 0.05$). The vertical green lines represent the fold-change limits. The volcano plot shows the passing features (coloured) and non-passing features (grey). Each square, coloured pale blue or orange, corresponds to a feature that has successfully passed the significance test yet failed to meet the fold-change cut-off criteria. During the fermentation treatment of the mushrooms, pairwise group comparisons were conducted by comparing treated and untreated samples as a bound or free phenolic fraction. Each red square represents upregulated features, each blue square represents downregulated features ($p < 0.05$ and FC $\geq 2.0$), the upper right and left corner green squares represent the features ($p < 0.001$ and FC ≥ 2.0), and grey squares reveal metabolites that have no significant difference between different fermentation time point samples.



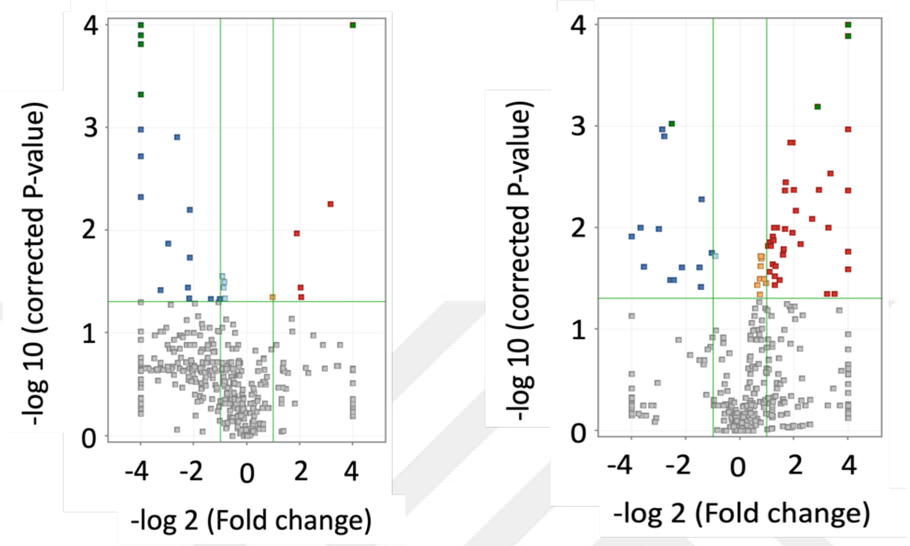
(a)

(b)

**Figure 5.3 :** Principal component analysis (PCA) plots with PC 1 and PC 2: (a) PCA plot of *L. edodes*; (b) PCA plot of *La. deliciosus*. The triangle represents bound phenolics, and the square represents free phenolics. Red colour represents untreated mushrooms, caramel colour represents treated mushrooms.

A total of 468 features were evaluated in pairwise groups of fermented *L. edodes*. After the volcano plot analysis with $p < 0.05$ and FC ≥ 2.0, 31 and 65 out of 468 features were significantly different in *L. edodes* samples for bound and free phenolic fractions, respectively (Figures 5.4a) ($p < 0.05$). In addition, 89 and 77 of the 469 potential features with significantly different amounts were screened within the pairwise groups for *La. deliciosus* for bound and free phenolic fractions, respectively (Figure 5.4b) ($p < 0.05$). These findings suggest that the LAB fermentation procedure can alter both the composition and concentration of phenolic compounds, highlighting the fact that free phenolics are predominantly affected by treatment. This phenomenon may be linked to lower pH, which has the potential to influence the stability of phenolic compounds, leading to degradation or structural alterations (X. H. Wang et al., 2022).

(**a**) 1) BPs; Treated vs Untreated 2) FPs; Treated vs Untreated



**(b)** 1) BPs; Treated vs Untreated 2) FPs; Treated vs Untreated

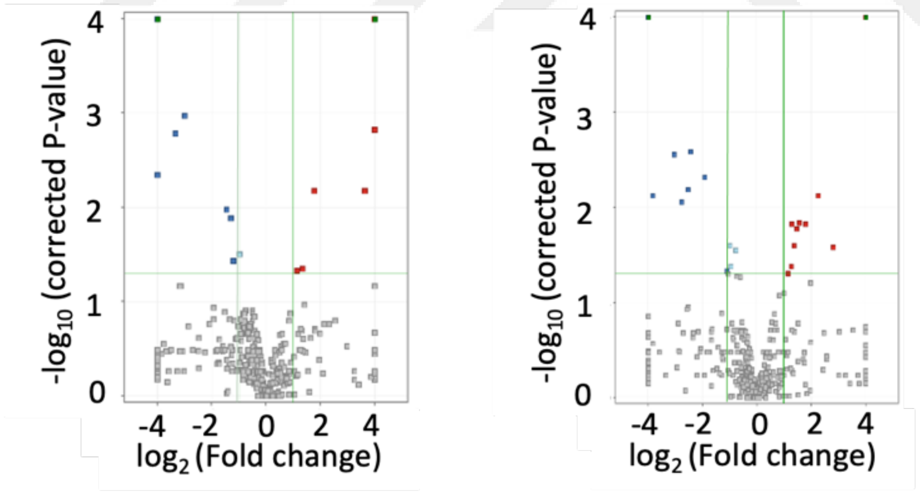


**Figure 5.4 :** Volcanic plots of differential metabolites in pairwise comparisons between treated and untreated: (**a**) *L. edodes*; (**b**) *La. deliciosus*. BPs; bound phenolics, FPs; free phenolics

Moreover, fermentation contributes to the diversification of free phenolic compounds, which further enriches the nutritional profile. Li et al. (Z. Li et al., 2019b) also observed that the phenolic composition of apple juice could undergo variations during fermentation with *Lb. plantarum*. This alteration may result from the removal or hydrolysis of structural moieties in various phenolic compounds, leading to alterations in phenolic profiles.

### 5.5 Conclusions

Although no studies have specifically examined the impact of fermentation on the bioaccessibility of phenolic compounds in mushrooms, *Lentinula edodes* and *Lactarius deliciosus* were successfully fermented using *Lactiplantibacillus plantarum* for 24 hours. Bioaccessibility was assessed by measuring the phenolic content released during various digestion phases, along with antioxidant activity and protein hydrolysis during in vitro digestion. TPC of the fermented mushrooms during the in vitro digestion was higher than that during the unfermented digestion stage, indicating that fermentation potentially enhances the bioaccessibility of phenolic compounds through the metabolic activities of the intestinal microbiota. This increase in phenolic content post-fermentation and digestion is likely due to the enzymatic breakdown of bound phenolic compounds, facilitated by enzymatic action and the acidic conditions during fermentation. Although the fermented mushroom samples displayed stable or reduced TPC after simulated intestinal digestion (SID) compared to simulated gastric digestion (SGD), the highest antioxidant activity was observed in samples following SID. This disparity could be attributed to the ongoing degradation and regeneration by LAB fermentation of certain functionally bioactive compounds, such as the transformation of phenolic compounds and hydrolysis of proteins, potentially resulting in the generation of antioxidant peptides. To sum up, LAB fermentation significantly enhances the antioxidant activity of mushrooms through in-vitro digestion.

# 6. CONCLUSIONS

## 6.1 Status and Main Outcomes of This Study

### 6.1.1 Valorization of mushroom by-products

Mushroom production involves a diverse range of by-products with applications in various fields. Although research on bioactive compounds derived from mushroom by-products is still in its early stages, it holds great promise for use in nutraceuticals and food products. Bioactive compounds have been isolated and analysed from mushroom by-products, including fruiting bodies (caps and stipes), SMS, mycelium, and fermentation broth. From the comparative studies, it cannot be concluded that there is an ideal solvent or method to obtain polyphenols from mushroom by-products, as it depends on the combination of different chosen parameters. Given the extensive literature on various extraction methods, such as ultrasound, pulsed electric fields, and microwave-assisted extraction techniques (*Chapter 2*), this study did not explore these methods further (Gogoi et al., 2019). Instead, by selecting the most appropriate extraction technique based on recent research, we streamlined the process of extracting and characterising phenolic compounds from *L. edodes* and *La. deliciosus*. This approach saved time and resources and allowed for a more focused study progression.

Mushrooms contain both soluble and insoluble polysaccharides. After the extraction of water-soluble polysaccharides, the residue is still rich in several bioactive compounds and is worth exploring. In this thesis, mushroom by-products were still available for lab fermentation. They even exhibited a higher viable cell count than the mushrooms themselves, likely due to a steadier increase in viable cells that prevented a sharp drop in pH, thus maintaining favourable conditions for lactic acid bacteria. Furthermore, because of the lack of soluble polysaccharides, lactic acid bacteria metabolise the compounds found in mushroom by-products, thus influencing the bacteria's substrate metabolism, consequently leading to protein degradation. Besides, this degradation affects the product's functional properties and contributes partially to its antioxidant activity.

Despite the potential variability in proximate composition, such as protein, polysaccharide content, and phenolic compound ratios, which may affect the nutritional value between mushrooms and their by-products (*Chapter 2*), the presence of natural compounds in the by-products was crucial for successful fermentation through substrate modification (*Chapter 3*). The effective fermentation of mushroom by-products resulted in these by-products possessing phenolic content, antioxidant activity, and water-holding capacity similar to those of mushrooms at the end of fermentation. Additionally, although the mushroom by-products initially showed a lower level of protein hydrolysis and oil-holding capacity than the mushrooms, these properties were enhanced in the by-products before fermentation, clearly highlighting the beneficial impact of fermentation on the nutritional value of the mushroom by-products.

### 6.1.2 Biotransformation of phenolic compounds

Phenolic compounds can be found in food in both free and bound forms. The bound phenolic compounds are covalently linked to the cell wall matrices. They cannot be absorbed in the small intestine, resulting in lower bioavailability than that of free phenolic compounds. To increase the bioavailability of phenolic compounds, additional processing techniques are required to release the bound phenolic compounds and make them available for absorption, similar to free phenolic compounds. This would also enhance the health benefits of mushrooms (Yeo et al., 2021). Lactic acid bacteria are known to convert sugars into lactic acid during food fermentation, which can significantly enhance the sensory qualities of food. Besides, this process also degrades macronutrients such as carbohydrates and proteins, which can change the nutritional composition of the food product (Tao et al., 2022).

During fermentation, lactic acid bacteria can release antioxidants and alter the phenolic compounds. This can lead to food biotransformation, which can change the content and profile of the bioactive compounds. In this study, LAB fermentation could change the content and profile of bioactive compounds, modify the parent phenolic compounds by changing their nutritional value and bioaccessibility, and structurally break down the mushroom cell walls (Rocchetti et al., 2022).

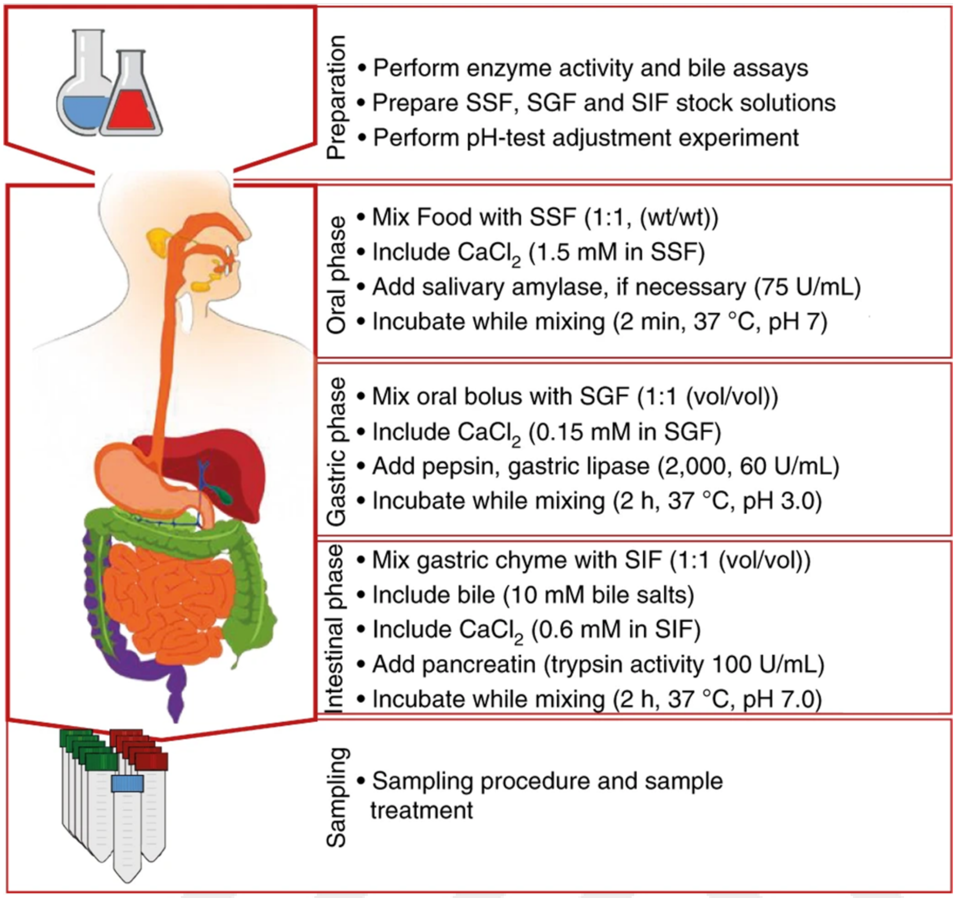
The metabolic activity of lactic acid bacteria can also affect the levels of specific compounds. For instance, we found that some compounds became undetectable or reduced within free phenolic fractions during fermentation. However, fermentation increased the levels of specific components that could break down large molecules in favour of phenolic compounds in mushrooms. This includes the breakdown of cell wall polysaccharides and proteins, wherein bound phenolics are linked (Zhang et al., 2022).

The bound fractions' phenolic content decreased, while free phenolics increased. Thus, LAB fermentation can enhance biological activity by converting bound phenolics into a free state. Additionally, it can biotransform complex phenolic compounds and flavonoids into simple bioactive compounds such as phenolic acids, thereby increasing their bioaccessibility and bioavailability.

### 6.1.3 In vitro digestion of phenolic compounds

The human digestive system consists of the mouth, stomach, small intestine, and large intestine (Figure 6.1). The INFOGEST method has several advantages: simplicity, affordability, ease of evaluation, and reliable reproducibility (Brodkorb et al., 2019). However, it had some limitations. Since it is a static model, it cannot accurately replicate the dynamic nature of digestive processes. For example, it maintains constant pH and digestion time during the gastric phase, ignoring progressive gastric emptying. Additionally, it simplifies the intestinal phase into a single stage instead of distinguishing between duodenal, jejunal, and ileal phases, which have different dilution, mineral content, pH, enzyme activity, and microbial diversity and concentration. Furthermore, the INFOGEST method does not consider the potential physiological interactions between food substrates and the gut microbiota, such as insoluble bound phenolic compounds in the colon.

During gastric digestion, phenolic compounds are broken down through enzymatic activity and low pH conditions. However, differences in the survival and activity of lactic acid bacteria strains can cause variations in phenol content. This, in turn, can affect the metabolism of phenolic compounds. Although the survival rate of LAB was assessed in the fermented samples, the quantification of LAB populations during gastrointestinal transit was not conducted in this study. Additionally, the decrease in the phenolic content during intestinal digestion may be attributed to the transition from acidic to alkaline conditions.



