REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

MINISTERE DE L'ENSEIGNEMENT SUPERIEURE ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE DE MEDEA

FACULTE DES SCIENCES

Mémoire

Presented to obtain the Master's degree In
Natural and Life Sciences

Spécialité : applied microbiology

by :Fekhar Adlane

Thème :

Valorization of biotechnological and microbial products for the improvement of baker's yeast

Soutenue publiquement le : 25 /06 /2024., Devant le jury composé de :

Hmaza Imane	**MCB**	U. Médéa	Président
Ainas Mahfoud	**MCA**	U. Médéa	Examinateur
Zergoug Amina	**MCA**	U. Médéa	Encadreur
ALI AGHA Hamza	MCA	U.Médéa	Mambre d'incubateur

REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

MINISTERE DE L'ENSEIGNEMENT SUPERIEURE ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE DE MEDEA

FACULTE DES SCIENCES

Mémoire

Presented to obtain the Master's degree In
Natural and Life Sciences

Spécialité : applied microbiology

by :Fekhar Adlane

Thème :

Valorization of biotechnological and microbial products for the improvement of baker's yeast

Soutenue publiquement le : 25 /06 /2024., Devant le jury composé de :

Hmaza Imane	**MCB**	U. Médéa	Président
Ainas Mahfoud	**MCA**	U. Médéa	Examinateur
Zergoug Amina	**MCA**	U. Médéa	Encadreur
ALI AGHA Hamza	MCA	U.Médéa	Mambre d'incubateur

Année universitaire 2023/2024

ACKNOWLEDGEMENTS

FIRST AND FOREMOST, THANKS AND PRAISE TO GOD ALMIGHTY «الله» FOR GIVING US TH ENESESRY KNOWLEDGE ,STRAINTH AND HELP IN EVREY WEY THROUGH THE ENTIRE PROJECT

First of all, i thank my supervisor Mrs. Zergoug Amina

doctor at the university of yahia fares médéa for believing in my ideas and giving me the chance to fulfill them

I thank Mr Khames Mammar, doctor at the University of Yahia Fares Médéa for the honor he gave us by agreeing to chair the jury

i also thank Mr Ainas Mahfoud, doctor at Yahia Fares Medea University

for agreeing to review this work.

A special thanks goes to mr. nabi Ibrahim and khames mammer for teaching me a lot morales and giving me different instructions that shaped my work and mind , another thanks goes to mr,latrach Soufiane /nabile touzout /boubkri kamel for offering a complete support and help at any time .

a finale thanks goes to my friends whom i studied with in this period of 5 years, wish the best of luck

Dedication :

I didicate this work to my parents whom have been such big support and a source of inspiration , a mother who didn't stope for one moment in beleving in her son and never stoped on puching him to his best and to become a man .

A father who took all of the blur out of my eyes and made the image of this world clear, showing the correct path of whom men should take

So to my parents :

سبحان الذي سخر الارزاق وجعلكما اياي احسنها وما

ايكما اتمنى اني كنت خـيـر هـا

Table des matières

Chapter 1 : Bread

1 Introduction to bread : 16
1.1 Historical background : 16
1.2 Cultural Significance : 16
1.3 Bread in Modern Cuisine : 17
2 Ingredients of Bread 17
2.1 Flour : 17
2.2 Yeast : 18
2.3 Sugar : 18
3 The Chemistry of Bread Making : 18
3.1 **Role of Gluten in Bread:** 18
3.2 The process of fermentation : 18
3.3 Maillard Reaction and Crust Formation : 20
4 Types of Bread : 20
4.1 **Leavened Bread**: 20
4.2 **Unleavened Bread**: 20
4.3 Based on wheat composition : 20
4.4 Based on Regional Varieties : 21
5 bread making process : 22
5.1 Step One: Sieving and Weighing Ingredients : 22
5.2 Step Two: Mixing the Dough 22
5.3 Step Three: Fermenting the Dough 23
5.4 Step Four: Dough Make-Up 24
5.5 Step five: Baking 25

<u>chapter 2: Yeast</u>

6 Introduction to yeast : 27
6.1 Sourdough starter in history: 27
6.2 Different names of yest in the magharibane region : 28
7 Methods of making yeast at home (starter) : 28
8 Starter types : 29
9 Compostion of a starter : 30
9.1 The source of microorganisms in starter : 31
9.2 Sourdough compostion : 31
10 Sourdough Benefits : 32
10.1 Shelf Life and Spoilage Prevention : 32

10.2 Aroma and Flavor Enhancement : 32
10.3 Texture Improvement : 33
10.4 Nutritional Value and Digestibility : 33
10.5 Gastrointestinal Health : 33
10.6 Gluten Reduction : 33
10.7 Prebiotics and Probiotics : 34
10.8 Glycaemic Index Control : 34
10.9 Antioxidant and Antihypertensive Benefits : 34
11 The evolution of modern yeast : 34
11.1 Modern yeast (Saccharomyces cerevisiae) : 34
11.2 Life cycle : 35
12 Natural Habitat and Isolation of S. cerevisiae : 36
chapter 3:materiels and methodes
13 Materiels and methodes : 39
13.1 Materiels : 39
13.2 Raw materiales selection : 39
13.3 methodes : 39
chapter 4: results and descution
14 Results and discussion 51
14.1 Step 1 :dehydration 51
14.2 Step 2 : harmful bacteria test 55
14.3 Step 3 : cultivation and dry freezing 60
15 conclusion and perspective : 63
16 Bibliographic References 65

Abreviations list

LAB : lactic acid bacteria

G : Gram

Ml : millimeter

VRBL : Violet Red Bile Agar

MRS : de Man, Rogosa, Sharpe

H : Hours

PH : Potential hydrogen.

RPM : Rotations per minute.

C : Celçus degree.

% : percentage.

CRAPC : SCIENTIFIC AND TECHNICAL RESEARCH CENTER IN PHYSICAL – CHEMICAL ANALYSIS

UFC : colony-forming units

Figures list :

Figure 1: The Life Cycle and Mating Behaviors of Saccharomyces cerevisiae(Bai et al., 2022).....36
Figure 2: cut in half and ready to dehydrate.....40
Figure 3: weight of D1 product (original ,2024).....40
Figure 4: D1 cut into quarters after first dehydration and ready to be redehydarted (original,2024)...40
Figure 6 :D1 cut and soread in a spesfic order
(original,2024).....41
Figure 5 :D1 blended and ready to be redehydrated (original,2024) 41
Figure 7 :the 342.602 g of the B product cleaned and cut ready to be bland (original,2024).....41
Figure 8 : grated B product ready for dehydration (original,2024) 42
Figure 9: the final blending step of product B (original,2024) 42
Figure 10 : G21 cut into quarters and ready to dehydrate (original,2024).....43
Figure 11: G21 sample blended into small pieces and ready to be dehydrated (original,2024) 43
Figure 12: weighting approx 300g of the G21 (original,2024).....44
Figure 13 : approx 300g of boiled G21 (original,2024).....44
Figure 14: preparation of MRS agare medium (original,2024).....45
Figure 15: poring 15 ml of medium in petrie plates and conserving at 4°C after autoclaving at 121°C for 15min
(original,2024).....46
Figure 16 : cultivating isolated colonie in broth MRS at 37°C for 24h (original,2024).....46
Figure 17 :a representation of condition of water in different pressure condition and temperature. ..47
Figure 18: chamber 2 cover (original,2024).....47
Figure 19: chamber 2 cover (original,2024 47
Figure 20: chamber 2 cover (original,2024 47
Figure 21 :chamber 1 cover (original,2024).....48
Figure 22 :chamber 2 at the right temperature (original,2024).....48
Figure 23: chamber 1 at the right temperature (original,2024).....48
Figure 24: testing the product at a locale bakery (original,2024) 49
Figure 25 :the finale product of dehydration D1 (method 2) (original,2024).....51
Figure 26: the finale product of dehydration of product B (method 2) (original,2024) 52
Figure 27 : G21 after being treated with method 1 (original,2024) 54
Figure 28: G21 after being treated with method 2 and 3 (original,2024) 54
Figure 29 : G21 after being treated with method 4 (original,2024) 55
Figure 30:results of different concentration of product B in VRBL medium after 48H incubation at 30°C (original,2024)
.....55
Figure 31: results of different concentration of product B in VRBL medium after 48H incubation at 44°C (original,2024)
.....56
Figure 32: results of different concentration of product G21 in VRBL medium after 24H incubation at 30°C (original,2024)
.....57
Figure 33: results of different concentration of product G21 in VRBL medium after 24H incubation at 44°C (original,2024)
.....58
Figure 34: results of different concentration of product D1 in VRBL medium after 24H incubation at 44°C (original,2024)
.....59
Figure 35 : lactic acide bacteria isolated in MRS medium (original,2024).....60
Figure 36 : cultivated and washed LAB preserved at -40°C (original,2024).....60
Figure 37 :lactic acid bacteria after freeze drying process (original,2024).....61

tables list

Table 1: comparison between different methods used to make D1 product according on 3 different criteria 51
Table 2: comparison between different methods used to make product B powder according on 3 different criteria 52
Table 3 : comparison between different methodes used to make G21 powder useing 3 different criteria 53

Abstract

Bread, a staple food throughout history, is known for its taste, texture, smell, and health benefits. Bread-making has evolved through three major eras. The first era produced flatbreads with good taste and health benefits but minimal aroma. The second era introduced fermented loaf bread, notable for its strong texture, rich aroma, delicious taste, and health benefits from rich microflora. The third era, starting with the Industrial Revolution, brought diverse bread types, many detrimental to health and lacking in taste, texture, and aroma.