- Perform enzyme activity and bile assays
- Prepare SSF, SGF and SIF stock solutions
- Perform pH-test adjustment experiment

- Mix Food with SSF (1:1, (wt/wt))
- Include $CaCl_2$ (1.5 mM in SSF)
- Add salivary amylase, if necessary (75 U/mL)
- Incubate while mixing (2 min, 37 °C, pH 7)

- Mix oral bolus with SGF (1:1 (vol/vol))
- Include $CaCl_2$ (0.15 mM in SGF)
- Add pepsin, gastric lipase (2,000, 60 U/mL)
- Incubate while mixing (2 h, 37 °C, pH 3.0)

- Mix gastric chyme with SIF (1:1 (vol/vol))
- Include bile (10 mM bile salts)
- Include $CaCl_2$ (0.6 mM in SIF)
- Add pancreatin (trypsin activity 100 U/mL)
- Incubate while mixing (2 h, 37 °C, pH 7.0)

- Sampling procedure and sample treatment

**Figure 6.1 :** Schematic representation of the INFOGEST static model adapted from Brodkorb et al. (2019).

### 6.2 Future Perspectives

Reducing food waste and generating value-added products are critical factors in valorising valuable compounds from by-products. However, such recovery processes can increase the total input required in the value chain. To ensure commercial success, economically viable valorisation processes that minimise input costs and maximise output profits are essential. An integrated extraction strategy that employs a combination of novel approaches to yield multiple value-added products may be a practical approach for a sustainable and integrated valorisation of agro-industrial by-products. This strategy offers several benefits, including enhanced nutritional value, reduced waste production, and a more varied range of outputs rather than focusing on a single component.

Compared to other methods, the valorisation of mushrooms through lactic acid bacteria fermentation offers several advantages: (1) It is green and safe, toxin-free. (2) It increases the nutritional value offered by such foods as it enhances the bioavailability of vitamins and minerals, accompanied by other beneficially synthesised compounds, namely B vitamins and bioactive peptides (Ravyts et al., 2012). (3) It can easily enhance digestibility and bioavailability; for example, LAB fermentation can degrade complex carbohydrates, proteins, and fats into simple, readily digestible molecules. So, it not only improves digestibility but bioavailability as well (Castellone et al., 2021). (4) It develops flavours and textures by developing various types of esters, fatty acids, ketones, aldehydes, alcohols, and other compounds. They produce organic acids, alcohols, and other substances that give food the characteristic taste and aroma of fermentation products. (5) It enhances food safety by producing organic acids, hydrogen peroxide, and bacteriocins. It often results in the inhibition of pathogenic and spoilage microorganisms. This antimicrobial activity helps extend the shelf-life of food products and ensures safety from all these harmful microorganisms (Bains et al., 2021).

However, there are some challenges in developing a lab fermentation method: (1) Time-consuming: LAB fermentation methods are often slower than microwave or ultrasound extraction methods, making them inefficient for mass production. (2) Variable results: The effectiveness of LAB fermentation is influenced by various factors, such as the type of LAB strain, the composition of the substrate, and fermentation conditions. These factors can lead to inconsistent quality of the final product (Suwannarach et al., 2022). (3) Less control: Low or unpredictable enzyme activity can hinder the ability to achieve desired modifications in the end product. (4) Limited extraction of certain compounds: While LAB fermentation can be effective in bioconversion, it may not be as efficient as physical or chemical methods for extracting certain compounds like some polysaccharides or antioxidants (Raman et al., 2022). (5) Production of Biogenic Amines: While LAB is beneficial in many ways, some LAB strains can produce toxic metabolites, such as biogenic amines, depending on the type of food and fermentation conditions (Barbieri et al., 2019). (6) Operational and Scale-up Challenges: The major operational and engineering problems facing LAB fermentation from the laboratory to the industrial level include temperature, pH, and overall substrate quality consistency. These problems could limit the wider applicative scope of LAB fermentation (Hölker et al., 2004)

Several key insights have been identified based on the analysis of the collected data and the review of the benefits and potential of the emerging approaches discussed in this study. These insights suggest the need for additional research to complement the findings of this study. Furthermore, they indicate the importance of exploring alternative and unique topics that align with the observations and knowledge presented in this study.

*In-depth analysis of nutritional composition and other functional properties*: The prebiotic potential of mushroom extracts could be detailed. In the present thesis, when the residue was characterised (*Chapter 3*), it was observed that the level of lactic acid bacteria was higher in the residue than in the fermented mushroom itself. Our discussion focuses on the effect of pH on lab survival. Besides, since the bound phenolic content was higher than the free phenolic content in mushrooms (*Chapter 4*), observing these bound phenolic compounds' prebiotic potential would help a deeper understanding of digestion.

*Bioaccessibility and bioavailability issues:* To the best of our knowledge, the literature does not contain a comprehensive study about the bound phenolics and, most importantly, the bioavailability of phenolics in fermented mushrooms (*Chapter 5*). Even though information about fermented mushrooms' phenolic compounds is available, in vitro digestion of the fermented mushroom is absent. With the potential of the prebiotic influence of mushrooms explained above, further digestion analysis would be performed. Therefore, the behaviour of fermented mushrooms and by-product mushrooms, which contain insoluble fractions and lactic acid bacteria, can be analysed in greater detail using in vitro dynamic models such as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), TNO Gastro-Intestinal Model-2 (TIM-2), and Simulator of the Gastro-Intestinal tract (SIMGISI). These dynamic models provide a more accurate simulation of the physiology and microbial ecology of the intestinal phase, particularly the large intestine phase. Dynamic models usually consist of several reactors that simulate the stomach, small intestine, ascending colon, transverse colon, and descending colon, with varying intestinal microbiota compositions and physiological conditions (Figure 6.2) (Kleiveland et al., 2019).

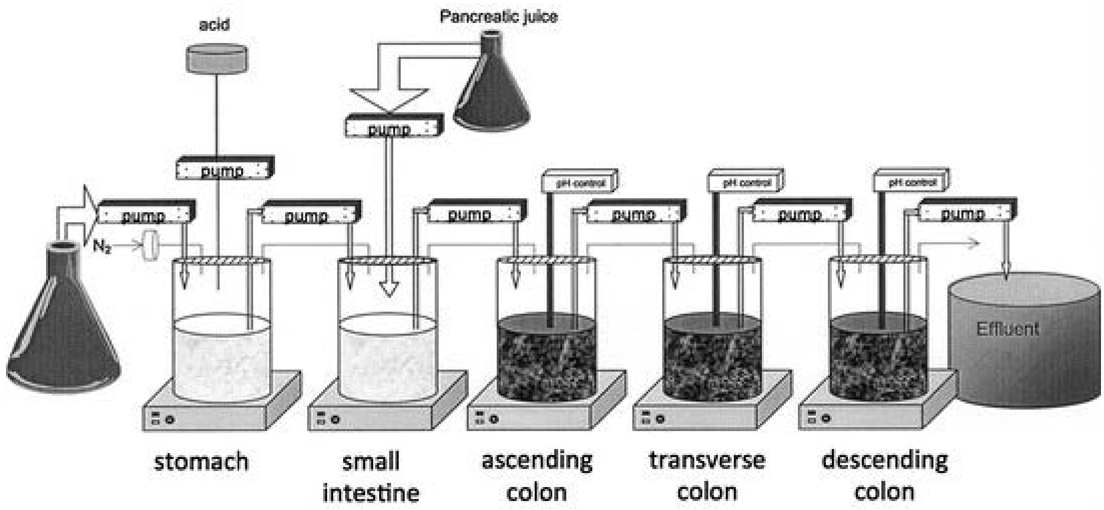


**Figure 6.2 :** Schematic representation of the SHIME$^{\circledR}$ (de Wiele Tomand Van den Abbeele, 2015).

*Improving other properties of mushrooms:* Fermentation changes food's structure, activity, and composition and alters its taste. Although the study approaches fermentation as a biotransformation and extraction helper technique, the literature clarifies the effect of fermentation on taste (*Chapter 2*). During fermentation, bacteria can produce many compounds that give a rich umami taste by breaking down proteins into amino acids, which produce a range of aromatic compounds and esters that contribute to complex flavours not found in unfermented foods. Fermentation also helps reduce the bitterness of some foods because it breaks down bitter compounds and produces organic acids. In this study, we observed the breakdown of proteins into amino acids in fermented mushrooms (*Chapters 3* and *Chapter 5*). Moreover, we discovered that the breakdown process was even more robust in fermented mushroom by-products (*Chapter 3*). This indicates the possibility of creating functional foods with well-accepted and improved taste compositions.

# REFERENCES

**Abdelshafy, A. M., Belwal, T., Liang, Z., Wang, L., Li, D., Luo, Z., & Li, L.** (2022). A comprehensive review on phenolic compounds from edible mushrooms: Occurrence, biological activity, application and future prospective. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 22, pp. 6204–6224). Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2021.1898335

**Abreu, H., Zavadinack, M., Smiderle, F. R., Cipriani, T. R., Cordeiro, L. M. C., & Iacomini, M.** (2021a). Polysaccharides from *Pleurotus eryngii*: Selective extraction methodologies and their modulatory effects on THP-1 macrophages. *Carbohydrate Polymers, 252*. https://doi.org/10.1016/j.carbpol.2020.117177

**Abreu, H., Zavadinack, M., Smiderle, F. R., Cipriani, T. R., Cordeiro, L. M. C., & Iacomini, M.** (2021b). Polysaccharides from *Pleurotus eryngii*: Selective extraction methodologies and their modulatory effects on THP-1 macrophages. *Carbohydrate Polymers, 252*. https://doi.org/10.1016/j.carbpol.2020.117177

**Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O.** (2014). Bound phenolics in foods, a review. In *Food Chemistry* (Vol. 152, pp. 46–55). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2013.11.093

**Adebo, O. A., & Medina-Meza, I. G.** (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. In *Molecules* (Vol. 25, Issue 4). MDPI AG. https://doi.org/10.3390/molecules25040927

**Adler-Nissen.** (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food chemistry*, 27(6), 1256-1262.

**Aguiló-Aguayo, I., Walton, J., Viñas, I., & Tiwari, B. K**. (2017). Ultrasound assisted extraction of polysaccharides from mushroom by-products. *LWT*, *77*, 92–99. https://doi.org/10.1016/j.lwt.2016.11.043

**Ahire, J. J., Jakkamsetty, C., Kashikar, M. S., Lakshmi, S. G., & Madempudi, R. S**. (2021). In Vitro Evaluation of Probiotic Properties of *Lactobacillus plantarum UBLP40* Isolated from Traditional Indigenous Fermented Food. *Probiotics and Antimicrobial Proteins, 13*(5), 1413–1424. https://doi.org/10.1007/s12602-021-09775-7

**Alves, G., Lobo, L. A., Domingues, R. M. C. P., Monteiro, M., & Perrone, D**. (2021). Bioaccessibility and Gut Metabolism of Free and Melanoidin-Bound Phenolic Compounds From Coffee and Bread. *Frontiers in Nutrition, 8*. https://doi.org/10.3389/fnut.2021.708928

**Andrade, J. K. S., Barros, R. G. C., Pereira, U. C., Gualberto, N. C., de Oliveira, C. S., Shanmugam, S., & Narain, N.** (2022). α-Amylase inhibition, cytotoxicity and influence of the in vitro gastrointestinal digestion on the bioaccessibility of phenolic compounds in the peel and seed of *Theobroma grandiflorum*. *Food Chemistry*, *373*. https://doi.org/10.1016/j.foodchem.2021.131494

**Antognoni, F., Mandrioli, R., Potente, G., Taneyo Saa, D. L., & Gianotti, A.** (2019). Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. *Food Chemistry*, *292*, 211–216. https://doi.org/10.1016/j.foodchem.2019.04.061

**Antunes, F., Marçal, S., Taofiq, O., Morais, A. M. M. B., Freitas, A. C., Ferreira, I. C. F. R., & Pintado, M.** (2020). Valorization of mushroom by-products as a source of value-added compounds and potential applications. *Molecules*, *25*(11). https://doi.org/10.3390/molecules25112672

**Assemie, A., & Abaya, G**. (2022). The Effect of Edible Mushroom on Health and Their Biochemistry. In *International Journal of Microbiology* (Vol. 2022). Hindawi Limited. https://doi.org/10.1155/2022/8744788

**Attri, S., Singh, N., Singh, T. R., & Goel, G**. (2017). Effect of in vitro gastric and pancreatic digestion on antioxidant potential of fruit juices. *Food Bioscience*, *17*, 1–6. https://doi.org/10.1016/j.fbio.2016.10.003

**Ayala-Ninõ, A., Rodríguez-Serrano, G. M., Jiménez-Alvarado, R., Bautista-Avila, M., Sánchez-Franco, J. A., González-Olivares, L. G., & Cepeda-Saez, A.** (2019). Bioactivity of Peptides Released during Lactic Fermentation of Amaranth Proteins with Potential Cardiovascular Protective Effect: An in Vitro Study. *Journal of Medicinal Food*, *22*(10), 976–981. https://doi.org/10.1089/jmf.2019.0039

**Ayser, M., Tonny, W., Hernandez, I. S., Kuriakose, R., Smith, J. D., Wallaert, S. J., Karim, A., Robertson, M. L., & Balan, V.** (2023). Fractionating Chitin-Glucan Complex and Coproducts from *Pleurotus Ostreatus* Mushrooms. *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-023-02364-5

**Baeva, E., Bleha, R., Lavrova, E., Sushytskyi, L., Čopíková, J., Jablonsky, I., Klouček, P., & Synytsya, A.** (2019). Polysaccharides from Basidiocarps of Cultivating Mushroom *Pleurotus ostreatus*: Isolation and Structural Characterization. *Molecules (Basel, Switzerland)*, *24*(15). https://doi.org/10.3390/molecules24152740

**Bains, A., Chawla, P., Kaur, S., Najda, A., Fogarasi, M., & Fogarasi, S.** (2021). Bioactives from mushroom: Health attributes and food industry applications. In *Materials* (Vol. 14, Issue 24). MDPI. https://doi.org/10.3390/ma14247640

**Barbieri, F., Montanari, C., Gardini, F., & Tabanelli, G.** (2019). Biogenic amine production by lactic acid bacteria: A review. In *Foods* (Vol. 8, Issue 1). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods8010017

**Bartkiene, E., Zarovaite, P., Starkute, V., Mockus, E., Zokaityte, E., Zokaityte, G., Rocha, J. M., Ruibys, R., & Klupsaite, D.** (2023). Changes in Lacto-Fermented Agaricus bisporus (White and Brown Varieties) Mushroom Characteristics, including Biogenic Amine and Volatile Compound Formation. *Foods*, *12*(13). https://doi.org/10.3390/foods12132441

**Bartkiene, E., Zokaityte, E., Starkute, V., Mockus, E., Klupsaite, D., Lukseviciute, J., Bogomolova, A., Streimikyte, A., & Ozogul, F.** (2022). Biopreservation of Wild Edible Mushrooms (*Boletus edulis, Cantharellus, and Rozites caperata*) with Lactic Acid Bacteria Possessing Antimicrobial Properties. *Foods, 11*(12). https://doi.org/10.3390/foods11121800

**Bedlovičová, Z., Strapáč, I., Baláž, M., & Salayová, A.** (2020). A brief overview on antioxidant activity determination of silver nanoparticles. In *Molecules* (Vol. 25, Issue 14). MDPI AG. https://doi.org/10.3390/molecules25143191

**Behera, S. S., Ray, R. C., & Zdolec, N.** (2018). *Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. In *BioMed Research International* (Vol. 2018). Hindawi Limited. https://doi.org/10.1155/2018/9361614

**Borremans, A., Bußler, S., Sagu, S. T., Rawel, H., Schlüter, O. K., & Leen, V. C.** (2020). Effect of blanching plus fermentation on selected functional properties of mealworm (*Tenebrio molitor*) powders. *Foods*, *9*(7). https://doi.org/10.3390/foods9070917

**Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I.** (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

**Büntgen, U., Kauserud, H., & Egli,** S. (2012). Linking climate variability to mushroom productivity and phenology. *Frontiers in Ecology and the Environment*, *10*(1), 14–19. https://doi.org/10.1890/110064

**Carvalho, D. O., & Guido, L. F.** (2022). A review on the fate of phenolic compounds during malting and brewing: Technological strategies and beer styles. In *Food Chemistry* (Vol. 372). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2021.131093

**Castellanos-Reyes, K., Villalobos-Carvajal, R., & Beldarrain-Iznaga, T.** (2021). Fresh mushroom preservation techniques. In *Foods* (Vol. 10, Issue 9). MDPI. https://doi.org/10.3390/foods10092126

**Castellone, V., Bancalari, E., Rubert, J., Gatti, M., Neviani, E., & Bottari, B.** (2021). Eating fermented: Health benefits of lab-fermented foods. In *Foods* (Vol. 10, Issue 11). MDPI. https://doi.org/10.3390/foods10112639

**Castro-Alves, V. C., & do Nascimento, J. R. O.** (2016). Speeding up the Extraction of Mushroom Polysaccharides. *Food Analytical Methods*, *9*(9), 2429–2433. https://doi.org/10.1007/s12161-016-0406-9

**Cateni, F., Gargano, M. L., Procida, G., Venturella, G., Cirlincione, F., & Ferraro, V.** (2022). Mycochemicals in wild and cultivated mushrooms: nutrition and health. In *Phytochemistry Reviews* (Vol. 21, Issue 2, pp. 339–383). Springer Science and Business Media B.V. https://doi.org/10.1007/s11101-021-09748-2

**Çayan, F., Deveci, E., Tel-Çayan, G., & Duru, M. E**. (2020). Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC–DAD. *Journal of Food Measurement and Characterization*, *14*(3), 1690–1698. https://doi.org/10.1007/s11694-020-00417-0

**Chang, S. T., & Buswell, J. A.** (1996). Mushroom nutriceuticals. In *World Journal of Microbiology 8 Biotechnology* (Vol. 12).

**Chen, P., Liu, H. P., Ji, H. H., Sun, N. X., & Feng, Y. Y.** (2019). A cold-water soluble polysaccharide isolated from Grifola frondosa induces the apoptosis of HepG2 cells through mitochondrial passway. *International Journal of Biological Macromolecules*, *125*, 1232–1241. https://doi.org/10.1016/j.ijbiomac.2018.09.098

**Chen, Z., Fang, X., Wu, W., Chen, H., Han, Y., Yang, H., & Gao, H.** (2021). Effects of fermentation with *Lactiplantibacillus plantarum GDM1.191* on the umami compounds in shiitake mushrooms (*Lentinus edodes*). *Food Chemistry*, *364*. https://doi.org/10.1016/j.foodchem.2021.130398

**Chen, Z., Gao, H., Wu, W., Chen, H., Fang, X., Han, Y., & Mu, H.** (2021). Effects of fermentation with different microbial species on the umami taste of Shiitake mushroom (*Lentinus edodes*). *LWT*, *141*. https://doi.org/10.1016/j.lwt.2021.110889

**Cheong, C. B., Peh, G. R., Wei, Y., Rehka, T., Ang, E. L., Zhao, H., Zhang, C., & Lim, Y. H.** (2023). A Spirobicyclo Terpene from the Investigation of Sesquiterpene Synthases from *Lactarius deliciosus*. *ACS Chemical Biology*, *18*(1), 134–140. https://doi.org/10.1021/acschembio.2c00760

**Chopra, H., Mishra, A. K., Baig, A. A., Mohanta, T. K., Mohanta, Y. K., & Baek, K. H.** (2021). Narrative review: Bioactive potential of various mushrooms as the treasure of versatile therapeutic natural product. In *Journal of Fungi* (Vol. 7, Issue 9). MDPI. https://doi.org/10.3390/jof7090728

**Chu, M., Khan, R. D., Zhou, Y., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R.** (2023). LC-ESI-QTOF-MS/MS Characterization of Phenolic Compounds in Common Commercial Mushrooms and Their Potential Antioxidant Activities. *Processes*, *11*(6). https://doi.org/10.3390/pr11061711

**Chun, S., Gopal, J., & Muthu, M.** (2021). Antioxidant activity of mushroom extracts/polysaccharides —their antiviral properties and plausible antiCOVID-19 properties. In *Antioxidants* (Vol. 10, Issue 12). MDPI. https://doi.org/10.3390/antiox10121899

**Cui, F. J., Qian, L. S., Sun, W. J., Zhang, J. S., Yang, Y., Li, N., Zhuang, H. N., & Wu, D.** (2018). Ultrasound-assisted extraction of polysaccharides from Volvariella volvacea: Process optimization and structural characterization. *Molecules*, *23*(7). https://doi.org/10.3390/molecules23071706

**Cuvas-Limon, R. B., Ferreira-Santos, P., Cruz, M., Teixeira, J. A., Belmares, R., & Nobre, C.** (2022). Effect of Gastrointestinal Digestion on the Bioaccessibility of Phenolic Compounds and Antioxidant Activity of Fermented Aloe vera Juices. *Antioxidants*, *11*(12). https://doi.org/10.3390/antiox11122479

**Davis, R. M., Sommer, R., & Menge, J. A.** (2012). *Field Guide to Mushrooms of Western North America*. University of California Press.

**De Beer, D., Tobin, J., Walczak, B., Van Der Rijst, M., & Joubert, E.** (2019). Phenolic composition of rooibos changes during simulated fermentation: Effect of endogenous enzymes and fermentation temperature on reaction kinetics. *Food Research International*, *121*, 185–196. https://doi.org/10.1016/j.foodres.2019.03.041

**De Montijo-Prieto, S., Razola-Díaz, M. del C., Barbieri, F., Tabanelli, G., Gardini, F., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M.** (2023a). Impact of Lactic Acid Bacteria Fermentation on Phenolic Compounds and Antioxidant Activity of Avocado Leaf Extracts. *Antioxidants*, *12*(2). https://doi.org/10.3390/antiox12020298

**De Montijo-Prieto, S., Razola-Díaz, M. del C., Barbieri, F., Tabanelli, G., Gardini, F., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M.** (2023b). Impact of Lactic Acid Bacteria Fermentation on Phenolic Compounds and Antioxidant Activity of Avocado Leaf Extracts. *Antioxidants*, *12*(2). https://doi.org/10.3390/antiox12020298

**de Wiele Tom and Van den Abbeele, P. and O. W. and P. S. and M. M.** (2015). The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). In P. and L.-E. I. and K. C. and L. T. and M. A. and R. T. and S. D. and W. H. Verhoeckx Kitty and Cotter (Ed.), *The Impact of Food Bioactives on Health: in vitro and ex vivo models* (pp. 305–317). Springer International Publishing. https://doi.org/10.1007/978-3-319-16104-4_27

**Dedousi, M., Melanouri, E. M., Karayannis, D., Kaminarides, E. I., & Diamantopoulou, P.** (2024). Utilization of spent substrates and waste products of mushroom cultivation to produce new crops of *Pleurotus ostreatus*, *Pleurotus eryngii* and *Agaricus bisporus*. *Carbon Resources Conversion*, *7*(2). https://doi.org/10.1016/j.crcon.2023.08.001

**Degrain, A., Manhivi, V., Remize, F., Garcia, C., & Sivakumar, D.** (2020). Effect of lactic acid fermentation on color, phenolic compounds and antioxidant activity in african nightshade. *Microorganisms*, *8*(9), 1–12. https://doi.org/10.3390/microorganisms8091324

**Dogan, A., Uyar, A., Hasar, S., & Keles, O. F.** (2022). The protective effects of the *Lactarius deliciosus* and *Agrocybe cylindracea* mushrooms on histopathology of carbon tetrachloride induced oxidative stress in rats. *Biotechnic and Histochemistry*, *97*(2), 143–151. https://doi.org/10.1080/10520295.2021.1918349

**Ekowati, N., Kasiamdari, R. S., Pusposendjojo, N., & Soegihardjo, C. J.** (2011). Daya Antımıkroba Metabolit Bioaktıf Jamur Shiitake (*Lentinula Edodes (Berk.) Pegler*) Yang Dıkultur Pada Tıga Jenıs Medıum Fermentası Antimicrobial Activity Of Bioactive Metabolites Of (*Lentinula Edodes (Berk.) Pegler*) Cultured Onthree Types Of Fermentation Media. In *Majalah Obat Tradisional* (Vol. 16, Issue 3).

**Emkani, M., Oliete, B., & Saurel, R.** (2021). Pea protein extraction assisted by lactic fermentation: Impact on protein profile and thermal properties. *Foods*, *10*(3). https://doi.org/10.3390/foods10030549

**Erjavec, J., Kos, J., Ravnikar, M., Dreo, T., & Sabotič, J.** (2012). Proteins of higher fungi - from forest to application. In *Trends in Biotechnology* (Vol. 30, Issue 5, pp. 259–273). https://doi.org/10.1016/j.tibtech.2012.01.004

**Faithong, N., Benjakul, S., Phatcharat, S., & Binsan, W.** (2010). Chemical composition and antioxidative activity of Thai traditional fermented shrimp and krill products. *Food Chemistry*, *119*(1), 133–140. https://doi.org/10.1016/j.foodchem.2009.06.056

**Feher, I., Floare-Avram, C. V., Covaciu, F. D., Marincas, O., Puscas, R., Magdas, D. A., & Sârbu, C.** (2021). Evaluation of mushrooms based on ft-ir fingerprint and chemometrics. *Applied Sciences (Switzerland)*, *11*(20). https://doi.org/10.3390/app11209577

**Feussi Tala, M., Qin, J., Ndongo, J. T., & Laatsch, H.** (2017). New Azulene-Type Sesquiterpenoids from the Fruiting Bodies of Lactarius deliciosus. *Natural Products and Bioprospecting*, *7*(3), 269–273. https://doi.org/10.1007/s13659-017-0130-1

**Food and Agriculture Organization of the United Nations, Statistics Division (FAOSTAT).** Crops Production. Available online: http://faostat.fao.org.

**Finimundy, T. C., Gambato, G., Fontana, R., Camassola, M., Salvador, M., Moura, S., Hess, J., Henriques, J. A. P., Dillon, A. J. P., & Roesch-Ely, M.** (2013). Aqueous extracts of *Lentinula edodes and Pleurotu*s sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity. *Nutrition Research*, *33*(1), 76–84. https://doi.org/10.1016/j.nutres.2012.11.005

**Fry, S. C. (1986).** Cross-Linking Of Matrix Polymers in The Growing Cell Walls Of Angiosperms. In *Ann. Rev. Plant Physiol* (Vol. 37).

**Fukushima-Sakuno, E.** (2020). Bioactive small secondary metabolites from the mushrooms Lentinula edodes and Flammulina velutipes. In *Journal of Antibiotics* (Vol. 73, Issue 10, pp. 687–696). Springer Nature. https://doi.org/10.1038/s41429-020-0354-x

**Gao, Y., Padhiar, A. A., Wang, J., Zhang, W., Zhong, M., Liu, B., Kang, Z., Wang, X., Li, X., & Huang, M.** (2018). Recombinant latcripin 11 of *Lentinula edodes* C91-3 suppresses the proliferation of various cancer cells. *Gene*, *642*, 212–219. https://doi.org/10.1016/j.gene.2017.10.080

**Gaur, G., & Gänzle, M. G.** (2023). Conversion of (poly)phenolic compounds in food fermentations by lactic acid bacteria: Novel insights into metabolic pathways and functional metabolites. In *Current Research in Food Science* (Vol. 6). Elsevier B.V. https://doi.org/10.1016/j.crfs.2023.100448

**Geller, A., & Yan, J.** (2020). Could the Induction of Trained Immunity by β-Glucan Serve as a Defense Against COVID-19? *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.01782

**Gil-Ramírez, A., Aldars-García, L., Palanisamy, M., Jiverdeanu, R. M., Ruiz-Rodríguez, A., Marín, F. R., Reglero, G., & Soler-Rivas, C.** (2013). Sterol enriched fractions obtained from *Agaricus bisporus* fruiting bodies and by-products by compressed fluid technologies (PLE and SFE). *Innovative Food Science and Emerging Technologies*, *18*, 101–107. https://doi.org/10.1016/j.ifset.2013.01.007

**Gogoi, P., Chutia, P., Singh, P., & Mahanta, C. L.** (2019). Effect of optimized ultrasound-assisted aqueous and ethanolic extraction of Pleurotus citrinopileatus mushroom on total phenol, flavonoids and antioxidant properties. *Journal of Food Process Engineering*, *42*(6). https://doi.org/10.1111/jfpe.13172

**Gonzales, G. B., Raes, K., Coelus, S., Struijs, K., Smagghe, G., & Van Camp, J.** (2014). Ultra(high)-pressure liquid chromatography-electrospray ionization-time-of-flight-ion mobility-high definition mass spectrometry for the rapid identification and structural characterization of flavonoid glycosides from cauliflower waste. *Journal of Chromatography A*, *1323*, 39–48. https://doi.org/10.1016/j.chroma.2013.10.077

**Gonzales, G. B., Smagghe, G., Raes, K., & Van Camp, J.** (2014). Combined alkaline hydrolysis and ultrasound-assisted extraction for the release of nonextractable phenolics from cauliflower (Brassica oleracea var. botrytis) waste. *Journal of Agricultural and Food Chemistry*, *62*(15), 3371–3376. https://doi.org/10.1021/jf500835q

**Grimm, D., & Wösten, H. A. B.** (2018). Mushroom cultivation in the circular economy. In *Applied Microbiology and Biotechnology* (Vol. 102, Issue 18, pp. 7795–7803). Springer Verlag. https://doi.org/10.1007/s00253-018-9226-8

**Gu, L., Peng, N., Chen, S., McClements, D. J., Tang, Z., Li, J., Chang, C., Su, Y., & Yang, Y.** (2024). Bioactive peptides derived from quinoa protein: fabrication, antioxidant activities, and in vitro digestion profiles. *Journal of Food Measurement and Characterization*, *18*(2), 894–903. https://doi.org/10.1007/s11694-023-02212-z

**Gulsunoglu, Z., Karbancioglu-Guler, F., Raes, K., & Kilic-Akyilmaz, M.** (2019). Soluble and insoluble-bound phenolics and antioxidant activity of various industrial plant wastes. *International Journal of Food Properties*, *22*(1), 1501–1510. https://doi.org/10.1080/10942912.2019.1656233