This study aims to optimize the quality of bread by incorporating various natural products into industrial dry yeast and combining it with a kind of beneficial bacteria named lactic acid bacteria. The objective of this study is to enhance the taste, texture, smell, and health benefits of bread in the modern era, counteracting the negative effects often associated with industrial bread production.

The research involves the dehydration of various natural products to create powders using different methods to ensure quality and efficiency, which was successfully achieved through creating products D1/B/G21, resulting in a great replacement for sugar in bread. Additionally, the isolation of lactic acid bacteria and their culture on MRS broth medium was performed, along with building a custom freeze dryer to apply the freeze-drying process for the cultivated bacteria, wish was also achieved. The final step involves mixing the dehydrated natural product powders and freeze-dried lactic acid bacteria with industrial dry yeast to create a superior bread-making product.

The primary goal of our study was to create a high-quality natural product that improves bread quality while avoiding the negative aspects of current commercial bread. This objective was successfully achieved.

Key words : Yeast-bread-lactic acid bacteria-dehydration-cultivation

Résumé

Le pain, un aliment de base tout au long de l'histoire, est connu pour son goût, sa texture, son odeur et ses bienfaits pour la santé. La fabrication du pain a évolué à travers trois grandes époques. La première époque a produit des pains plats avec un bon goût et des bienfaits pour la santé, mais une arôme minimale. La deuxième époque a introduit le pain fermenté en miche, remarquable pour sa texture solide, son arôme riche, son goût délicieux et ses bienfaits pour la santé grâce à une microflore abondante. La troisième époque, débutant avec la Révolution industrielle, a vu l'apparition de divers types de pain, dont beaucoup sont néfastes pour la santé et manquent de goût, de texture et d'arôme.

La présente étude vise à optimiser la qualité du pain en incorporant divers produits naturels dans la levure sèche industrielle et en la combinant avec des bactéries bénéfiques, à savoir les bactéries lactiques. L'objectif de cette étude est donc d'apporter une amélioration au niveau du goût, de la texture, de l'arôme et des bienfaits pour la santé du pain à l'ère moderne, en contrant les effets négatifs souvent associés à la production industrielle de pain.

La démarche entreprise lors de cette recherche implique de déshydrater divers produits naturels afin de créer des poudres en utilisant différentes méthodes pour assurer la qualité et l'efficacité, ce qui a été réussi grâce à la création des produits D1/B/G21, aboutissant à un excellent substitut pour le sucre dans le pain. De plus, l'isolement des bactéries lactiques et leur culture sur milieu MRS, puis l'élaboration d'un lyophilisateur afin d'obtenir des bactéries cultivées lyophilisées, le souhait a également été réalisé. L'étape finale consiste à mélanger les poudres de produits naturels déshydratés et les bactéries lactiques lyophilisées avec de la levure sèche industrielle afin de créer un produit de panification de qualité supérieure.

L'objectif principal de notre étude était de créer un produit naturel de haute qualité qui améliore la qualité du pain tout en évitant les aspects négatifs du pain commercial actuel. Cet objectif a été atteint avec succès.

Mots-clés : levure – pain - bactéries lactiques – déshydratation - cultivation

ملخص

يعتبر الخبز، غذاء أساسي عبر التاريخ، معروف بمذاقه، قوامه، رائحته، وفوائده الصحية. تطورت صناعة الخبز عبر ثلاث حقب رئيسية. الحقبة الأولى أنتجت الخبز المسطح بمذاق جيد وفوائد صحية ولكن برائحة قليلة. الحقبة الثانية تميزت بالخبز المخمر على شكل أرغفة، المشهور بقوامه القوي، رائحته الغنية، مذاقه اللذيذ، وفوائده الصحية الناتجة عن تعدد الكائنات الدقيقة. الحقبة الثالثة، التي بدأت مع الثورة الصناعية، شهدت ظهور أنواع متنوعة من الخبز، الكثير منها ضار بالصحة ويفتقر إلى المذاق، القوام، والرائحة.

تهدف هذه الدراسة إلى تحسين جودة الخبز عن طريق دمج منتجات طبيعية مختلفة مع الخميرة الجافة الصناعية ودمجها مع بكتيريا حمض لكتيك .

تشمل العمليات تجفيف مختلف منتجات طبيعية لإنشاء مساحيق باستخدام طرق مختلفة لضمان الجودة والكفاءة وقد تم تحقيق هذا الهدف بنجاح من خلال إنشاء المنتجات (د1/ب/ج21) مما أسفر عن بديل ممتاز للسكر في الخبز

وعزل بكتريا حمض لكتيك وزراعتها في وسط مخصص لها لتكاثرها .وبناء جهاز تجميد لتطبيق عملية التجفيف بالتجميد للبكتيريا المزروعة , وقد تم الوصول لهذه الرغبة أيضا.

تتضمن الخطوة النهائية مزج مساحيق المنتجات الطبيعية المجففة وبكتيريا حمض لكتيك المجففة بالتجميد مع الخميرة الجافة الصناعية لخلق منتج خبز متفوق.

تهدف هذه الدراسة إلى تعزيز الطعم ونسيج الخبز مع رائحة زكية وتعزيز الفوائد الصحية للخبز في العصر الحديث، مواجهة لتأثيرات السلبية المرتبطة غالباً بإنتاج الخبز الصناعي.

كان الهدف الرئيسي من دراستنا هو إنشاء منتج طبيعي عالي الجودة يحسن جودة الخبز مع تجنب الجوانب السلبية للخبز التجاري الحالي. وقد تم تحقيق هذا الهدف بنجاح.

الكلمات المفتاحية: خميرة - الخبز- بكتيريا حمض لكتيك -التجفيف - الاستنبات

Introduction

Introduction

Bread has historically been a staple in global diets due to its significant nutritional value, providing essential nutrients, energy, and dietary balance. Traditional bread, crafted from whole grains and natural ingredients, was rich in vitamins, minerals, fiber, and protein (Lau et al., 2021)However, in modern times, the nutritional content of bread has markedly declined due to the industrialization of production processes. Many commercially available breads now utilize refined flours, added sugars, preservatives, and artificial additives, which diminish the bread's essential nutrient content. This shift has resulted in bread that often lacks the fiber, vitamins, and minerals found in traditional varieties, potentially compromising its health benefits and, in some cases, causing harm (Lau et al., 2021)

One of the critical alterations has been the replacement of traditional starters (natural fermenting agents containing a diverse community of microorganisms) with isolated yeast strains (*Saccharomyces cerevisiae*). This change overlooks the beneficial contributions of lactic acid bacteria and other microorganisms present in traditional starters, further reducing the nutritional richness of modern bread (Lau et al., 2021).

In response to these challenges, our study aims to restore the nutritional value of bread. We propose a novel approach by combining various strains of lactic acid bacteria and incorporating natural products into the fermentation process. These additions are intended to provide essential nutrients that support the growth and activity of diverse microbial communities during bread fermentation. By creating an optimal environment for fermentation, we seek to enhance the nutritional profile of bread, revitalizing its original integrity as a nourishing staple in the human diet.

This manuscript is divided into three chapters:

- The first chapter consists of a bibliographical review that covers the general state of knowledge about bread. It explores its history with humans and its development over time, highlighting its various forms and variations. The chapter then narrows its focus to yeast, detailing its impact on bread throughout history.
- The second chapter will concern the experimental and technical part.
- The third is essentially devoted to the presentation of the results obtained from our research and investigations.

A discussion concerning the obtained results is proposed with analysis of the results having been recorded during this work, as well as a conclusion and perspectives.

Chapter 1
Bread

1 Introduction to bread :

1.1 Historical background :

Bread, often considered one of the oldest processed foods, has a rich and extensive history dating back to prehistoric times. Early forms of bread were quite different from the loaves we recognize today, with the earliest evidence of bread-like foodstuffs dating back to around 14,000 years ago. This is significantly earlier than the advent of Neolithic agriculture, as demonstrated by the charred remains discovered at Shubayqa 1 in northeastern Jordan, which were produced by Natufian hunter-gatherers (Arranz-Otaegui et al., 2018). These early forms of bread were likely simple, flat, and unleavened, made from wild grass seeds that were crushed, mixed with water, and baked on hot stones. The transition to more complex bread-making techniques coincided with the rise of agriculture in the Neolithic period, particularly in Europe and Southwest Asia, where fully fledged agricultural societies began cultivating wheat and other grains. Over millennia, these early bread-making practices evolved with the understanding of fermentation and the development of gluten proteins in wheat, eventually leading to the sophisticated bread varieties crafted by bakers around the world today. The continuous experimentation and refinement by bakers, combined with scientific advancements, have resulted in the diverse and high-quality bread products we enjoy in the modern era. **(Cauvain and Young 2007).**

1.2 Cultural Significance :

Bread holds immense cultural significance worldwide, serving not only as a staple food but also as a symbol of tradition, unity, and identity.

In France, bread, particularly the baguette, is a symbol of national pride and an essential part of daily life and cuisine. The act of buying a fresh baguette every morning is a common routine for many French people, showcasing bread's integral role in French culture

In Mexico, bread is woven into cultural and religious traditions. For example, "pan de muerto" (bread of the dead) is baked for Day of the Dead celebrations. This bread is often elaborately decorated and symbolizes the connection between the living and the deceased, serving as an offering to ancestors .**(Luminaire, 2023)**

In Ethiopia and Eritrea, injera, a spongy flatbread made from teff flour, is central to the diet and is used as both a plate and utensil. This bread is integral to communal eating practices, highlighting the importance of sharing meals in these cultures.**(Luminaire, 2023)**

Historically, bread has also symbolized sustenance and resilience. For instance, Irish soda bread, which uses baking soda instead of yeast, became a staple during times of economic hardship, reflecting the resourcefulness of the Irish people **(Luminaire, 2023)**

The cultural significance of bread extends to its ability to foster unity and community. The act of breaking bread together is a universal symbol of hospitality and friendship, creating moments of connection and shared humanity **(Luminaire, 2023)**

Overall, bread's cultural importance is reflected in its diverse forms and uses across the globe, representing everything from daily sustenance and culinary tradition to religious ritual and social unity **(Luminaire, 2023)**

1.3 Bread in Modern Cuisine :

In modern cuisine, the widespread use of commercial dry yeast in bread production, as opposed to traditional starters, has several negative implications. Scientific studies indicate that bread made with commercial yeast lacks the complex microbial diversity found in traditional sourdough starters, which can lead to reduced nutritional benefits, such as lower levels of beneficial vitamins, minerals, and antioxidants. This simplified fermentation process can also result in increased gastrointestinal problems for some individuals, including bloating and discomfort, due to the rapid fermentation and lack of pre-digestion of gluten and other proteins. Furthermore, the reliance on commercial yeast has diminished the unique flavors and textures that traditional methods offer, impacting the sensory quality of bread. These changes not only affect the healthiness and quality of bread but also reflect broader issues in contemporary dietary habits, where convenience often overrides nutritional value and culinary heritage. This shift has broader sustainability implications, as traditional methods often utilize locally sourced and diverse grains, promoting ecological balance and food biodiversity

2 Ingredients of Bread

2.1 Flour :

Flour is the foundational ingredient in bread making, providing the structure and bulk of the dough. Traditionally, whole grain flours were used, which include the bran, germ, and endosperm of the grain, offering a rich source of fiber, vitamins, and minerals. The traditional stone milling process preserved these nutrients by grinding the whole grain slowly and at a cooler temperature. In contrast, modern refined flours, produced through roller milling, strip away the bran and germ, leaving only the starchy endosperm. This process results in a finer texture but significantly reduces the nutritional content of the flour, leading to what is often termed "empty calories." The shift from whole grain to refined flours has had considerable nutritional implications. Whole grains, as highlighted by **(Slavin, 2004)** ,provide essential nutrients and have been associated with a lower risk of chronic diseases such as heart disease and diabetes. **(Seal and Brownlee, 2015)**further emphasize that whole-grain foods contribute to better overall health and are crucial in preventing chronic conditions. Thus, the transition from

traditional whole grain flours to modern refined flours represents a significant shift in both the nutritional quality of bread and its health implications.

2.2 Yeast :

Yeast plays a pivotal role in leavening bread by fermenting sugars present in the dough, producing carbon dioxide gas that causes the dough to rise. This process creates air pockets, contributing to the bread's light and airy texture. Traditional sourdough starters harness wild yeast strains alongside lactic acid bacteria, which impart distinct flavors and textures to bread due to their metabolic activities. **(De Vuyst and Neysens, 2005)**emphasize the biodiversity and metabolic interactions within sourdough microflora, highlighting how these organisms influence bread's final taste profile. In contrast, commercial dry yeast consists of a single strain of Saccharomyces cerevisiae, chosen for its predictable and rapid fermentation capabilities. **(Arendt et al., 2007)**, note that sourdough fermentation also enhances bread texture by improving dough extensibility and crumb structure. Flavor-wise, sourdough bread tends to be tangier and more complex due to the production of acids during fermentation, while commercial yeast yields a milder taste. Nutritionally, sourdough fermentation breaks down gluten and phytates, potentially increasing mineral bioavailability compared to bread made with commercial yeast. Thus, the choice between sourdough and commercial yeast impacts not only flavor and texture but also the nutritional composition of the bread.

2.3 Sugar :

Sugar plays a crucial role in bread by contributing to the Maillard reaction, which enhances flavor and browning. Additionally, it aids in moisture retention, extending shelf life, and helps in feeding yeast for better fermentation **("The Science of Sugar & How it Plays a Vital Role in Baking | Imperial Sugar," 2016).** However, the health implications of excessive sugar consumption cannot be overlooked. Excessive sugar consumption is associated with an increased risk of type 2 diabetes, as it can lead to elevated blood glucose levels and insulin resistance **(Lau et al., 2021)**. In many bakeries in our country, excessive amounts of sugar are added to bread to enhance sweetness, accelerate yeast activity, and reduce rising time. Unfortunately, such practices are often undertaken without adequate regulatory oversight. This excessive sugar intake can lead to elevated blood glucose levels and insulin resistance, increasing the risk of type 2 diabetes among frequent consumers of these sugary breads.

3 The Chemistry of Bread Making :

3.1 Role of Gluten in Bread:

- Composition and Structure: Gluten is composed primarily of two protein fractions, gliadins and glutenins, which play a critical role in bread making. Gliadins contribute to the dough's viscosity and extensibility, while glutenins provide strength and elasticity (Ye et al., 2023)

- Dough Formation: During dough mixing, gluten proteins absorb water and form a network. This gluten network is essential for trapping the carbon dioxide gas produced by yeast during fermentation, which helps the dough to rise and develop a light, airy structure.

- Baking Performance: The viscoelastic properties of gluten are key to the dough's ability to stretch and expand without breaking. This elasticity allows the dough to expand during proofing and baking, resulting in bread with good volume and a desirable crumb texture (“Bread Rises And Falls With Gluten,” 2015)

- Quality Attributes: The quality of the gluten network directly impacts the texture, volume, and overall quality of the bread. Strong gluten networks lead to high-quality bread with good volume, fine crumb structure, and resilience. Weak gluten networks can result in dense, poorly risen bread (Ye et al., 2023)

- Nutritional and Functional Aspects: Beyond its functional properties in baking, gluten also contributes to the nutritional value of bread. It is a significant source of protein in wheat-based diets. The balance between gliadins and glutenins, as well as their interaction with other wheat components, influences the nutritional and functional properties of the final bread product (Plessas, 2021)("Gluten," 2017).

3.2 The process of fermentation :

The fermentation process in bread making is a critical stage that transforms simple dough into a flavorful and nutritious staple. Initially, yeast and lactic acid bacteria initiate fermentation by metabolizing sugars present in the dough, producing carbon dioxide and ethanol. This gas is trapped within the dough’s gluten network, causing it to rise and develop its characteristic airy structure. As a result, the bread gains its light texture and soft crumb. Simultaneously, the metabolic activities of yeast and bacteria yield a spectrum of organic acids and volatile compounds, enhancing the bread’s aroma and taste complexity **(Plessas, 2021)**

Moreover, as fermentation progresses, the dough undergoes acidification, creating a hostile environment for spoilage organisms and contributing to natural preservation **(Plessas, 2021)**. Nutritionally, fermentation breaks down complex nutrients, increases mineral bioavailability, and generates beneficial compounds like peptides and amino acids **(Borsuk et al., 2021)**. As a result, the bread not only delights the senses but also offers improved digestibility and enhanced nutritional benefits, making it a wholesome choice for consumers seeking both flavor and health benefits.

3.3 Maillard Reaction and Crust Formation :

The Maillard reaction is a complex chemical reaction that occurs between amino acids and reducing sugars when exposed to heat. This reaction is responsible for the browning of foods, such as bread crusts, and contributes significantly to their flavor and aroma profile. It begins with the condensation of a reducing sugar, such as glucose or fructose, with an amino group from an amino acid or protein. This initial step forms a Schiff base, which undergoes rearrangement and dehydration to form Amadori products. Further reactions involving dehydration, cyclization, and fragmentation lead to the formation of a wide range of compounds known as melanoidins. These melanoidins are responsible for the characteristic brown color of baked goods and contribute to their desirable flavor attributes. The Maillard reaction is not only important for sensory properties but also impacts the nutritional quality and functional characteristics of foods. However, it can also lead to the formation of potentially harmful compounds like acrylamide in certain conditions, highlighting the dual role of this reaction in food chemistry.**("Chemistry of Food Browning," n.d.)**

4 Types of Bread :

We can classify bread into leavened and unleavened, wheat composition, and regional varieties:

4.1 Leavened Bread:

This type of bread includes the use of leavening agents such as yeast, baking soda, or baking powder to cause the dough to rise, resulting in a light and airy texture. Yeast-leavened bread, for instance, relies on the fermentation of sugars by yeast to produce carbon dioxide gas, which makes the dough rise.