**Guo, J., Zhang, M., & Fang, Z.** (2022). Valorization of mushroom by-products: a review. In *Journal of the Science of Food and Agriculture*. John Wiley and Sons Ltd. https://doi.org/10.1002/jsfa.11946

**Hamauzu, Y., & Suwannachot, J.** (2019). Non-extractable polyphenols and in vitro bile acid-binding capacity of dried persimmon (Diospyros kaki)fruit. *Food Chemistry*, *293*, 127–133. https://doi.org/10.1016/j.foodchem.2019.04.092

**Hashemi Gahruie, H., & Niakousari, M.** (2017). Antioxidant, antimicrobial, cell viability and enzymatic inhibitory of antioxidant polymers as biological macromolecules. In *International Journal of Biological Macromolecules* (Vol. 104, pp. 606–617). Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2017.06.021

**He, C., Sampers, I., & Raes, K.** (2021). Dietary fiber concentrates recovered from agro-industrial by-products: Functional properties and application as physical carriers for probiotics. *Food Hydrocolloids*, *111*. https://doi.org/10.1016/j.foodhyd.2020.106175

**Heleno, S. A., Prieto, M. A., Barros, L., Rodrigues, A., Barreiro, M. F., & Ferreira, I. C. F. R.** (2016). Optimization of microwave-assisted extraction of ergosterol from *Agaricus bisporus L.* by-products using response surface methodology. *Food and Bioproducts Processing*, *100*, 25–35. https://doi.org/10.1016/j.fbp.2016.06.006

**Hole, A. S., Rud, I., Grimmer, S., Sigl, S., Narvhus, J., & Sahlstrøm, S.** (2012). Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic *Lactobacillus acidophilus, Lactobacillus johnsonii*, and *Lactobacillus reuteri*. *Journal of Agricultural and Food Chemistry*, *60*(25), 6369–6375. https://doi.org/10.1021/jf300410h

**Hölker, U., Höfer, M., & Lenz, J.** (2004). Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. In *Applied Microbiology and Biotechnology* (Vol. 64, Issue 2, pp. 175–186). https://doi.org/10.1007/s00253-003-1504-3

**Hou, Y., Liu, L., Ding, X., Zhao, D., & Hou, W.** (2016). Structure elucidation, proliferation effect on macrophage and its mechanism of a new heteropolysaccharide from *Lactarius deliciosus Gray*. *Carbohydrate Polymers*, *152*, 648–657. https://doi.org/10.1016/j.carbpol.2016.07.064

**Huang, Q., & Chen, L.** (2016). Effect of Different Drying Method on Volatile Flavor Compounds of Lactarius deliciosus. *Journal of Food Processing & Technology*, *7*(8). https://doi.org/10.4172/2157-7110.1000615

**Jabłońska-Ryś, E., Skrzypczak, K., Sławińska, A., Radzki, W., & Gustaw, W.** (2019). Lactic Acid Fermentation of Edible Mushrooms: Tradition, Technology, Current State of Research: A Review. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 18, Issue 3, pp. 655–669). Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12425

**Jabłońska-Ryś, E., Sławińska, A., Skrzypczak, K., & Goral, K.** (2022). Dynamics of Changes in pH and the Contents of Free Sugars, Organic Acids and LAB in Button Mushrooms during Controlled Lactic Fermentation. *Foods*, *11*(11). https://doi.org/10.3390/foods11111553

**Jabłońska-Ryś, E., Sławińska, A., & Szwajgier, D.** (2016). Effect of lactic acid fermentation on antioxidant properties and phenolic acid contents of oyster (*Pleurotus ostreatus*) and chanterelle (*Cantharellus cibarius*) mushrooms. *Food Science and Biotechnology*, *25*(2), 439–444. https://doi.org/10.1007/s10068-016-0060-4

**Jafarzadeh, S., Mohammadi Nafchi, A., Salehabadi, A., Oladzad-abbasabadi, N., & Jafari, S. M.** (2021). Application of bio-nanocomposite films and edible coatings for extending the shelf life of fresh fruits and vegetables. In *Advances in Colloid and Interface Science* (Vol. 291). Elsevier B.V. https://doi.org/10.1016/j.cis.2021.102405

**Ji, H. yu, Chen, P., Yu, J., Feng, Y. ying, & Liu, A. jun**. (2019). Effects of Heat Treatment on the Structural Characteristics and Antitumor Activity of Polysaccharides from *Grifola frondosa*. *Applied Biochemistry and Biotechnology*, *188*(2), 481–490. https://doi.org/10.1007/s12010-018-02936-5

**Jia, J., Ji, B., Tian, L., Li, M., Lu, M., Ding, L., Liu, X., & Duan, X.** (2021). Mechanism study on enhanced foaming properties of individual albumen proteins by *Lactobacillus* fermentation. *Food Hydrocolloids*, *111*. https://doi.org/10.1016/j.foodhyd.2020.106218

**Kalogeropoulos, N., Yanni, A. E., Koutrotsios, G., & Aloupi, M.** (2013a). Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food and Chemical Toxicology*, *55*, 378–385. https://doi.org/10.1016/j.fct.2013.01.010

**Kalogeropoulos, N., Yanni, A. E., Koutrotsios, G., & Aloupi, M.** (2013b). Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food and Chemical Toxicology*, *55*, 378–385. https://doi.org/10.1016/j.fct.2013.01.010

**Kashyap, P., Riar, C. S., & Jindal, N.** (2022). Effect of extraction methods and simulated in vitro gastrointestinal digestion on phenolic compound profile, bio-accessibility, and antioxidant activity of Meghalayan cherry (*Prunus nepalensis*) pomace extracts. *LWT*, *153*. https://doi.org/10.1016/j.lwt.2021.112570

**Khan, S. A., Zhang, M., Liu, L., Dong, L., Ma, Y., Wei, Z., Chi, J., & Zhang, R.** (2020). Co-culture submerged fermentation by lactobacillus and yeast more effectively improved the profiles and bioaccessibility of phenolics in extruded brown rice than single-culture fermentation. *Food Chemistry*, *326*. https://doi.org/10.1016/j.foodchem.2020.126985

**Kim, J. S., Lee, Y. H., Kim, Y. I., Ahmadi, F., Oh, Y. K., Park, J. M., & Kwak, W. S.** (2016). Effect of microbial inoculant or molasses on fermentative quality and aerobic stability of sawdust-based spent mushroom substrate. *Bioresource Technology*, *216*, 188–195. https://doi.org/10.1016/j.biortech.2016.05.056

**Kim, M. Y., Seguin, P., Ahn, J. K., Kim, J. J., Chun, S. C., Kim, E. H., Seo, S. H., Kang, E. Y., Kim, S. L., Park, Y. J., Ro, H. M., & Chung, I. M**. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *Journal of Agricultural and Food Chemistry*, *56*(16), 7265–7270. https://doi.org/10.1021/jf8008553

**Kim, S. P., Park, S. O., Lee, S. J., Nam, S. H., & Friedman, M.** (2013). A polysaccharide isolated from the liquid culture of *Lentinus edodes* (Shiitake) mushroom mycelia containing black rice bran protects mice against a Salmonella lipopolysaccharide-induced endotoxemia. *Journal of Agricultural and Food Chemistry*, *61*(46), 10987–10994. https://doi.org/10.1021/jf403173k

**Kind, T., Tsugawa, H., Cajka, T., Ma, Y., Lai, Z., Mehta, S. S., Wohlgemuth, G., Barupal, D. K., Showalter, M. R., Arita, M., & Fiehn, O.** (2018). Identification of small molecules using accurate mass MS/MS search. In *Mass Spectrometry Reviews* (Vol. 37, Issue 4, pp. 513–532). John Wiley and Sons Inc. https://doi.org/10.1002/mas.21535

**Kosanić, M., Ranković, B., Rančić, A., & Stanojković, T.** (2016). Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms Lactarius deliciosus and Macrolepiota procera. *Journal of Food and Drug Analysis*, *24*(3), 477–484. https://doi.org/10.1016/j.jfda.2016.01.008

**Kour, H., Kour, D., Kour, S., Singh, S., Jawad Hashmi, S. A., Yadav, A. N., Kumar, K., Sharma, Y. P., & Ahluwalia, A. S.** (2022). Bioactive compounds from mushrooms: Emerging bioresources of food and nutraceuticals. In *Food Bioscience* (Vol. 50). Elsevier Ltd. https://doi.org/10.1016/j.fbio.2022.102124

**Krahe, J., Krahe, M. A., & Naumovski, N.** (2021). The implications of post-harvest storage time and temperature on the phytochemical composition and quality of japanese-styled green tea grown in australia: A food loss and waste recovery opportunity. *Beverages*, *7*(2). https://doi.org/10.3390/beverages7020025

**Krüzselyi, D., Móricz, Á. M., & Vetter, J.** (2020). Comparison of different morphological mushroom parts based on the antioxidant activity. *LWT*, *127*. https://doi.org/10.1016/j.lwt.2020.109436

**Kumar, K., Mehra, R., Guiné, R. P. F., Lima, M. J., Kumar, N., Kaushik, R., Ahmed, N., Yadav, A. N., & Kumar, H.** (2021). Edible mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects. In *Foods* (Vol. 10, Issue 12). MDPI. https://doi.org/10.3390/foods10122996

**Kumar, P., Eid, E. M., Al-Huqail, A. A., Širić, I., Adelodun, B., Abou Fayssal, S., Valadez-Blanco, R., Goala, M., Ajibade, F. O., Choi, K. S., & Kumar, V.** (2022). Kinetic Studies on Delignification and Heavy Metals Uptake by Shiitake (*Lentinula edodes*) Mushroom Cultivated on Agro-Industrial Wastes. *Horticulturae*, *8*(4). https://doi.org/10.3390/horticulturae8040316

**Kumaran, A., & Joel Karunakaran, R.** (2007). In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT*, *40*(2), 344–352. https://doi.org/10.1016/j.lwt.2005.09.011

**Kwak, A. M., Lee, I. K., Lee, S. Y., Yun, B. S., & Kang, H. W**. (2016). Oxalic acid from lentinula edodes culture filtrate: Antimicrobial activity on phytopathogenic bacteria and qualitative and quantitative analyses. *Mycobiology*, *44*(4), 338–342. https://doi.org/10.5941/MYCO.2016.44.4.338

**Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Wu, M., Sackey, A. S., Xiao, L., & Tahir, H. E.** (2018). Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. *Food Chemistry*, *250*, 148–154. https://doi.org/10.1016/j.foodchem.2018.01.009

**Lee, M. A., Choi, Y. J., Lee, H., Hwang, S., Lee, H. J., Park, S. J., Chung, Y. B., Yun, Y. R., Park, S. H., Min, S., Kwon, L. S., & Seo, H. Y.** (2021). Influence of salinity on the microbial community composition and metabolite profile in Kimchi. *Fermentation*, *7*(4). https://doi.org/10.3390/fermentation7040308

**Leonard, W., Zhang, P., Ying, D., Adhikari, B., & Fang, Z.** (2021). Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. In *Biotechnology Advances* (Vol. 49). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2021.107763

**Leong, Y. K., Ma, T. W., Chang, J. S., & Yang, F. C.** (2022). Recent advances and future directions on the valorization of spent mushroom substrate (SMS): A review. In *Bioresource Technology* (Vol. 344). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2021.126157

**Leong, Y. K., Yang, F. C., & Chang, J. S.** (2021). Extraction of polysaccharides from edible mushrooms: Emerging technologies and recent advances. In *Carbohydrate Polymers* (Vol. 251). Elsevier Ltd. https://doi.org/10.1016/j.carbpol.2020.117006

**Li, H., Gao, J., Zhao, F., Liu, X., & Ma, B.** (2023). Bioactive Peptides from Edible Mushrooms—The Preparation, Mechanisms, Structure—Activity Relationships and Prospects. In *Foods* (Vol. 12, Issue 15). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods12152935

**Li, M., Chen, X., Deng, J., Ouyang, D., Wang, D., Liang, Y., Chen, Y., & Sun, Y.** (2020). Effect of thermal processing on free and bound phenolic compounds and antioxidant activities of hawthorn. *Food Chemistry*, *332*. https://doi.org/10.1016/j.foodchem.2020.127429

**Li, X., Song, L., Xu, X., Wen, C., Ma, Y., Yu, C., Du, M., & Zhu, B.** (2020). One-step coextraction method for flavouring soybean oil with the dried stipe of *Lentinus edodes* (Berk.) sing by supercritical CO2 fluid extraction. *LWT*, *120*. https://doi.org/10.1016/j.lwt.2019.108853

**Li, Z., Teng, J., Lyu, Y., Hu, X., Zhao, Y., & Wang, M**. (2019b). Enhanced antioxidant activity for apple juice fermented *with Lactobacillus plantarum ATCC14917*. *Molecules*, *24*(1). https://doi.org/10.3390/molecules24010051

**Lin, P. H., Huang, S. Y., Mau, J. L., Liou, B. K., & Fang, T. J.** (2010). A novel alcoholic beverage developed from shiitake stipe extract and cane sugar with various *Saccharomyces* strains. *LWT*, *43*(6), 971–976. https://doi.org/10.1016/j.lwt.2010.02.006

**Liu, X., Hou, R., Wang, D., Mai, M., Wu, X., Zheng, M., & Fu, J**. (2019). Comprehensive utilization of edible mushroom *Auricularia auricula* waste residue—Extraction, physicochemical properties of melanin and its antioxidant activity. *Food Science and Nutrition*, *7*(11), 3774–3783. https://doi.org/10.1002/fsn3.1239

**Liu, X., Wang, A., Zhu, L., Guo, W., Guo, X., Zhu, B., & Yang, M.** (2023). Effect of additive cellulase on fermentation quality of whole-plant corn silage ensiling by a Bacillus inoculant and dynamic microbial community analysis. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1330538

**Liu, Y. W., Liong, M. T., & Tsai, Y. C.** (2018). New perspectives of *Lactobacillus plantarum* as a probiotic: The gut-heart-brain axis. In *Journal of Microbiology* (Vol. 56, Issue 9, pp. 601–613). Microbiological Society of Korea. https://doi.org/10.1007/s12275-018-8079-2

**Liu, Y., Xie, X. X., Ibrahim, S. A., Khaskheli, S. G., Yang, H., Wang, Y. F., & Huang, W.** (2016). Characterization of *Lactobacillus pentosus* as a starter culture for the fermentation of edible oyster mushrooms (*Pleurotus spp*.). *LWT*, *68*, 21–26. https://doi.org/10.1016/j.lwt.2015.12.008

**lkay Koca, A. K. İ., & Gençcelep, H.** (2011). Antioxidant Properties of Wild Edible Mushrooms. *Journal of Food Processing & Technology*, *02*(06). https://doi.org/10.4172/2157-7110.1000130

Lowry O H, Rosebrough N J, Farr A L, & Randall R J. (1951). *A1977DM02300001*.