4.2 Unleavened Bread:

These breads do not contain leavening agents and rely on other means, such as steam or baking, to achieve their texture. Common examples include flatbreads like tortillas, matzah, and pita.

4.3 Based on wheat composition :

White Bread: Made from refined flour, which has been stripped of the bran and germ, leaving mainly the starchy endosperm, this bread is lighter in texture and flavor but lower in nutrients compared to whole wheat bread. .**(Bond, 2023)**

Whole Wheat Bread: Made using flour that contains all parts of the wheat kernel, including the bran, germ, and endosperm, this type of bread is higher in fiber, vitamins, and minerals compared to refined flour breads.**(Bond, 2023)**

Rye Bread: Made using rye flour, either entirely or mixed with wheat flour, rye bread tends to be denser and darker with a slightly sour taste, often associated with European varieties such as pumpernickel.**("Types of Bread," n.d.)**

Multigrain Bread: Made using two or more types of grain flours, such as wheat, barley, oats, and millet. This type of bread often contains whole grains and seeds, offering a variety of textures and enhanced nutritional benefits.**(Bond, 2023)**

Gluten-Free Bread: Made using alternative flours such as rice, almond, or tapioca, designed for those with gluten intolerance or celiac disease. These breads often include additional ingredients like xanthan gum or psyllium husk to mimic the texture of gluten.**(Bond, 2023)**

4.4 Based on Regional Varieties :

Sourdough: Originating from ancient Egypt but now popular worldwide, sourdough bread uses naturally occurring lactobacilli and yeast to ferment the dough. This results in a tangy flavor and chewy texture.**(Bond, 2023)**

Focaccia: An Italian flatbread similar to pizza dough, typically seasoned with olive oil, salt, and sometimes herbs. It is known for its rich flavor and soft, airy texture.**(Bond, 2023)**

Baguettes: A type of French bread that is long and cylindrical, made from flour, water, yeast, and salt, and baked in steam ovens, which gives it a thin and crispy crust and a pillowy interior. Baguettes originated in France, and their legacy has had such an impression on the world that UNESCO granted them World Heritage Status in 2022.**("Types of Bread," n.d.)**

Naan: A type of leavened flatbread that is soft, chewy, and textured, made from flour, water, sugar, yeast, yogurt, vegetable oil, and salt. Naan originated in many parts of Western Asia, South Asia, Indonesia, and Malaysia. It is baked in a tandoor, a hot clay oven that can reach temperatures of 900 degrees Fahrenheit. Naan is usually served alongside West Asian and South Asian dishes such as regional curries.**("Types of Bread," n.d.)**

chapati: A type of Indian flatbread that is made from wheat flour and toasted over a griddle until freckled gold. Chapati can be eaten as is or with cooked lentil soup, vegetable dishes like Indian curry, or even used to make sandwich wraps. It is usually made from whole wheat flour and cooked without oil, making it a healthier choice than other flatbreads like naan or parathas. Sometimes chapatis come buttered, and in such cases, it's better to use an unsaturated spread rather than butter or ghee.**("An A-Z of breads from around the world," n.d.)**

5 bread making process :

Over time, the global market has become inundated with a vast array of bread varieties, each crafted through various bread-making processes. Despite the diversity, most methods share the common goal of transforming raw flour into a leavened food product primarily through fermentation. The standard process for making pan or loaf-type bread involves several key sequential operations..**(Garcia-Vaquero et al., 2023)**

5.1 Step One: Sieving and Weighing Ingredients :

The first step in the bread-making process involves both sieving and weighing the ingredients. Sieving the flour and other dry ingredients aims to eliminate any extraneous matter and coarse particles, making the flour more homogeneous and free of lumps by partially incorporating air. After sieving, the ingredients are weighed according to the formulated recipe, ensuring the appropriate ratios are used. This formulation varies from bakery to bakery, influenced by factors such as ingredient costs and consumer preferences regarding bread quality. Ingredients like salt, sugar, and flour improvers (such as oxidizing agents) are typically added in solution form, while yeast is incorporated as a suspension. To optimize the dough mixing process and reduce mixing time, salt is usually added after. This combined step ensures that all ingredients are properly prepared and measured, setting the foundation for the subsequent stages of bread-making .**(Garcia-Vaquero et al., 2023)**

5.2 Step Two: Mixing the Dough

The mixing process aims to uniformly distribute all the ingredients and develop gluten in the dough, ensuring it has optimal physical characteristics such as elasticity, resistance to deformation, extensibility, viscosity, and stickiness. These factors influence the pore structure of the bread. As the ingredients are mixed with water, hydration occurs at the surface of the flour particles due to the mechanical energy input from progressive mixing. This combined action of hydration and continued mixing leads to the gradual formation of the gluten network, which depends on the applied shear and elongation forces during the process.**(Therdthai and Zhou, 2014) ,(Garcia-Vaquero et al., 2023)**

Conventional mechanical mixers deliver combined deformation flows through blending, stretching, combining, pushing, compressing, and folding the dough **(Therdthai and Zhou 2014**). At a chemical level, mixing allows the polymerization and cross-linking of proteins present in the wheat flour (glutenin and gliadin), generating a 3D gluten network. As mixing continues, the dough becomes more cohesive, forming a viscoelastic mass with elastic and viscous properties attributed to glutenin and gliadin, respectively. **(Xu et al., 2007),(Garcia-Vaquero et al., 2023)**

However, excessive shear and mechanical energy from prolonged mixing can break down the stable molecular interactions between gluten-forming proteins, depolymerize large gluten aggregates, and disrupt the dough network. Conversely, under-mixing may leave small unmixed patches that interfere during the proofing stage. Mixing also incorporates air, essential for oxidation and yeast activity, and generates gas bubble nuclei for the carbon dioxide released by yeast fermentation. The gluten network helps retain this carbon dioxide during baking.**(Garcia-Vaquero et al., 2023; Rosell, 2011)**

Industrial mixing can be performed using various equipment that mimics hand-mixing actions like compressing and stretching (kneading) at high speed, reducing the time to a few minutes Common commercial mixers include low-speed, spiral mixers (allowing both fast and slow speed mixing), and high-speed mixers (planetary, horizontal, and continuous type mixers).**(Garcia-Vaquero et al., 2023)**

5.3 Step Three: Fermenting the Dough

The mixed dough should be fermented for an appropriate length of time to produce a light, aerated, and porous loaf of bread. Fermentation is achieved by adding yeast cultures (Saccharomyces cerevisiae) or other cultures, such as lactic acid bacteria (LAB) in the case of sourdough, at an appropriate ratio and mixing them into the dough. Yeast plays a crucial role as a leavening agent, where the enzymes in the flour and yeast break down starch into sugars. These sugars are then fermented by the yeast primarily into CO2 and alcohol, along with small amounts of glycerol and various compounds (alcohols, esters, aldehydes, and organic acids) that contribute to the flavor and aroma of fermented bread dough. The CO2 gas bubbles produced during fermentation leaven the dough into a foam structure, which, when baked, generates the aerated structure of bread crumb.**(Garcia-Vaquero et al., 2023; Struyf et al., 2017).**

The leavening process relies on sufficient gas production, which is influenced by the availability of soluble, fermentable sugars, and on the retention of that gas in the dough, which depends mainly on the properties of the dough. The fermentation process duration can vary from 2 to 6 hours or even longer, depending on the concentration of yeast added, baking method, and type of flour used. Temperature and relative humidity conditions are critical for yeast activity, with optimal temperatures ranging from 30–35 °C and relative humidity of 85% or higher. Yeast performance decreases above 40 °C. The optimum pH range for yeast activity is 4–6; below pH 4, yeast activity diminishes and is inactivated below pH 3.**(Bhatia, 2016; Garcia-Vaquero et al., 2023)**

During fermentation, multiple and interlinked chemical and physical changes occur:

Hydrolysis of starch to simpler sugars: The enzyme diastase in wheat flour begins to break down starch into maltose. Maltase converts maltose into glucose, invertase transforms any added sucrose into glucose and fructose, and the zymase complex converts glucose and fructose into carbon dioxide gas, alcohol, and other flavor and

aroma compounds. CO2 release is accompanied by the growth of air bubbles incorporated during mixing, and most of the alcohol evaporates during baking.**(Garcia-Vaquero et al., 2023)**

Acid production by lactic acid bacteria: LAB produces acids from sugars, lowering the dough's pH from 5.5 to 4.7, as observed in sourdough .**(Garcia-Vaquero et al., 2023)**

Activity of proteolytic enzymes: Proteolytic enzymes from flour and yeast increase the extensibility and elasticity of gluten, aiding in gas retention as the gluten network expands without rupturing .**(Garcia-Vaquero et al., 2023)**

5.4 Step Four: Dough Make-Up

The dough make-up stage transforms the fermented bulk dough into appropriately molded portions that, after final proofing, yield bread with desirable properties. This stage involves several operations:

Dividing (Scaling): The bulk dough is divided into uniform individual pieces of predetermined weight and size. Dividing should be done quickly to ensure uniform weight and prevent any changes in dough density due to CO2 production. If there is a delay, corrective steps like degassing the dough may be necessary. Various methods like piston dividing and extrusion dividing are used, with some dividers coupled with pressure compensators to prevent dough bubble structure breakdown. Different dough types require specific dividers to optimize accuracy and minimize compression damage.**(Garcia-Vaquero et al., 2023)**

Rounding : After dividing, the dough pieces are irregular in shape with sticky cut surfaces through which gas can easily diffuse. Rounding shapes the dough into balls and creates a thick, continuous surface skin to retain the gas and ease handling. Traditional hand rounding involves kneading with a rotary motion to produce a ball-shaped piece. Industrial equipment for high-speed rounding includes conical rounders, belt rounders, and cylindrical rounders, which use various methods to shape the dough into spherical loaves.**(Garcia-Vaquero et al., 2023)**

Intermediate Proofing : Dividing and rounding can reduce the dough's pliability, elasticity, and extensibility, making it prone to tearing. Intermediate proofing allows the dough to rest and recover from the strains of previous operations, restoring its properties through continued fermentation and gluten relaxation. This short rest period, typically 2–20 minutes, is critical for achieving the desired final bread cell structure. Optimal proofing conditions include temperatures of 26.7–29.4 °C and a relative humidity of 75%. Commercial equipment like pocket-type and conveyor provers are used for large-scale production to ensure proper intermediate proofing.**(Garcia-Vaquero et al., 2023)**

Moulding : Moulding is the final step of dough make-up, shaping the dough pieces according to the bread variety being produced and making them ready for baking. This process includes:

Sheeting: The dough is passed through rollers to flatten it, reducing its thickness and increasing its surface area. Sheeting helps subdivide large gas bubbles, improving bubble distribution and yielding a fine breadcrumb structure.

Curling: The sheeted dough is rolled into a cylindrical form using a belt conveyor and a flexible mesh chain. The rolling operation creates a tight curl, preventing air entrapment and eliminating gas pockets.

Final Moulding: The dough pieces are shaped into their final loaf-type or cylindrical shape by exerting downward and outward pressure. This de-gassing process results in uniform, tight, and sealed cylinders. Modern high-speed moulders use variable speed drives to accommodate different production rates.

Final Proofing/Proving : During final proofing, the degassed dough continues to ferment in the baking pan, achieving the desired dough height. This period allows for flavor development and increased shelf life through organic acid formation. Final proofing is typically performed at 30–35 °C and 85% relative humidity, with time and conditions adjusted based on factors like flour strength, dough formulation, and the desired product type.**(Garcia-Vaquero et al., 2023)**

5.5 Step five: Baking

Before the final step, baking, the dough can undergo certain pre-treatments, such as glazing and scoring. Glazing involves applying a surface coating to enhance the quality of bakery products by increasing shine or modifying the crust's color. Glazes applied before baking typically consist of whole eggs, skim milk, shortening, and may include various sugars, gums, and starches. Scoring, the process of making cuts on the dough's surface, allows carbon dioxide to escape during oven spring, leading to greater expansion and increased loaf volume. The choice of technique depends on the dough type, bread type, and desired final appearance.**(Garcia-Vaquero et al., 2023)**

Chapter 2
Yeast

6 Introduction to yeast :

Developing aroma, texture, and other aspects of bread requires manipulating at least one of the essential products in the bread-making process. In our case, this involves yeast, which plays a crucial role in fermenting sugars in the flour into small amounts of ethanol and, most importantly, carbon dioxide gas (CO2) that causes the dough to rise during baking. The use of yeast in bread production dates back more than 14,000 years. Throughout this period, we find different names for it originating from various civilizations and times. It is essential to keep in mind that the yeast that existed in ancient times did not have the same composition as the yeast existing today. Modern yeast is a pure strain of one specific microorganism, while ancient yeast was a mix of lactic acid bacteria and yeast, each having its own role in the bread-making process.**(Bai et al., 2022)**

Later, with the development and changes in human needs and various studies in microscopy, microbiology, enzymology, and biochemistry performed by pioneering scientists, including Antonie van Leeuwenhoek, Joseph Gay-Lussac, and Louis Pasteur, the role of Saccharomyces cerevisiae as an agent of fermentation was discovered. The isolation of pure yeast cultures in 1888 by Emil Hansen, who perfected Louis Pasteur's method, paved the way for the extensive use of S. cerevisiae in biological research, **(Bai et al., 2022)**. When this research crossed paths with the Industrial Revolution, it gave birth to the industrial baker's yeast we find today. This innovation responded to human needs and necessities for speed and quantities, with the great cost of losing taste, health benefits, aroma, and texture.

6.1 Sourdough starter in history:

Egypt: Evidence of yeast existence in ancient Egypt is substantiated through molecular archaeological analyses of residues found in early wine jars, specifically dated to 3150 B.C. These jars, discovered in the tomb of Scorpion I at Abydos, contained solid material presumed to include dead yeast cells and cellular debris, indicating the use of yeast in winemaking. DNA extractions and amplifications from the lees of these jars confirmed the presence of yeast DNA, with a significant quantity recovered due to the excellent preservation conditions in the tomb. These findings, supported by studies conducted by Cavalieri and colleagues, reveal that yeast was an integral part of ancient Egyptian wine production, contributing to the biocultural evolution of early societies by enabling the fermentation process necessary for creating alcoholic beverages.**(Cavalieri et al., 2003)**

Iran: The existence of yeast in ancient Iran is substantiated by the archaeological discovery of early winemaking practices in the region, particularly at the site of Hajji Firuz Tepe in the northern Zagros Mountains. Chemical analyses of residue found in pottery jars from this site, dating back to around 5400 B.C., revealed biomarkers consistent with fermented grape products, indicative of the use of Saccharomyces cerevisiae, the yeast responsible for alcohol fermentation. This evidence highlights the advanced state of viticulture and winemaking in ancient Iran, where yeast played a crucial role in transforming grape juice into wine. The historical and molecular analysis of grapevine cultivars, using techniques such as microsatellite markers, further underscores the sophisticated

agricultural practices in ancient Iran, reinforcing the significance of yeast in the region's early biotechnological developments.**(McGovern et al., 1996)**

China: China, like other ancient civilizations, has its share of evidence regarding the existence of yeast in its history, primarily through ancient beverages found in sealed bronze vessels from the Shang and Western Zhou Dynasties of the second millennium BCE. These findings shed light on the significance of fermented beverages in ancient Chinese society, revealing their importance in social, religious, and medical contexts. Further evidence comes from chemical analyses of ancient organics absorbed into pottery jars from the early Neolithic village of Jiahu in Henan province, China. These analyses revealed that a mixed fermented beverage made from rice, honey, and fruit (such as hawthorn fruit and/or grape) was being produced as early as 7000 BC.**(McGovern et al., 2004)**

Mesopotamia : One of the first regions to develop agricultural practices, including the cultivation of grains and the production of fermented beverages like beer, which explains the rich history of evidence about yeast existence, from texts such as the Sumerian Hymn to Ninkasi (the goddess of beer), detailing the brewing process that inherently involves yeast as a fermenting agent, to chemical analyses of residues found in pottery vessels from sites like Godin Tepe, dated to around 3500-3100 BCE**.(McGovern et al., 2004)**

- With all this evidence from different eras and regions throughout history, our purpose is to paint a small picture of the product we are about to manipulate. Yet, we still need one piece of information: yeast history in our region, the Maghreb region. Sadly, we do not find much research conducted in this context. However, with a little effort of collecting information from a few people aged 70 and older, we find out that yeast and the fermentation process for bread and other beverages have actually existed until very recently. The Algerian and Maghrebi population stopped making yeast at home as a result of industrial yeast being imported to the market. Yet, we still find that it has been called by different names and has different ways of making it.

6.2 Different names of yest in the magharibane region :

The different names and methods for the yeast in this region returns to the different accsec to materiales used in making the yeast ,according to dr,saoudi karima specialist in nutrition the west algeriane call the home made yeast (الحامضة) whish translate to (the acidique) as a reffrance for the aciditi of the product in the center of the country we find the name (خميرة الدار) (home yeast) and one of the most commen names we find in all the maghariban cuntrys is (الخميرة البلدية) yet there is a lot of lost names that we don’t know that could be expalind by the existance many different tribes through this region .