**Lu, X., Hou, H., Fang, D., Hu, Q., Chen, J., & Zhao, L.** (2022). Identification and characterization of volatile compounds in *Lentinula edodes* during vacuum freeze-drying. *Journal of Food Biochemistry*, *46*(6). https://doi.org/10.1111/jfbc.13814

**Lubbers, R. J. M., Dilokpimol, A., Visser, J., Mäkelä, M. R., Hildén, K. S., & de Vries, R. P.** (2019). A comparison between the homocyclic aromatic metabolic pathways from plant-derived compounds by bacteria and fungi. In *Biotechnology Advances* (Vol. 37, Issue 7). Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2019.05.002

**Ma, L., Zhao, C., Chen, J., & Zheng, J.** (2021). Effects of Anaerobic Fermentation on Black Garlic Extract by Lactobacillus: Changes in Flavor and Functional Components. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.645416

**Ma, Y., Yang, Y., Gao, J., Feng, J., Shang, Y., & Wei, Z.** (2020). Phenolics and antioxidant activity of bamboo leaves soup as affected by in vitro digestion. *Food and Chemical Toxicology*, *135*. https://doi.org/10.1016/j.fct.2019.110941

**Mandha, J., Shumoy, H., Devaere, J., Schouteten, J. J., Gellynck, X., de Winne, A., Matemu, A. O., & Raes, K.** (2021). Effect of lactic acid fermentation of watermelon juice on its sensory acceptability and volatile compounds. *Food Chemistry*, *358*. https://doi.org/10.1016/j.foodchem.2021.129809

**Manowan, K., Wongputtisin, P., Tassanaudom, U., Sassa-Deepaeng, T., & Chomsri, N. O.** (2020). Quality characteristics of fermented mushroom and vegetable product using a mixed starter of lactic acid bacteria. *Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering*, *2020*(1), 25–31.

**Mantzourani, I., Kazakos, S., Terpou, A., Alexopoulos, A., Bezirtzoglou, E., Bekatorou, A., & Plessas, S.** (2019). Potential of the probiotic *Lactobacillus plantarum ATCC 14917* strain to produce functional fermented pomegranate juice. *Foods*, *8*(1). https://doi.org/10.3390/foods8010004

**Mantzourani, I., Terpou, A., Alexopoulos, A., Bezirtzoglou, E., Bekatorou, A., & Plessas, S.** (2019). Production of a potentially synbiotic fermented Cornelian cherry (*Cornus mas L.)* beverage using *Lactobacillus paracasei K5* immobilized on wheat bran. *Biocatalysis and Agricultural Biotechnology*, *17*, 347–351. https://doi.org/10.1016/j.bcab.2018.12.021

**Mantzourani, I., Terpou, A., Bekatorou, A., Mallouchos, A., Alexopoulos, A., Kimbaris, A., Bezirtzoglou, E., Koutinas, A. A., & Plessas, S.** (2020). Functional pomegranate beverage production by fermentation with a novel synbiotic *L. paracasei* biocatalyst. *Food Chemistry*, *308*. https://doi.org/10.1016/j.foodchem.2019.125658

**Martínez-Miranda, J. G., Chairez, I., & Durán-Páramo, E.** (2022). Mannitol Production by Heterofermentative Lactic Acid Bacteria: a Review. In *Applied Biochemistry and Biotechnology* (Vol. 194, Issue 6, pp. 2762–2795). Springer. https://doi.org/10.1007/s12010-022-03836-5

**Mashitoa, F. M., Akinola, S. A., Manhevi, V. E., Garcia, C., Remize, F., Slabbert, R. M., & Sivakumar, D**. (2021). Influence of fermentation of pasteurised papaya puree with different lactic acid bacterial strains on quality and bioaccessibility of phenolic compounds during in vitro digestion. *Foods*, *10*(5). https://doi.org/10.3390/foods10050962

**Melini, F., & Melini, V**. (2021). Impact of fermentation on phenolic compounds and antioxidant capacity of quinoa. In *Fermentation* (Vol. 7, Issue 1). MDPI AG. https://doi.org/10.3390/fermentation7010020

**Mohd Hanafi, F. H., Rezania, S., Mat Taib, S., Md Din, M. F., Yamauchi, M., Sakamoto, M., Hara, H., Park, J., & Ebrahimi, S. S.** (2018). Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): an overview. In *Journal of Material Cycles and Waste Management* (Vol. 20, Issue 3, pp. 1383–1396). Springer Tokyo. https://doi.org/10.1007/s10163-018-0739-0

**Morales, D., Piris, A. J., Ruiz-Rodriguez, A., Prodanov, M., & Soler-Rivas, C.** (2018). Extraction of bioactive compounds against cardiovascular diseases from *Lentinula edodes* using a sequential extraction method. *Biotechnology Progress*, *34*(3), 746–755. https://doi.org/10.1002/btpr.2616

**Muñoz-Castiblanco, T., Mejía-Giraldo, J. C., & Puertas-Mejía, M. Á**. (2022). *Lentinula edodes*, a Novel Source of Polysaccharides with Antioxidant Power. *Antioxidants*, *11*(9). https://doi.org/10.3390/antiox11091770

**Muszyńska, B., Grzywacz-Kisielewska, A., Kała, K., & Gdula-Argasińska, J.** (2018). Anti-inflammatory properties of edible mushrooms: A review. In *Food Chemistry* (Vol. 243, pp. 373–381). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2017.09.149

**Mwangi, R. W., Macharia, J. M., Wagara, I. N., & Bence, R. L.** (2022). The antioxidant potential of different edible and medicinal mushrooms. In *Biomedicine and Pharmacotherapy* (Vol. 147). Elsevier Masson s.r.l. https://doi.org/10.1016/j.biopha.2022.112621

**Myo, H., Nantarat, N., & Khat-Udomkiri, N.** (2021). Changes in bioactive compounds of coffee pulp through fermentation-based biotransformation using lactobacillus plantarum tistr 543 and its antioxidant activities. *Fermentation*, *7*(4). https://doi.org/10.3390/fermentation7040292

**Naczk, M., & Shahidi, F.** (2004). Extraction and analysis of phenolics in food. In *Journal of Chromatography A* (Vol. 1054, Issues 1–2, pp. 95–111). https://doi.org/10.1016/j.chroma.2004.08.059

**Naher, L., Mustaffa Bakri, N. A., Muhammad Sukhri, S. A. N., Nik Hassan, N. R., Mohd Firdaus Ganga, H., Md Zain, N., Abdul Rahman, N., Ch'ng, H. Y., & Mokhtar, S. I.** (2024). Economical substrate formulation for mushroom cultivation and food production of mushroom crackers to reduce postharvest waste. *Studies in Fungi*, *9*. https://doi.org/10.48130/sif-2024-0003

**Nam, M., Choi, J. Y., & Kim, M. S.** (2021a). Metabolic profiles, bioactive compounds, and antioxidant capacity in lentinula edodes cultivated on log versus sawdust substrates. *Biomolecules*, *11*(11). https://doi.org/10.3390/biom11111654

**Nam, M., Choi, J. Y., & Kim, M. S.** (2021b). Metabolic profiles, bioactive compounds, and antioxidant capacity in lentinula edodes cultivated on log versus sawdust substrates. *Biomolecules*, *11*(11). https://doi.org/10.3390/biom11111654

**Nascimento Santos, M. da S., Magalhães, J. E. de M., Will Castro, L. S. E. P., Pinheiro, T. de S., Sabry, D. A., Nobre, L. T. D. B., Santos Lima, J. P. M., Baseia, I. G., & Leite, E. L.** (2014). Effect of glucans from *Caripia montagnei* mushroom on TNBS-induced colitis. *International Journal of Molecular Sciences*, *15*(2), 2368–2385. https://doi.org/10.3390/ijms15022368

**Ng, Z. X., & Rosman, N. F.** (2019). In vitro digestion and domestic cooking improved the total antioxidant activity and carbohydrate-digestive enzymes inhibitory potential of selected edible mushrooms. *Journal of Food Science and Technology*, *56*(2), 865–877. https://doi.org/10.1007/s13197-018-3547-6

**Nie, Y., Yu, M., Zhou, H., Zhang, P., Yang, W., & Li, B.** (2020). Effect of boiling time on nutritional characteristics and antioxidant activities of *Lentinus edodes* and its broth. *CYTA - Journal of Food*, *18*(1), 543–550. https://doi.org/10.1080/19476337.2020.1799077

**Ojha, A. K.** (2021). Structural Analysis of a Polysaccharide Isolated from the Alkali Extract of an Edible Mushroom, *Pleurotus sajor-caju,* Cultivar Black Japan. *Journal of Scientific Research*, *13*(3), 1025–1041. https://doi.org/10.3329/jsr.v13i3.53502

**Onbaşili, D., Yuvali Çelik, G., Katircioğlu, H., & Narin, İ.** (2015). Antimicrobial, Antioxidant Activities and Chemical Composition of *Lactarius deliciosus (L.)* Collected from Kastamonu Province of Turkey. In *Journal of Forestry Faculty* (Vol. 15, Issue 1).

**Ozdemir, M., Gungor, V., Melikoglu, M., & Aydiner, C.** (2024). Solvent selection and effect of extraction conditions on ultrasound-assisted extraction of phenolic compounds from galangal (*Alpinia officinarum*). *Journal of Applied Research on Medicinal and Aromatic Plants*, *38*. https://doi.org/10.1016/j.jarmap.2023.100525

**Palacios, I., García-Lafuente, A., Guillamón, E., & Villares, A**. (2012). Novel isolation of water-soluble polysaccharides from the fruiting bodies of *Pleurotus ostreatus* mushrooms. *Carbohydrate Research*, *358*, 72–77. https://doi.org/10.1016/j.carres.2012.06.016

**Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M. A., Martínez, J. A., García-Lafuente, A., Guillamón, E., & Villares, A.** (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, *128*(3), 674–678. https://doi.org/10.1016/j.foodchem.2011.03.085

**Papoutsis, K., Grasso, S., Menon, A., Brunton, N. P., Lyng, J. G., Jacquier, J. C., & Bhuyan, D. J.** (2020). Recovery of ergosterol and vitamin D2 from mushroom waste - Potential valorization by food and pharmaceutical industries. In *Trends in Food Science and Technology* (Vol. 99, pp. 351–366). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.03.005

**Pau, S., Tan, L. C., Arriaga, S., & Lens, P. N. L.** (2022). Lactic acid fermentation of food waste at acidic conditions in a semicontinuous system: effect of HRT and OLR changes. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-022-03201-w

**Peerajan, S., Chaiyasut, C., Sirilun, S., Chaiyasut, K., Kesika, P., & Sivamaruthi, B. S.** (2016). Enrichment of nutritional value of *Phyllanthus emblica* fruit juice using the probiotic bacterium, *Lactobacillus paracasei HII01* mediated fermentation. *Food Science and Technology (Brazil)*, *36*(1), 116–123. https://doi.org/10.1590/1678-457X.0064

**Karakas, F. P., Turker, A. U., & Bozat, B. G.** (2023). Phenolic Content, Antibacterial and Antioxidant Potential of Several Edible Agaricomycetes Mushrooms Sold in Public Bazaar in Bolu, Turkey. *International Journal of Medicinal Mushrooms*, 25(1).

**Pérez-Bassart, Z., Fabra, M. J., Martínez-Abad, A., & López-Rubio, A.** (2023). Compositional differences of β-glucan-rich extracts from three relevant mushrooms obtained through a sequential extraction protocol. *Food Chemistry*, *402*. https://doi.org/10.1016/j.foodchem.2022.134207

**Pérez-Chávez, A. M., Mayer, L., & Albertó, E.** (2019). Mushroom cultivation and biogas production: A sustainable reuse of organic resources. In *Energy for Sustainable Development* (Vol. 50, pp. 50–60). Elsevier B.V. https://doi.org/10.1016/j.esd.2019.03.002

**Perveen, I., Bukhari, B., Sarwar, A., Aziz, T., Koser, N., Younis, H., Ahmad, Q. ul ain, Sabahat, S., Tzora, A., & Skoufos, I.** (2023). Applications and efficacy of traditional to emerging trends in lacto-fermentation and submerged cultivation of edible mushrooms. In *Biomass Conversion and Biorefinery*. Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s13399-023-04694-9

**Plessas, S.** (2022). Advancements in the use of fermented fruit juices by lactic acid bacteria as functional foods: Prospects and challenges of *Lactiplantibacillus (Lpb.) plantarum subsp. plantarum* application. In *Fermentation* (Vol. 8, Issue 1). MDPI. https://doi.org/10.3390/fermentation8010006

**Qian, G. M., Pan, G. F., & Guo, J. Y.** (2012). Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom Cordyceps sinensis. *Natural Product Research*, *26*(24), 2358–2362. https://doi.org/10.1080/14786419.2012.658800

**Radzki, W., Ziaja-Sołtys, M., Nowak, J., Topolska, J., Bogucka-Kocka, A., Sławińska, A., Michalak-Majewska, M., Jabłońska-Ryś, E., & Kuczumow, A**. (2019). Impact of processing on polysaccharides obtained from button mushroom (*Agaricus bisporus*). *International Journal of Food Science and Technology*, *54*(4), 1405–1412. https://doi.org/10.1111/ijfs.14084

**Raghavendra, S. N., Rastogi, N. K., Raghavarao, K. S. M. S., & Tharanathan, R. N.** (2004). Dietary fiber from coconut residue: Effects of different treatments and particle size on the hydration properties. *European Food Research and Technology*, *218*(6), 563–567. https://doi.org/10.1007/s00217-004-0889-2

**Rahi, D. K., & Malik, D**. (2016). Diversity of Mushrooms and Their Metabolites of Nutraceutical and Therapeutic Significance. *Journal of Mycology*, *2016*, 1–18. https://doi.org/10.1155/2016/7654123

**Raman, J., Kim, J. S., Choi, K. R., Eun, H., Yang, D., Ko, Y. J., & Kim, S. J.** (2022). Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations. In *International Journal of Molecular Sciences* (Vol. 23, Issue 14). MDPI. https://doi.org/10.3390/ijms23147784

**Ravyts, F., Vuyst, L. De, & Leroy, F**. (2012). Bacterial diversity and functionalities in food fermentations. In *Engineering in Life Sciences* (Vol. 12, Issue 4, pp. 356–367). https://doi.org/10.1002/elsc.201100119

**Razola-Díaz, M. del C., De Montijo-Prieto, S., Aznar-Ramos, M. J., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M.** (2023a). Effect of Lactic Acid Bacteria Fermentation on the Polar Compounds Content with Antioxidant and Antidiabetic Activity of Avocado Seed Extracts. *Fermentation*, *9*(5). https://doi.org/10.3390/fermentation9050420

**Razola-Díaz, M. del C., De Montijo-Prieto, S., Aznar-Ramos, M. J., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M.** (2023b). Effect of Lactic Acid Bacteria Fermentation on the Polar Compounds Content with Antioxidant and Antidiabetic Activity of Avocado Seed Extracts. *Fermentation*, *9*(5). https://doi.org/10.3390/fermentation9050420

**Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C.** (1999). *Original Contribution Antioxidant Activity Applying An Improved Abts Radical Cation Decolorization Assay*.

**Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. F. R.** (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology*, *50*(5), 1201–1207. https://doi.org/10.1016/j.fct.2012.02.013

**Ren, G., Xu, L., Lu, T., & Yin, J.** (2018). Structural characterization and antiviral activity of lentinan from *Lentinus edodes* mycelia against infectious hematopoietic necrosis virus. *International Journal of Biological Macromolecules, 115,* 1202–1210. https://doi.org/10.1016/j.ijbiomac.2018.04.132

**Rinaudo, M.** (2006). Chitin and chitosan: Properties and applications. In *Progress in Polymer Science (Oxford)* (Vol. 31, Issue 7, pp. 603–632). https://doi.org/10.1016/j.progpolymsci.2006.06.001

**Rocchetti, G., Gregorio, R. P., Lorenzo, J. M., Barba, F. J., Oliveira, P. G., Prieto, M. A., Simal-Gandara, J., Mosele, J. I., Motilva, M. J., Tomas, M., Patrone, V., Capanoglu, E., & Lucini, L.** (2022). Functional implications of bound phenolic compounds and phenolics–food interaction: A review. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 21, Issue 2, pp. 811–842). John Wiley and Sons Inc. https://doi.org/10.1111/1541-4337.12921

**Roszczyk, A., Turło, J., Zagożdżon, R., & Kaleta, B.** (2022). Immunomodulatory Properties of Polysaccharides from *Lentinula edodes*. In *International Journal of Molecular Sciences* (Vol. 23, Issue 16). MDPI. https://doi.org/10.3390/ijms23168980

**Ruiz-Ruiz, J. C., Matus-Basto, A. J., Acereto-Escoffié, P., & Segura-Campos, M. R**. (2017). Antioxidant and anti-inflammatory activities of phenolic compounds isolated from Melipona beecheii honey. *Food and Agricultural Immunology, 28*(6), 1424–1437. https://doi.org/10.1080/09540105.2017.1347148

**Salar, R. K., Purewal, S. S., & Bhatti, M. S.** (2016). Optimization of extraction conditions and enhancement of phenolic content and antioxidant activity of pearl millet fermented with *Aspergillus awamori MTCC-548*. *Resource-Efficient Technologies, 2*(3), 148–157. https://doi.org/10.1016/j.reffit.2016.08.002

**Santana Andrade, J. K., Chagas Barros, R. G., Gualberto, N. C., Santos de Oliveira, C., Shanmugam, S., & Narain, N.** (2022). Influence of in vitro gastrointestinal digestion and probiotic fermentation on the bioaccessibility of gallic acid and on the antioxidant potential of Brazilian fruit residues. *LWT, 153*. https://doi.org/10.1016/j.lwt.2021.112436

**Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B**. (2014). Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rizhopus oryzae. Food Chemistry, 146,* 371–377. https://doi.org/10.1016/j.foodchem.2013.09.101

**Shahidi, F., & Yeo, J. D.** (2016). Insoluble-bound phenolics in food. In *Molecules* (Vol. 21, Issue 9). MDPI AG. https://doi.org/10.3390/molecules21091216

**Sheng, K., Wang, C., Chen, B., Kang, M., Wang, M., Liu, K., & Wang, M.** (2021). Recent advances in polysaccharides from *Lentinus edodes* (Berk.): Isolation, structures and bioactivities. In *Food Chemistry* (Vol. 358). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2021.129883

**Shi, Y., Singh, A., Kitts, D. D., & Pratap-Singh, A.** (2021). Lactic acid fermentation: A novel approach to eliminate unpleasant aroma in pea protein isolates. *LWT*, *150*. https://doi.org/10.1016/j.lwt.2021.111927

**Siemińska-Kuczer, A., Szymańska-Chargot, M., & Zdunek, A.** (2022). Recent advances in interactions between polyphenols and plant cell wall polysaccharides as studied using an adsorption technique. In *Food Chemistry* (Vol. 373). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2021.131487

**Sillapachaiyaporn, C., Nilkhet, S., Ung, A. T., & Chuchawankul, S.** (2019). Anti-HIV-1 protease activity of the crude extracts and isolated compounds from Auricularia polytricha. *BMC Complementary and Alternative Medicine*, *19*(1). https://doi.org/10.1186/s12906-019-2766-3

**Singleton, V. L., Orthofer, R., & Lamuela-Ravent6s, R. M.** (1999). *52 Polyphenols and Flavonoids. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent*.

**Skrzypczak, K., Gustaw, K., Jabłońska-Ryś, E., Sławińska, A., Gustaw, W., & Winiarczyk, S.** (2020). Spontaneously Fermented Fruiting Bodies *of Agaricus bisporus* as a Valuable Source of New Isolates of Lactic Acid Bacteria with Functional Potential. *Foods*, *9*(11). https://doi.org/10.3390/foods9111631

**Soua, L., Koubaa, M., Barba, F. J., Fakhfakh, J., Ghamgui, H. K., & Chaabouni, S. E.** (2020). Water-Soluble Polysaccharides from Ephedra alata Stems: Structural characterization, functional properties, and antioxidant activity. *Molecules*, *25*(9). https://doi.org/10.3390/molecules25092210

**Stamets, P.** (2011). Growing Gourmet and Medicinal Mushrooms; Ten Speed Press: Berkeley, CA, USA.

**Stein, S.** (2012). Mass spectral reference libraries: An ever-expanding resource for chemical identification. *Analytical Chemistry*, *84*(17), 7274–7282. https://doi.org/10.1021/ac301205z

**Su, D., Lv, W., Wang, Y., Wang, L., & Li, D**. (2020). Influence of microwave hot-air flow rolling dry-blanching on microstructure, water migration and quality of *Pleurotus eryngii* during hot-air drying. *Food Control*, *114*. https://doi.org/10.1016/j.foodcont.2020.107228

**Sun, J., He, H., & Bi, J. X**. (2004). Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum. Journal of Agricultural and Food Chemistry, 52*(21), 6646–6652. https://doi.org/10.1021/jf0495136

**Suwannarach, N., Kumla, J., Zhao, Y., & Kakumyan, P**. (2022). Impact of Cultivation Substrate and Microbial Community on Improving Mushroom Productivity: A Review. In *Biology* (Vol. 11, Issue 4). MDPI. https://doi.org/10.3390/biology11040569

**Suzuki, N., Takimoto, Y., Suzuki, R., Arai, T., Uebaba, K., Nakai, M., Strong, J. M., & Tokuda, H.** (2013). Efficacy of oral administration of lentinula eododes mycelia extract for breast cancer patients undergoing postoperative hormone therapy. *Asian Pacific Journal of Cancer Prevention*, *14*(6), 3469–3472. https://doi.org/10.7314/APJCP.2013.14.6.3469

**Synytsya, A., Míčková, K., Synytsya, A., Jablonský, I., Spěváček, J., Erban, V., Kováříková, E., & Čopíková, J.** (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, *76*(4), 548–556. https://doi.org/10.1016/j.carbpol.2008.11.021

**Synytsya, A., & Novák, M.** (2013). Structural diversity of fungal glucans. In *Carbohydrate Polymers* (Vol. 92, Issue 1, pp. 792–809). https://doi.org/10.1016/j.carbpol.2012.09.077

**Szliszka, E., Czuba, Z. P., Domino, M., Mazur, B., Zydowicz, G., & Krol, W.** (2009). Ethanolic Extract of Propolis (EEP) Enhances the Apoptosis-Inducing Potential of TRAIL in Cancer Cells. *Molecules*, *14*(2), 738–754. https://doi.org/10.3390/molecules

**Tan, P. W., Tan, C. P., & Ho, C. W.** (2011). Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). In *International Food Research Journal* (Vol. 18).

**Tao, R., Chen, Q., Li, Y., Guo, L., & Zhou, Z.** (2022). Physicochemical, nutritional, and phytochemical profile changes of fermented citrus puree from enzymatically hydrolyzed whole fruit under cold storage. *LWT*, *169*. https://doi.org/10.1016/j.lwt.2022.114009

**Taofiq, O., Calhelha, R. C., Heleno, S., Barros, L., Martins, A., Santos-Buelga, C., Queiroz, M. J. R. P., & Ferreira, I. C. F. R.** (2015). The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: Screening in phenolic extracts, individual parent molecules and synthesized glucuronated and methylated derivatives. *Food Research International*, *76*, 821–827. https://doi.org/10.1016/j.foodres.2015.07.044

**Taofiq, O., Corrêa, R. C. G., Barros, L., Prieto, M. A., Bracht, A., Peralta, R. M., González-Paramás, A. M., Barreiro, M. F., & Ferreira, I. C. F. R.** (2019). A comparative study between conventional and non-conventional extraction techniques for the recovery of ergosterol from *Agaricus blazei Murrill*. *Food Research International*, *125*. https://doi.org/10.1016/j.foodres.2019.108541

**Tavakoli, M., Habibi Najafi, M. B., & Mohebbi, M.** (2019). Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. *Heliyon*, *5*, e01204. https://doi.org/10.1016/j.heliyon.2019

**Teleky, B. E., Martău, G. A., & Vodnar, D. C.** (2020). Physicochemical effects of *Lactobacillus plantarum* and *Lactobacillus casei* cocultures on soy–wheat flour dough fermentation. *Foods*, *9*(12). https://doi.org/10.3390/foods9121894

**Thakur, M. P.** (2020). Advances in mushroom production: key to food, nutritional and employment security: A review. In *Indian Phytopathology* (Vol. 73, Issue 3, pp. 377–395). Springer. https://doi.org/10.1007/s42360-020-00244-9

**Tu, J., Brennan, M. A., Wu, G., Bai, W., Cheng, P., Tian, B., & Brennan, C. S.** (2021). Delivery of phenolic compounds, peptides and β-glucan to the gastrointestinal tract by incorporating dietary fibre-rich mushrooms into sorghum biscuits. *Foods*, *10*(8). https://doi.org/10.3390/foods10081812

**Ucar, T. M., & Karadag, A.** (2019). The effects of vacuum and freeze-drying on the physicochemical properties and in vitro digestibility of phenolics in oyster mushroom (*Pleurotus ostreatus*). *Journal of Food Measurement and Characterization*, *13*(3), 2298–2309. https://doi.org/10.1007/s11694-019-00149-w

**Umaña, M., Eim, V., Garau, C., Rosselló, C., & Simal, S.** (2020). Ultrasound-assisted extraction of ergosterol and antioxidant components from mushroom by-products and the attainment of a β-glucan rich residue. *Food Chemistry*, *332*. https://doi.org/10.1016/j.foodchem.2020.127390

**Valero-Cases, E., Nuncio-Jáuregui, N., & Frutos, M. J.** (2017). Influence of Fermentation with Different Lactic Acid Bacteria and in Vitro Digestion on the Biotransformation of Phenolic Compounds in Fermented Pomegranate Juices. *Journal of Agricultural and Food Chemistry*, *65*(31), 6488–6496. https://doi.org/10.1021/acs.jafc.6b04854

**Vergara, C. M. de A. C., Honorato, T. L., Maia, G. A., & Rodrigues, S.** (2010). Prebiotic effect of fermented cashew apple (*Anacardium occidentale L*) juice. *LWT*, *43*(1), 141–145. https://doi.org/10.1016/j.lwt.2009.06.009

**Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., ... & Wichers, H.** (2015). The impact of food bioactives on health: in vitro and ex vivo models.

**Verni, M., Verardo, V., & Rizzello, C. G.** (2019). How fermentation affects the antioxidant properties of cereals and legumes. In *Foods* (Vol. 8, Issue 9). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods8090362

**Vetter, J.** (2007). Chitin content of cultivated mushrooms *Agaricus bisporus, Pleurotus ostreatus* and *Lentinula edodes*. *Food Chemistry*, *102*(1), 6–9. https://doi.org/10.1016/j.foodchem.2006.01.037

**Vlassopoulou, M., Paschalidis, N., Savvides, A. L., Saxami, G., Mitsou, E. K., Kerezoudi, E. N., Koutrotsios, G., Zervakis, G. I., Georgiadis, P., Kyriacou, A., & Pletsa, V.** (2022). Immunomodulating Activity of *Pleurotus eryngii* Mushrooms Following Their In Vitro Fermentation by Human Fecal Microbiota. *Journal of Fungi*, *8*(4). https://doi.org/10.3390/jof8040329

**Wang, B., Zhao, N., Li, J., Xu, R., Wang, T., Guo, L., Ma, M., Fan, M., & Wei, X.** (2021). Selenium-enriched *Lactobacillus plantarum* improves the antioxidant activity and flavor properties of fermented *Pleurotus eryngii*. *Food Chemistry, 345*. https://doi.org/10.1016/j.foodchem.2020.128770

**Wang, C. Y.** (2020). A Review on the Potential Reuse of Functional Polysaccharides Extracted from the By-Products of Mushroom Processing. In *Food and Bioprocess Technology* (Vol. 13, Issue 2, pp. 217–228). Springer. https://doi.org/10.1007/s11947-020-02403-2

**Wang, J. J., Zhang, W. W., Guan, Z. J., Thakur, K., Hu, F., Rizwan Khan, M., Zhang, J. G., & Wei, Z. J.** (2023). Exploring the effects of the fermentation method on the quality of *Lycium barbarum* and *Polygonatum cyrtonema* compound wine based on LC-MS metabolomics. *Food Chemistry, 428*. https://doi.org/10.1016/j.foodchem.2023.136770

**Wang, K., Guo, J., Cheng, J., Zhao, X., Ma, B., Yang, X., & Shao, H.** (2021). Ultrasound-assisted extraction of polysaccharide from spent Lentinus edodes substrate: Process optimization, precipitation, structural characterization and antioxidant activity. *International Journal of Biological Macromolecules, 191,* 1038–1045. https://doi.org/10.1016/j.ijbiomac.2021.09.174

**Wang, M., & Zhao, R.** (2023). A review on nutritional advantages of edible mushrooms and its industrialization development situation in protein meat analogues. In *Journal of Future Foods* (Vol. 3, Issue 1, pp. 1–7). Beijing Academy of Food Sciences. https://doi.org/10.1016/j.jfutfo.2022.09.001

**Wang, W., Xia, W., Gao, P., Xu, Y., & Jiang, Q.** (2017). Proteolysis during fermentation of Suanyu as a traditional fermented fish product of China. *International Journal of Food Properties, 20,* S166–S176. https://doi.org/10.1080/10942912.2017.1293089

**Wang, X. H., Cheng, X. Du, Wang, D., Wu, Z., Chen, Y., & Wu, Q. X.** (2022). Antioxidant and anti-aging effects of polysaccharide LDP-1 from wild *Lactarius deliciosus* on Caenorhabditis elegans. *Food and Nutrition Research*, *66*. https://doi.org/10.29219/fnr.v66.8110

**Wang, X., He, X., Sun, C., Peng, M., Zhang, Q., Brennan, C. S., Guan, W., Wang, F., & Zhang, N.** (2022a). Extraction of bound phenolics from shiitake mushrooms (*Lentinus edodes*) by combined acid and base hydrolysis procedures and analysis of phenolic profiles and antioxidant capacities. *International Journal of Food Science and Technology, 57*(10), 6613–6620. https://doi.org/10.1111/ijfs.16004

**Wang, X., He, X., Sun, C., Peng, M., Zhang, Q., Brennan, C. S., Guan, W., Wang, F., & Zhang, N.** (2022b). Extraction of bound phenolics from shiitake mushrooms (*Lentinus edodes*) by combined acid and base hydrolysis procedures and analysis of phenolic profiles and antioxidant capacities. *International Journal of Food Science and Technology*, *57*(10), 6613–6620. https://doi.org/10.1111/ijfs.16004