7 Methods of making yeast at home (starter) :

Starter is a fermented mixture of flour and water, containing a complex community of microorganisms, including lactic acid bacteria (LAB) and yeasts. From a microbiological standpoint, starter presents a challenging

environment for these microorganisms, whose metabolic activities result in dough acidification, leavening, and the production of aroma and flavor compounds. As a leavening agent in bread making, starter produces bread that is more aromatic, has better texture and flavor, boasts an extended shelf life, and offers nutritional benefits by providing essential nutrients such as amino acids, vitamins, minerals, and dietary fiber.(Hernández-Parada et al., 2022)

Making a starter begins with grinding cereals, pseudo-cereals, or legumes, and then adding water to form dough, which eventually becomes starter over time. A symbiotic colony of lactic acid bacteria (LAB) and yeasts in our diverse ecosystem induces lactic acid fermentation, stabilizing the starter culture after many hours. This culture can be developed through three established methods, leading to the classification of starter into types I, II, and III.(Lau et al., 2021)

8 Starter types :

Type1:

Type I starter communities develop through spontaneous succession, much like how a forest grows from a fallow field. Environmental bacteria and yeasts colonize a flour-water mixture, fermenting the digestible carbohydrates and other nutrients. Over 5–15 days, the community is sustained by continuous refreshment with flour and water (a process known as backslopping or propagation), during which part of the mixture is discarded. Type I starters are commonly used in artisanal bakeries and are typically maintained at ambient temperatures (20–30 °C), though they can be refrigerated when not in use. These starters start with a near-neutral pH, which steadily declines as they mature, becoming highly acidic due to the organic acids produced by lactic acid bacteria (LAB) and acetic acid bacteria.(Calvert et al., 2021)

Macroscopic observations (i.e., amount of bubbles, volume/height, smell, flavor) are used for defining a mature Type I starter, but they are not well-documented in scientific literature, and true Type I starters have reportedly not been produced at an industrial scale. Traditionally, bakers determine a starter's maturity by evaluating its sensory characteristics, such as visual appearance and flavor. In scientific research, starter maturity is typically assessed by examining the stability of the starter's pH, its rising capability, and its microbiota composition, noting the consistent presence of specific bacterial and yeast species at certain stages.(Calvert et al., 2021)

Type 2:

Type II starters are created by inoculating specific bacteria (usually lactic acid bacteria, or LAB) and/or yeast (such as baker's yeast) into a flour-water mixture. While other species can also colonize these mixtures, they are typically less abundant, with the inoculated microorganisms eventually dominating. Type II starters are comparable to tree plantations, where a desired tree species is planted but other species might also colonize

randomly. These starters are usually fermented at higher temperatures for quicker development, favoring bacterial organic acid production and resulting in a lower pH.(Calvert et al., 2021)

Type II starters are advantageous for large-scale production due to their consistency, though they impart specific flavor profiles and can affect crumb texture, structure, and loaf volume. Specific bacteria are added to Type II starters to achieve unique properties, such as an extra sour taste and enhanced shelf life. To ensure a full rise, the dough is supplemented with baker's yeast or another acid-tolerant, high CO2-producing yeast.(Calvert et al., 2021)

In contrast, artisan, craft, and home bakers typically do not experiment with specific starter cultures. Instead, they create and maintain spontaneous starter cultures without detailed knowledge of the microbial makeup or its functions..(Calvert et al., 2021)

Type 3:

Type 3 starters are a little different from the second type as their type is a dried version of the type 2 starter. They do not have a direct analogue in terrestrial ecosystems; rather, they may be akin to bags of mixed seed or seed banks in fire- or drought-prone habitats. They acquire a unique advantage, which is the ability to be easily stored and transported in a dried format.

Type 4: Type 4 sourdough starters are inoculated starters that are maintained according to traditional methods. Though they are very similar to type 3, in type 4 we find the exemption of adjuncts addition, such as fruit or honey, which foster competition between inoculated microorganisms and those naturally present in the local sourdough environment. Altogether, the microbial ecology, organoleptic qualities, and leavening potential of Type III and Type IV starters, especially at the artisan scale, are less understood.(Calvert et al., 2021)

9 Compostion of a starter :

Sourdough starter, also known as natural yeast, provides a conducive habitat for microbes that can support the growth of more than 50 species of lactic acid bacteria (LAB) and more than 20 species of yeasts. Different species require specific conditions for optimal growth, leading to variations in the proportion of each species found in different sourdough starters. A sourdough starter is considered mature in the final stage of fermentation when the cell densities and abundances of both LAB and yeasts reach a stable plateau. We can observe differences in profiles from one sourdough starter to another due to variations in types of ingredients (mainly flour and water), number of sourdough propagation steps, fermentation time, fermentation temperature, dough hydration, leavening temperature, and baking environments.**(De Vuyst and Neysens, 2005)**

9.1 The source of microorganisms in starter :

The main source of microorganisms is the ingredients themselves used in making the starter, as flour is milled from raw cereals without prior sterilization, naturally inhabited by certain types of microorganisms.**(Corsetti et al., 2007)**, In a study by De Angelis et al. (2019), LAB were isolated from wheat and some non-conventional flours originating in Italy. These bacterial populations are considered pioneers of sourdough microbiota, as the lowest dissimilarity was found between microbial communities in flour and sourdough starters, compared to those from water sources, bakers' hands, and dust **(Lau et al., 2021)** These data confirm that microbial populations are significantly impacted by the varieties of flour used to make sourdough. Because the bacteria found in flour naturally prefer varied fermentation conditions, other factors impacting the sourdough microbiota are also strongly tied to the types of flour used. These factors include fermentation temperature and backslopping time.

Different results like these always raise the question of why and how. The distinction of microbial profiles found in sourdough starters can possibly be seen by comparing the farming conditions (climatic conditions, farming systems, parasitic or fungal attack, etc.) and the degree of milling of flours. However, studies directly relating farming systems and sourdough microbiome are still limited. According to Rizzello et al., sourdough starters made with flour from organically cultivated cereals typically have higher biodiversity than those made with conventionally grown cereals. These claims are complemented by a study of Gobbetti et al., which found that sourdough starter using conventionally grown flour only consisted of Leuconostoc, Lactococcus, and Lactobacillus, whereas in organically grown flour, other LAB such as Pediococcus were also found.**(Lau et al., 2021)**

The fact that each dough has its own microbiota has led bakers to use different methods, such as utilizing different types of flour during sourdough fermentation, which leads to diversity in wild yeast, for example, Saccharomyces cerevisiae, Candida holmii, C. humilis, and Kazachstania exigua. Unlike lactic acid bacteria, the microbial profile for yeast is more homogeneous as there are fewer yeast species**.(Lau et al., 2021)**

9.2 Sourdough compostion :

Sourdough, colonized by various LAB and yeasts, represents a dynamic and complex ecosystem that continuously evolves from the initial mixture of water and flour. The different microbial compositions, adaptations, and interactions can be studied and compared by manipulating technical, environmental, and ingredient parameters(Lau et al., 2021)

In order to study sourdough composition, reaching the optimal fermentation time, which is often crucial to achieve the best outcome in sourdough production, is necessary. However, there are challenges summarized by Oshiro et al., such as the uncontrollable succession of LAB due to spontaneous fermentation and technological limitations in quantifying them precisely and quickly. Despite these obstacles, the general process of understanding sourdough composition and succession during fermentation can still be described.

Microbial composition in sourdough starters typically occurs in three phases. During the first phase, there is a high abundance of microbes naturally inherited from cereals, such as Acinetobacter, Pseudomonas, Sphingomonas, and Enterobacter. The second phase, spanning days 2 to 5, sees the domination of Weissella spp. and Lb. lactis. By the third phase, after day 5, Leuconostoc spp. and Lb. sakei become the prevailing species in the microbiota of wheat sourdough.(Lau et al., 2021)

Non-specific sourdough species are significantly abundant during the early phases of fermentation but not in mature sourdough. Keep in mind that microbial profiles, acidifying, and leavening properties may not converge to similar values for all mature sourdough at the end of fermentation, as they vary from one type of flour to another.(Lau et al., 2021)

10 Sourdough Benefits :

10.1 Shelf Life and Spoilage Prevention :

Sourdough offers enhanced protection against spoilage in bread due to its acidic environment, which includes organic acids such as acetic, caproic, butyric, and propionic acids. These acids inhibit the growth of deleterious microbes, extending the bread's shelf life. Specifically, the release of organic acids and subsequent pH reduction by Lactobacillus sanfranciscensis CB1 can halt the growth of spoilage flora such as Aspergillus, Fusarium, Monilia, and Penicillium. Enhanced antifungal action is achieved with increased microbial complexity in sourdough, with optimal results observed with microbial combinations exceeding five LAB isolates. Additionally, studies by Jenkins, Torriani et al., and Fernández-Peláez et al. suggest that sourdough bread's prolonged shelf life and delayed staling are due to the decomposition of starch during lactic acid fermentation.(Lau et al., 2021)