**Wang, Y., Shao, S., Xu, P., Chen, H., Lin-Shiau, S. Y., Deng, Y. T., & Lin, J. K**. (2012). Fermentation process enhanced production and bioactivities of oolong tea polysaccharides. *Food Research International*, *46*(1), 158–166. https://doi.org/10.1016/j.foodres.2011.11.027

**Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W.** (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. In *Frontiers in Bioengineering and Biotechnology* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2021.612285

**Wasser, S.** (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. In *Applied Microbiology and Biotechnology* (Vol. 60, Issue 3, pp. 258–274). Springer Verlag. https://doi.org/10.1007/s00253-002-1076-7

**Wen, D., Li, C., Di, H., Liao, Y., & Liu, H**. (2005). A universal HPLC method for the determination of phenolic acids in compound herbal medicines. *Journal of Agricultural and Food Chemistry*, *53*(17), 6624–6629. https://doi.org/10.1021/jf0511291

**Wu, C., Li, T., Qi, J., Jiang, T., Xu, H., & Lei, H**. (2020). Effects of lactic acid fermentation-based biotransformation on phenolic profiles, antioxidant capacity and flavor volatiles of apple juice. *LWT*, *122*. https://doi.org/10.1016/j.lwt.2020.109064

**Xiaokang, W., Brunton, N. P., Lyng, J. G., Harrison, S. M., Carpes, S. T., & Papoutsis, K.** (2020). Volatile and non-volatile compounds of shiitake mushrooms treated with pulsed light after twenty-four hour storage at different conditions. *Food Bioscience*, *36*. https://doi.org/10.1016/j.fbio.2020.100619

**Xie, Y., Guo, J., Li, W., Wu, Z., & Yu, Z.** (2021). Effects of ferulic acid esterase-producing lactic acid bacteria and storage temperature on the fermentation quality, in vitro digestibility and phenolic acid extraction yields of sorghum (*Sorghum bicolor l.)* silage. *Microorganisms*, *9*(1), 1–12. https://doi.org/10.3390/microorganisms9010114

**Xu, Z., Fu, L., Feng, S., Yuan, M., Huang, Y., Liao, J., Zhou, L., Yang, H., & Ding, C.** (2019). Chemical composition, antioxidant and antihyperglycemic activities of the wild lactarius deliciosus from China. *Molecules*, *24*(7). https://doi.org/10.3390/molecules24071357

**Xue, Z., Chen, Y., Jia, Y., Wang, Y., Lu, Y., Chen, H., & Zhang, M.** (2019). Structure, thermal and rheological properties of different soluble dietary fiber fractions from mushroom *Lentinula edodes* (Berk.) Pegler residues. *Food Hydrocolloids*, *95*, 10–18. https://doi.org/10.1016/j.foodhyd.2019.04.015

**Yan, Y., Zhang, F., Chai, Z., Liu, M., Battino, M., & Meng, X.** (2019). Mixed fermentation of blueberry pomace with L. rhamnosus GG and L. plantarum-1: Enhance the active ingredient, antioxidant activity and health-promoting benefits. *Food and Chemical Toxicology*, *131*. https://doi.org/10.1016/j.fct.2019.05.049

**Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., Chen, S., Ye, X., & Wang, L**. (2023). Effects of Fermentation on Bioactivity and the Composition of Polyphenols Contained in Polyphenol-Rich Foods: A Review. In *Foods* (Vol. 12, Issue 17). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods12173315

**Yang, J., Gao, T., Ge, F., Sun, H., Cui, Z., Wei, Z., Wang, S., Show, P. L., Tao, Y., & Wang, W.** (2022). Porphyra yezoensis Sauces Fermented With Lactic Acid Bacteria: Fermentation Properties, Flavor Profile, and Evaluation of Antioxidant Capacity in vitro. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.810460

**Yang, J., Sun, Y., Chen, J., Cheng, Y., Zhang, H., Gao, T., Xu, F., Pan, S., Tao, Y., & Lu, J.** (2022a). Fermentation of ginkgo biloba kernel juice using Lactobacillus plantarum Y2 from the ginkgo peel: Fermentation characteristics and evolution of phenolic profiles, antioxidant activities in vitro, and volatile flavor compounds. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1025080

**Yang, J., Sun, Y., Chen, J., Cheng, Y., Zhang, H., Gao, T., Xu, F., Pan, S., Tao, Y., & Lu, J.** (2022b). Fermentation of ginkgo biloba kernel juice using Lactobacillus plantarum Y2 from the ginkgo peel: Fermentation characteristics and evolution of phenolic profiles, antioxidant activities in vitro, and volatile flavor compounds. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1025080

**Yang, J., Sun, Y., Gao, T., Wu, Y., Sun, H., Zhu, Q., Liu, C., Zhou, C., Han, Y., & Tao, Y.** (2022). Fermentation and Storage Characteristics of “Fuji” Apple Juice Using Lactobacillus acidophilus, Lactobacillus casei and Lactobacillus plantarum: Microbial Growth, Metabolism of Bioactives and in vitro Bioactivities. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.833906

**Yao, F., Gao, H., Yin, C. M., Shi, D. F., & Fan, X. Z**. (2023). Effect of Different Cooking Methods on the Bioactive Components, Color, Texture, Microstructure, and Volatiles of Shiitake Mushrooms. *Foods*, *12*(13). https://doi.org/10.3390/foods12132573

**Yeo, J. D., Tsao, R., Sun, Y., & Shahidi, F**. (2021). Liberation of insoluble-bound phenolics from lentil hull matrices as affected by Rhizopus oryzae fermentation: Alteration in phenolic profiles and their inhibitory capacities against low-density lipoprotein (LDL) and DNA oxidation. *Food Chemistry*, *363*. https://doi.org/10.1016/j.foodchem.2021.130275

**Yi, C., Li, Y., Zhu, H., Liu, Y., & Quan, K.** (2021). Effect of Lactobacillus plantarum fermentation on the volatile flavors of mung beans. *LWT*, *146*. https://doi.org/10.1016/j.lwt.2021.111434

**Yin, C., Fan, X., Fan, Z., Shi, D., & Gao, H.** (2018). Optimization of enzymes-microwave-ultrasound assisted extraction of Lentinus edodes polysaccharides and determination of its antioxidant activity. *International Journal of Biological Macromolecules*, *111*, 446–454. https://doi.org/10.1016/j.ijbiomac.2018.01.007

**Yin, H., Zhong, Y., Xia, S., Hu, J., Nie, S., Xiong, T., & Xie, M.** (2020). Effects of fermentation with Lactobacillus plantarum NCU137 on nutritional, sensory and stability properties of Coix (Coix lachryma-jobi L.) seed. *Food Chemistry, 314*. https://doi.org/10.1016/j.foodchem.2019.126037

**Zhang, B., Zhang, Y., Li, H., Deng, Z., & Tsao, R.** (2020). A review on insoluble-bound phenolics in plant-based food matrix and their contribution to human health with future perspectives. In *Trends in Food Science and Technology* (Vol. 105, pp. 347–362). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.09.029

**Zhang, D., Ye, Y., & Tan, B.** (2022). Comparative study of solid-state fermentation with different microbial strains on the bioactive compounds and microstructure of brown rice. *Food Chemistry, 397*. https://doi.org/10.1016/j.foodchem.2022.133735

**Zhao, D., & Shah, N. P.** (2016). Lactic acid bacterial fermentation modified phenolic composition in tea extracts and enhanced their antioxidant activity and cellular uptake of phenolic compounds following in vitro digestion. *Journal of Functional Foods, 20*, 182–194. https://doi.org/10.1016/j.jff.2015.10.033

**Zhao, H. M., Guo, X. N., & Zhu, K. X.** (2017). Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. *Food Chemistry, 217*, 28–36. https://doi.org/10.1016/j.foodchem.2016.08.062

**Zhao, Q., Wang, Z., Wang, X., Yan, X., Guo, Q., Yue, Y., Yue, T., & Yuan, Y.** (2023a). The bioaccessibility, bioavailability, bioactivity, and prebiotic effects of phenolic compounds from raw and solid-fermented mulberry leaves during in vitro digestion and colonic fermentation. *Food Research International, 165*. https://doi.org/10.1016/j.foodres.2023.112493

**Zhao, Q., Wang, Z., Wang, X., Yan, X., Guo, Q., Yue, Y., Yue, T., & Yuan, Y.** (2023b). The bioaccessibility, bioavailability, bioactivity, and prebiotic effects of phenolic compounds from raw and solid-fermented mulberry leaves during in vitro digestion and colonic fermentation. *Food Research International, 165*. https://doi.org/10.1016/j.foodres.2023.112493

**Zhao, R., Yang, W., Pei, F., Zhao, L., & Hu, Q.** (2018). In vitro fermentation of six kinds of edible mushrooms and its effects on fecal microbiota composition. *LWT, 96*, 627–635. https://doi.org/10.1016/j.lwt.2018.06.012

**Zhao, X., & Zheng, X.** (2009). A primary study on texture modification and proteolysis of mao-tofu during fermentation. *African Journal of Biotechnology, 8*(10), 2294–2300. http://www.academicjournals.org/AJB

**Zheng, H. G., Chen, J. C., & Ahmad, I.** (2018). Preservation of King Oyster Mushroom by the use of different fermentation processes. *Journal of Food Processing and Preservation*, *42*(1). https://doi.org/10.1111/jfpp.13396

**Zhou, Y., Chu, M., Ahmadi, F., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R.** (2023). A Comprehensive Review on Phytochemical Profiling in Mushrooms: Occurrence, Biological Activities, Applications and Future Prospective. In *Food Reviews International*. Taylor and Francis Ltd. https://doi.org/10.1080/87559129.2023.22027

# APPENDICES

## **APPENDIX A:** Chapter 4 Supporting Information

**APPENDIX A:** Chapter 4 Supporting Information

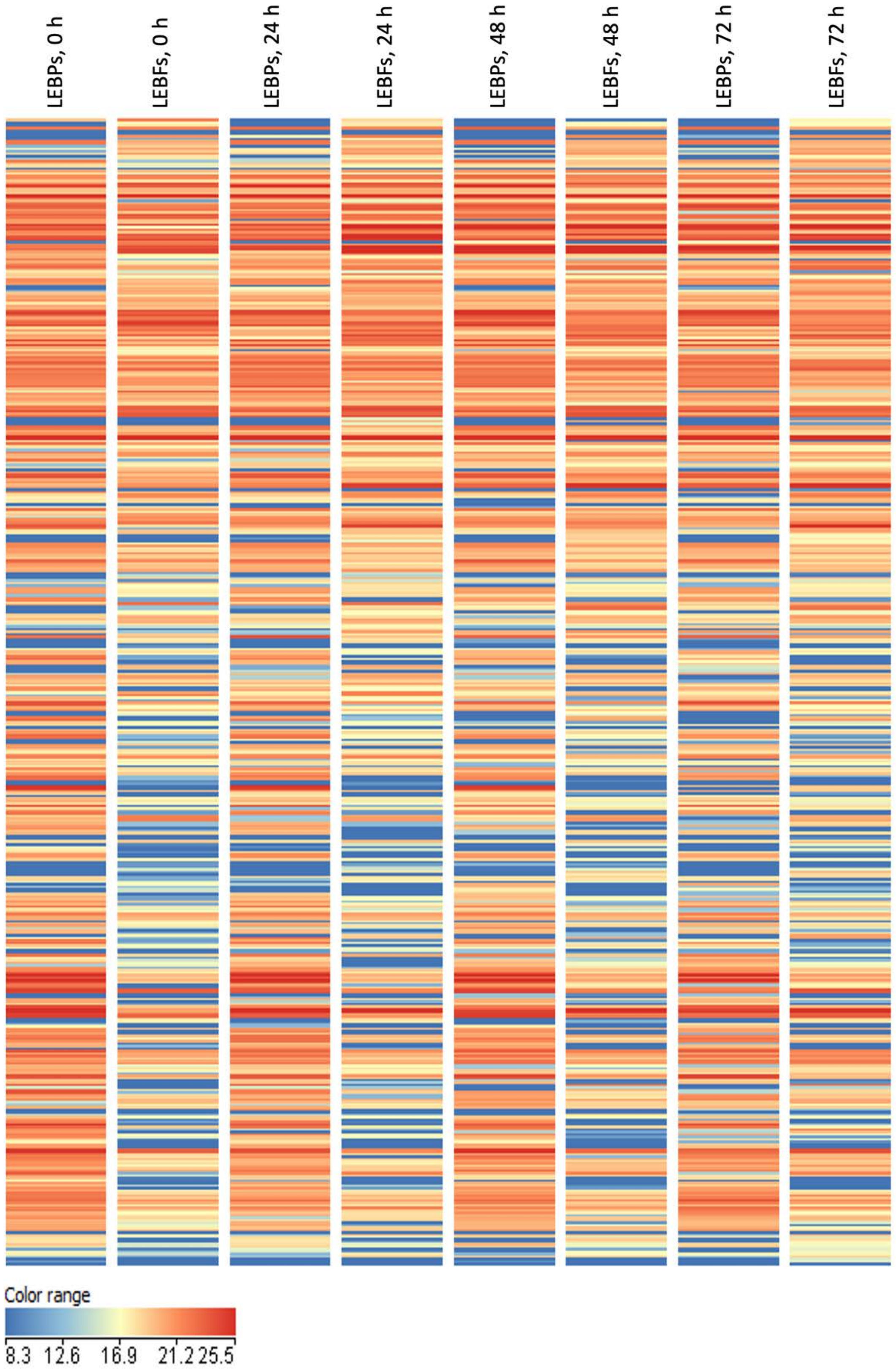


**Figure A.5 :** Heat map of fermented all 468 entities of *L.edodes* during fermentation.

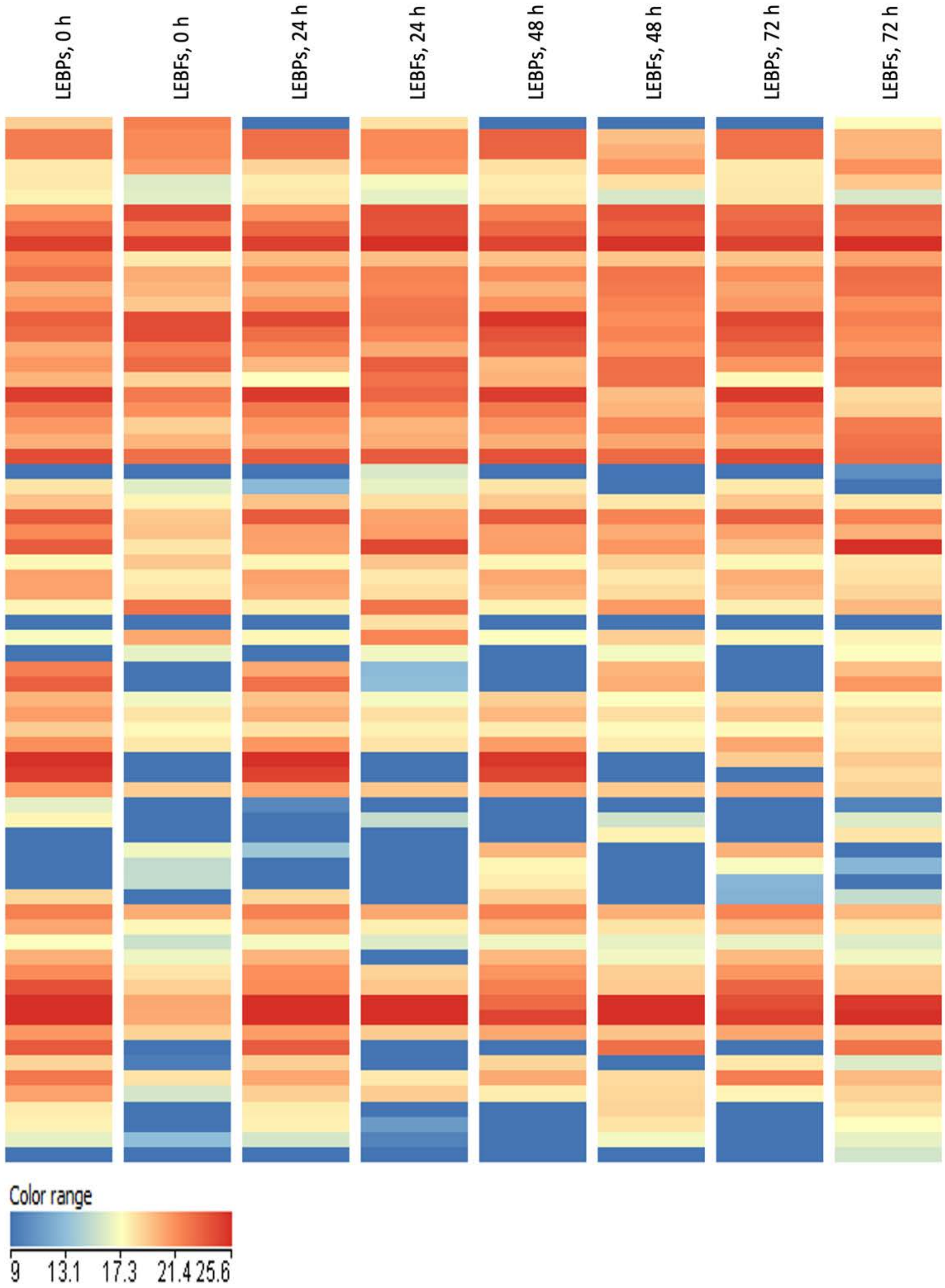


**Figure A.6 :** Heat map of fermented *L.edodes* during fermentation with 2-way ANOVA with time and fraction factor, 69 entities.