10.2 Aroma and Flavor Enhancement :

Sourdough fermentation significantly improves bread flavor, complexity, aroma, and texture. LAB enzymatic hydrolysis processes and the Maillard reaction during baking generate a rich array of flavor compounds (Lau et al., 2021). These include proteolytic processes of amino acids, conversion of glutamine to glutamate, and conversion of arginine to ornithine by LAB, resulting in a tangy and roasted taste. The formation of crumb aroma and flavor is due to microbial and enzymatic reactions such as lipid oxidation by cereal enzymes and the Ehrlich degradation of amino acids. For instance, lipid oxidation, initiated by enzymes like lipoxygenase, produces volatile compounds during baking that influence bread's aroma and flavor. Sourdough bread achieves higher scores in sensory evaluations compared to chemically acidified bread, partly due to the acetic acid formed during fermentation acting as a flavor enhancer.(De Angelis et al., 2002)**,(Hansen and Schieberle, 2005)**

10.3 Texture Improvement :

The presence of metabolites such as exopolysaccharides (EPS) produced by LAB in sourdough positively impacts bread texture. EPS have excellent water retention properties, reducing crumb hardness and contributing to the dough's viscoelastic properties, thereby increasing bread volume. β-glucans, a type of EPS, particularly enhance the texture and volume of sourdough bread. Studies by Bockwoldt et al. highlight that sourdough with Pediococcus claussenii achieves higher β-glucan concentrations more rapidly than sourdough with Lactobacillus brevis. The fermentation temperature significantly affects β-glucans production, with P. claussenii preferring 25°C and L. brevis thriving at 35°C.(Lau et al., 2021)

10.4 Nutritional Value and Digestibility :

Sourdough fermentation enhances the nutritional profile of bread by breaking down phytic acid, which can lead to gastrointestinal disorders, digestive discomfort, and flatulence,**(Crossland, 2015)** . The enzyme phytase, produced by LAB and yeast during fermentation, neutralizes phytic acid, improving the digestibility and nutritional content of bread. For instance, a study by **,**(De Vero et al., 2021) revealed that Lactobacillus brevis and Lactobacillus plantarum strains exhibit high phytase activity, significantly reducing phytic acid levels compared to conventional yeast fermentation. This process makes minerals such as iron, calcium, and zinc more bioavailable, enhancing the nutritional value of sourdough bread.(Lau et al., 2021)

10.5 Gastrointestinal Health :

Sourdough bread is beneficial for gastrointestinal health, particularly for individuals with irritable bowel syndrome (IBS) and other digestive disorders. LAB in sourdough break down non-digestible oligosaccharides, fructans, and raffinose, reducing digestive distress. For example, yeast Saccharomyces cerevisiae produces the enzyme invertase during fermentation, decreasing fructan levels by 50-80% in flour or wholemeal. This makes sourdough bread more tolerable for gastrointestinal patients and those with IBS.(Lau et al., 2021)

10.6 Gluten Reduction :

Sourdough fermentation can reduce the risk of gluten-related disorders. Selected sourdough lactobacilli, including *Lactobacillus sanfranciscensis, Lactobacillus rossiae, Lactobacillus plantarum,* and others, can modify immunogenic sequences, which are specific segments of proteins that can elicit an immune response in the body, also known as epitopes, making them less recognizable by the immune system of celiac disease patients. This involves the hydrolysis of glutenins and the disruption of gluten protein networks, facilitated by acidic conditions and the action of proline-specific peptidases from lactobacilli. Recent research found that *Lactobacillus paracasei* LPA4 exhibited the strongest gliadin degradation, highlighting the potential of sourdough in managing gluten intolerance.**(Cabrera-Chávez and Calderón De La Barca, 2010)**

10.7 Prebiotics and Probiotics :

Sourdough bread contains prebiotics and, to some extent, probiotics, which support gut health. Although probiotics may not survive the baking process, prebiotics released by LAB, such as β-glucan, remain beneficial. Prebiotics are compounds that promote the growth of beneficial gut bacteria, improving digestion and overall health. For example, Pérez-Ramos et al. demonstrated that β-glucan prebiotics released from LAB in sourdough boosted the viability of probiotic *Lactobacillus plantarum* (WCFS1) under starving conditions. Both probiotics and prebiotics share a crucial role in manipulating bacterial activities that colonize the digestive system in the human body.(Lau et al., 2021)

10.8 Glycaemic Index Control :

Sourdough bread has a lower glycemic index (GI) than conventional yeast bread, making it a healthier option for managing blood sugar levels. The presence of organic acids like lactic and acetic acid during fermentation slows down the digestion of carbohydrates, reducing the speed at which sugar enters the bloodstream. This lower GI is beneficial for people with type 2 diabetes and those looking to control their blood sugar levels.(Fardet et al., 2006)**,**(Lau et al., 2021)

10.9 Antioxidant and Antihypertensive Benefits :

Sourdough bread also exhibits antioxidant and antihypertensive activities, protecting body cells against free radicals and potentially reducing the risk of diseases and cancers. The fermentation process enhances the bioavailability of vitamins and minerals, contributing to overall health and well-being. Studies have shown that sourdough fermentation increases the solubility of minerals, making them more readily absorbed by the body.(Lau et al., 2021)

11 The evolution of modern yeast :

With the advent of the Industrial Revolution in the 18th century, numerous fields experienced significant transformation and development, profoundly impacting the utilization of yeast in bread-making processes. Advancements in microbiology and fermentation science during this era, particularly the contributions of scientists such as Louis Pasteur and Emil Hansen, facilitated the isolation and cultivation of pure yeast strains, notably Saccharomyces cerevisiae. Hansen's successful isolation of pure yeast cultures in 1888 was a pivotal achievement, allowing for consistent and controlled fermentation. This innovation addressed the increasing industrial demand for large-scale, efficient, and reliable bread production. Consequently, the widespread adoption of pure S. cerevisiae cultures revolutionized the baking industry by providing a uniform product that met the

requirements for speed and volume. However, this shift also led to a trade-off, as the diverse flavors and potential health benefits associated with traditional, mixed-culture fermentations were largely diminished. Thus, the Industrial Revolution not only transformed yeast production and usage but also reshaped the sensory and nutritional landscape of bread.

11.1 Modern yeast (*Saccharomyces cerevisiae*) :

Saccharomyces cerevisiae, a yeast species, preferentially metabolizes sugar through anaerobic fermentation to produce ethanol and CO2, even in the presence of oxygen, a phenomenon known as the Crabtree effect. This adaptive trait enables S. cerevisiae to outcompete other microbes in sugar-rich environments by rapidly consuming sugar and producing ethanol. In 1996, it became the first eukaryote to have its genome fully sequenced, greatly advancing the fields of functional genomics and synthetic biology. However, research has mainly focused on laboratory strains such as S288C, which are genetically atypical and do not represent the species' natural diversity. Recently, the discovery of genetically diverse wild lineages of S. cerevisiae has enhanced our understanding of its ecology, natural history, and evolution, offering new insights into its domestication and adaptation.(Bai et al., 2022)

11.2 Life cycle :

The life cycle of Saccharomyces cerevisiae is well-documented in laboratory settings, where it typically grows as a diploid in nutrient-rich media like YPD and reproduces clonally by budding at an optimal temperature of 30°C. Under nitrogen starvation, S. cerevisiae sporulates and undergoes meiosis, forming four haploid spores within an ascus, with two mating types (MATa and MATα). Haploid spores can mate within the ascus (intratetrad mating) or germinate to form haploid cells that reproduce mitotically. Haploid cells can mate with others of the opposite mating type from the same or different strain, or undergo mating-type switching via gene conversion at the MAT locus, facilitated by the HO endonuclease. This regulated mating-type switch allows for the formation of diploid cells through haplo-selfing or autodiploidization. The mating behaviors of S. cerevisiae, such as outcrossing and haplo-selfing, have different genetic and evolutionary consequences. Outcrossing, although rare, leads to heterozygous diploids, while haplo-selfing results in homozygous diploids. The high homozygosity in wild strains suggests frequent haplo-selfing in nature. This process may purge deleterious alleles through purifying selection but reduces allelic variation. The ecological and genetic implications of S. cerevisiae's life cycle in natural populations remain largely unknown, necessitating further ecological and population genetic studies to understand the species' reproduction in natural environments..(Bai et al., 2022)

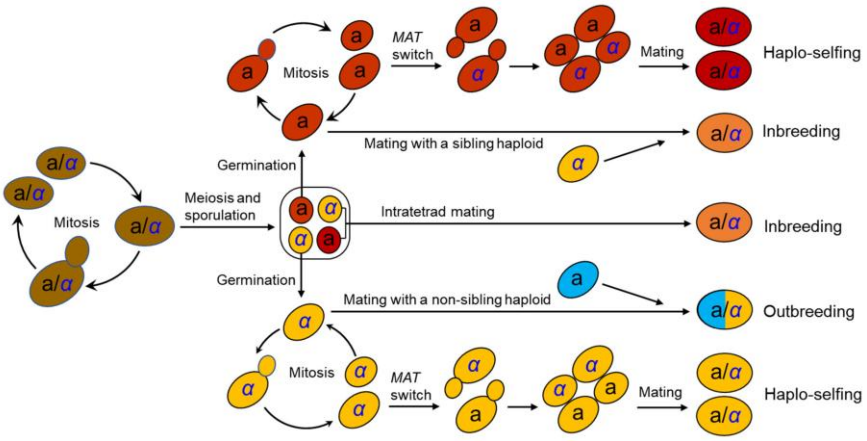


Figure 1: The Life Cycle and Mating Behaviors of Saccharomyces cerevisiae(Bai et al., 2022)