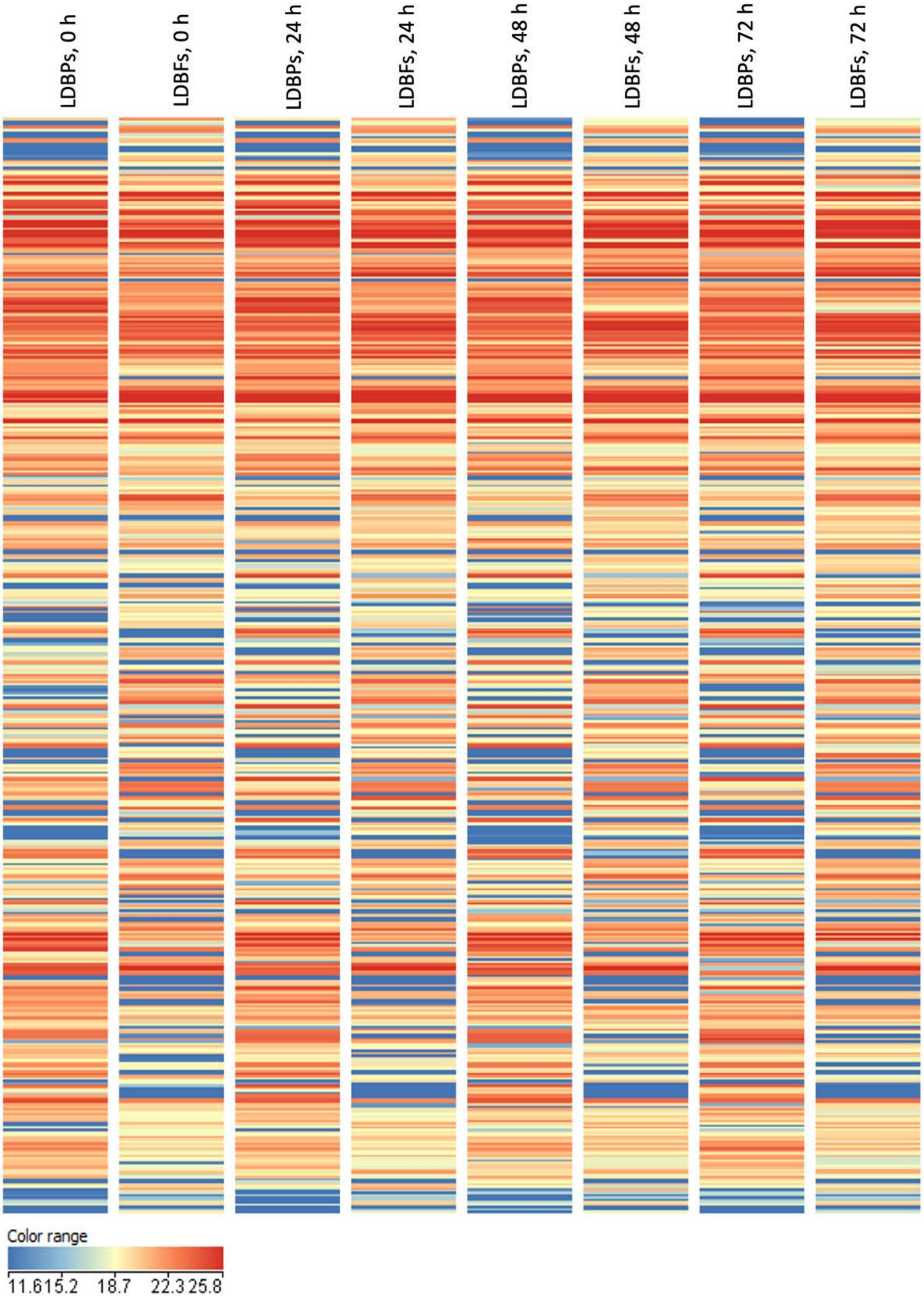


**Figure A.3 :** Heat map of fermented all 469 entities of *La. deliciosus* during fermentation.

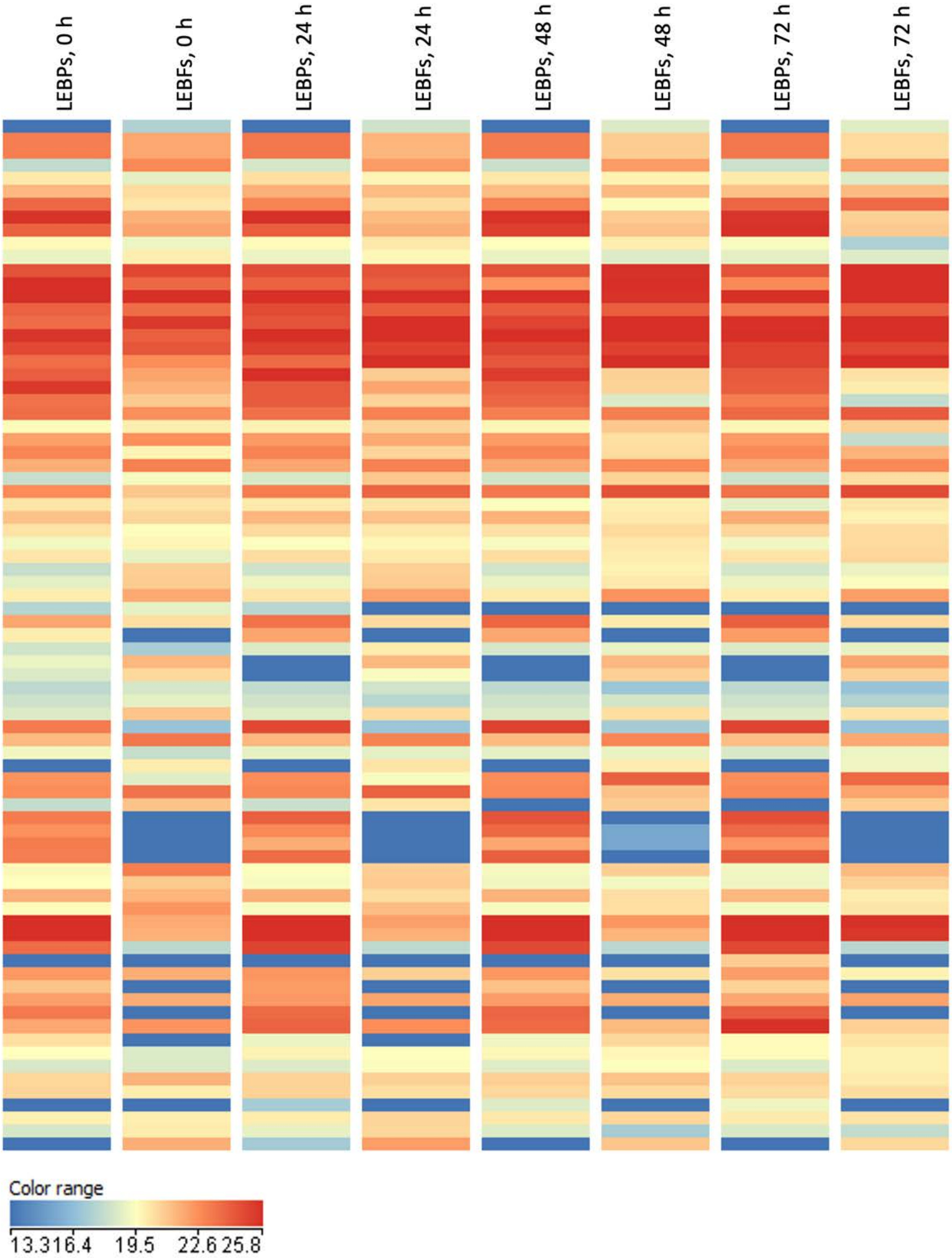


**Figure A.4 :** Heat map of fermented *La. deliciosus* during fermentation with 2-way ANOVA with time and fraction factor, 79 entities.

## CURRICULUM VITAE

**Name Surname** : Eda Nur AYAR SÜMER

**EDUCATION** :

- **B.Sc.** : 2013, Foggia University, Department of Agricultural Sciences, Food, Natural Resources and Engineering
  : 2015, Sakarya University, Faculty of Engineering, Department of Food Engineering
- **M.Sc.** : 2017, Istanbul Technical University, Chemical and Metallurgical Faculty, Food Engineering Department

**PROFESSIONAL EXPERIENCE AND REWARDS:**

- 2021-2022 Project management, Tubitak 2214/A Polysaccharide Extraction from Fermented Mushrooms: Investigation of Functional Properties and Its Use in the Development of Probiotic Encapsulation, The Scientific and Technological Research Council of Turkey (TUBITAK) with project number 1059B142100530. 15.10.2021 – 31.10.2022
- 2021-2022 Visiting Researcher, Ghent University (12 months)
- 2013-2014 Exchange Student, Foggia University (12 months)

**PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:**

- **Sumer-Ayar E.N.,** Verheust, Y., Özçelik, B., & Raes, K. (2024). Impact of lactic acid bacteria fermentation based on biotransformation of phenolic compounds and antioxidant capacity of mushrooms. *FOODS,* 13(11). https://doi.org/10.3390/foods13111616.

- **Sumer-Ayar E.N.,** Zou, Y., Özçelik, B., & Raes, K. Valorizatıon of Mushroom By-Products via Lactic Acid Fermentation: Functional and Structural Properties. Submitted.

- **Sumer-Ayar E.N.,** Verheust Y., Özçelik B., & Raes K., (2024). LC-Q-TOF-MS/MS characterisation of free and bound phenolic compounds of fermented *Lactarius deliciosus* and its potential antioxidant activities. International Food Innovation and Sustainability Congress, May 16-18 2024, Istanbul, Turkey (Oral presentation).

- **Ayar E.N.** & Raes K., (2022). Fermented mushrooms polysaccharides: investigation of functional properties and use in the probiotic encapsulation. 4th Symposium on Food Science, Masters in Food, 6 May 2022 (Oral presentation).
- **Ayar E.N.** & Özçelik B., (2020). Polyphenols as potential prebiotics. Journal of Gida Turk.
- **Ayar E.N.** & Ozcelik B., (2019). Two-way benefits of potential prebiotic polyphenols and gut microbiota. 3rd International Human Microbiota in Health and Diseases Congress, 7-9 November 2019, Istanbul, Turkey (Oral presentation).
- Altin, G., **Ayar, E.,** & Ozcelik, B. (2019). Phenolic-protein interaction: effects on functional properties of phenolics and advantages on phenolic delivery platform development. Glob. J. Med. Res. Nutr. Food Sci, 19, 1-11.

## OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- **Ayar-Sumer, E. N.,** Nyambe, C., Hashim, M. A., Altin-Yavuzarslan, G., El-Messery, T. M., & Ozçelik, B. (2024). Optimizing encapsulation of black carrot extract using complex coacervation technique: Maximizing the bioaccessibility and release kinetics in different food matrixes. *LWT*, 198, 115995.
- Yücetepe, A., Kirkin, C., Mertdinc, Z., **Ayar, E.N.,** … (2024). Chemical Composition and Nutrient Profiles of Nine Red Macroalgae Species. *Food Prod Process and Nutr* 6, 84.
- Yavuz-Düzgün, M., **Ayar, E. N.,** Şensu, E., Topkaya, C., & Özçelik, B. (2023). A comparative study on the encapsulation of black carrot extract in potato protein–pectin complexes. *Journal of Food Science and Technology*, *60*(10), 2628-2638.
- Şensu, E., **Ayar, E. N.**, Okudan, E. S., Özçelik, B., & Yücetepe, A. (2023). Characterization of Proteins Extracted from Ulva sp., Padina sp., and Laurencia sp. Macroalgae Using Green Technology: Effect of In Vitro Digestion on Antioxidant and ACE-I Inhibitory Activity. *ACS omega*, 8(51), 48689-48703.
- **Ayar E.N.,** Özçelik B., Özşahin E. and Aydın O. (2017). Bioavailability Enhancement of Lipids by Encapsulation. 5th International Conference on Food Digestion, INFOGEST, Rennes, France. (Poster presentation)
- **Ayar E.N.,** Kasapoglu K. & Ozcelik B. (2017). The use of organogel system in increasing bioavailability. Oil Science and Technology Association, 3rd Vegetable Oil Congress, İzmir, Turkey. (Poster presentation)
- **Ayar E.N.** & Özçelik B. (2016). Consumption of fresh and processed fruit and vegetables and consumer approaches in Turkey and the world. Food Metabolism & Health: Bioactive Components and Natural Additives Congress, İstanbul. (Poster presentation)
- **Ayar E.N.** & Ayar A. (2016). Evaluation of dietary fibers and flavoring substances in pudding and processing of ice cream with sensory analysis. Food Metabolism & Health: Bioactive Components and Natural Additives Congress, İstanbul, Turkey. (Poster presentation)
- Siçramaz, H., Ayar, A., & **Ayar, E. N.** (2016). The evaluation of some dietary fiber rich by-products in ice creams made from the traditional pudding–kesme muhallebi. J Food Technol, 3(2), 105-109.