12 Natural Habitat and Isolation of S. cerevisiae :

For a long time, Saccharomyces cerevisiae was considered an exclusively domesticated species due to its rarity in natural environments. Early isolates from the wild were thought to be escapees from domestic stocks. However, a field survey in Japan revealed frequent isolation of S. cerevisiae from forest materials, such as soil, decayed leaves, and tree bark, indicating its common occurrence in nature. Subsequent studies supported these findings, suggesting a broad distribution of S. cerevisiae in forest habitats and vineyards.(Bai et al., 2022)

The development of efficient selective isolation methods has facilitated field surveys of S. cerevisiae from the wild. While standard dilution plating methods are effective for isolating S. cerevisiae from alcoholic fermentation processes, they are less effective for natural substrates due to complex microbial communities and low yeast population densities. Enrichment media containing ethanol have been successfully used to isolate S. cerevisiae from vineyard grapes and uncultivated habitats. This method has been adapted with minor modifications to isolate Saccharomyces species from various natural substrates.(Bai et al., 2022)

A comprehensive field survey in China sampled a wide range of substrates across diverse climate zones, revealing that S. cerevisiae is ubiquitous in nature. S. cerevisiae strains were isolated from fruit, tree bark, soil, and rotten wood from orchards and forests in tropical to temperate regions. This survey demonstrated that S. cerevisiae exists in both human-influenced and remote natural environments.(Wang et al., 2012)**,** (Bai et al., 2022)

Despite its ubiquitous distribution, the specific ecological niches of S. cerevisiae in the wild remain uncertain. Although it is common in vineyards, S. cerevisiae is rarely isolated from grapefruit, and metagenomic sequencing shows it is rare on ripe grapes compared to other yeast species. Isolation success rates vary by substrate, with higher rates from tree bark, especially broad-leaved trees, compared to fruits. These findings suggest that fruit may not be the preferred niche of S. cerevisiae in the wild.(Bai et al., 2022)

S. cerevisiae and its sibling species *S. paradoxus* are frequently isolated from oak tree bark, leading to the belief that oak is a preferred niche. However, large-scale surveys indicate that broad-leaved trees, including various Fagaceae species and other broad-leaved trees, have higher isolation rates than oaks. Studies in Brazil, where

native oaks are absent, also show significant isolation rates from broad-leaved trees, supporting the preference of *S. cerevisiae* for these habitats.(Bai et al., 2022)

Overall, while S. cerevisiae is distributed widely in nature, more systematic surveys are needed to fully understand its ecological niches and habitat preferences.

Chapter 3
Materiel and Methodes

13 Materiels and methodes :

The study was conducted at Yahia Fares University in Medea (Ouzra Campus) over a period of 4 months with the aim of enhancing the value of baker's yeast. The objective was to leverage the benefits inherent in traditional starters, such as improved aroma, texture, taste, and health benefits, while also incorporating modern advantages like increased speed and volume in production.

13.1 Materiels :

In this study, three different products were transformed from their original states to desirable forms, referred to as D1, B, and G21. Additionally, a fourth product, referred to as B2, was produced to achieve the final product.

As mentioned earlier, three out of the four products (D1, B, G21) will undergo transformation. Each product requires specific raw materials, each selected based on distinct criteria.

13.2 Raw materiales selection :

Choosing the materials is based on three criteria (region/abundance/quality).

Region : The region for selecting products was based on being local (Algerian territory). Algerian farmers do not spray and use many chemical products on their harvest, resulting in good quality products.

Abundance : Considering that this study aimed to create a product with a future vision of industrial production, abundance is necessary to reach this goal and secure pricing from different suppliers.

Quality : The quality of the raw materials for the products does not necessarily need to be the highest. However, for the purposes of this study, premium quality materials were chosen.

For the final product (B2), only a reference and isolated lactic acid bacteria (LAB) strain are needed.

13.3 Methodes :

The methods section is divided into four steps: dehydration, pathogenicity testing, cultivation, and combining the ingredients in the correct proportions.

Step 1 (dehydration) :

The dehydration process took place to transform three materials (D1/B/G21) into powders to act as nutrition for the microflora (yeast and LAB).

D1: A 263 g sample of the product was cut in half and placed in a dehydrator at 50°C for 24 hours. After this initial drying period, the product was cut into quarters and subjected to another 24 hours of dehydration at 50°C.



Figure 3: weight of D1 product (original ,2024)



Figure 2: cut in half and ready to dehydrate



Figure 4: D1 cut into quarters after first dehydration and ready to be redehydarted (original,2024)

. Following the dehydration process, the product was blended and then rehydrated under the same temperature and duration conditions. Finally, the rehydrated product was blended one last time.

As a second approach, a 250 g sample of the product was cut in half, and with the seeds removed, it was spread over aluminum sheets with the interior facing up. This was dehydrated at 50°C for 48 hours. Subsequently, the product was blended and rehydrated for 24 hours at 50°C, followed by a final blending step.



Figure 6 :D1 cut and soread in a spesfic order

(original,2024)



Figure 5 :D1 blended and ready to be redehydrated (original,2024)

B: Starting by peeling off the skin after washing, the product was cut into pieces. The total weight of 342.602 g,



Figure 7 :the 342.602 g of the B product cleaned and cut ready to be bland (original,2024)

was then blended to further reduce the size of the pieces. The resulting mixture was placed in a dehydrator at 53°C for 30 hours with a final blending step. As a different approach, after trimming the excess parts and washing, the product was grated into fine pieces without peeling off the skin.



Figure 8 : grated B product ready for dehydration (original,2024)

This was followed by dehydration at 50°C for 16 hours and then a blending step.



Figure 9: the final blending step of product B (original,2024)

G21: For this product, four different methods were employed to evaluate the effects of various preparation and dehydration techniques:

1. **First Method**: The G21 sample was punctured with a sterile fork to create small holes, then spread over aluminum sheets and dehydrated at 50°C for 48 hours. This method aimed to facilitate even drying by allowing better air circulation through the small holes
2. **Second Method**: The G21 sample was cut into quarters, and the pieces were placed on parchment sheets and dehydrated at 53°C for 48 hours.



Figure 10 : G21 cut into quarters and ready to dehydrate (original,2024)

3. **Third Method**: The G21 sample was blended into small pieces, spread on parchment sheets, and then dehydrated at 53°C for 48 hours.



Figure 11: G21 sample blended into small pieces and ready to be dehydrated (original,2024)

4. **Fourth Method**: Approximately 300 g sample of G21 was boiled, then placed in a dehydrator at 50°C for 48 hours, followed by a blending step and rehydration for 20 minutes at 50°C.



Figure 12: weighting approx 300g of the G21 (original,2024)



Figure 13 : approx 300g of boiled G21 (original,2024)

Step 2 : The second step, which is a test that consists of checking for harmful bacteria which may exist on the freshly made products, relies on VRBL medium which is based on the ability of coliforms to ferment lactose. The medium is made inhibitory to Gram-positive bacteria and certain Gram-negative bacteria by the simultaneous presence of crystal violet and bile salts. Neutral red is used as a pH indicator.

Protocol:

1g of each product was inoculated inside sterile tubes and added to it 9 ml of sterile distilled water, following it with decimal dilutions with heavy agitation to each tube reaching up to 10-4 and always negative control was added which is a tube with the same sterile distilled water, then 1 ml of each tube was inoculated in Petri plate alongside with 15ml of VRBL, mixed and let it to cool down. The Petri plates were incubated at 30°C for 24h for the detection and enumeration of coliforms. The same process was remade with the Petri plates being incubated at 44°C for the detection and enumeration of thermotolerant coliforms.

Step 3 : The third step involves the cultivation of lactic acid bacteria (LAB) after successful isolation in MRS (de Man, Rogosa, Sharpe) medium. The process begins with isolating a specific LAB strain using MRS agar medium prepared in the laboratory. The medium composition per liter includes: peptone 10 g, yeast extract 5 g, meat extract 10 g, glucose 20 g, Tween 80 1 g, sodium acetate 5 g, magnesium sulfate 0.1 g, manganese sulfate 0.05 g, disodium phosphate 2 g, and agar 15 g. The medium was autoclaved at 121°C for 15 minutes. After autoclaving, 15 ml of the medium was poured into sterile Petri plates and allowed to cool. A few milligrams of lyophilized LAB strain were rehydrated in 10 ml of sterile distilled water with vigorous agitation. After a few minutes, an inoculating loop sterilized with a Bunsen burner was dipped into the LAB solution and streaked onto the MRS agar plates. The plates were incubated at 37°C for 24 hours.



Figure 14: preparation of MRS agare medium (original,2024)



Figure 15: poring 15 ml of medium in petrie plates and conserving at 4°C after autoclaving at 121°C for 15min (original,2024)

Following isolation, a 3-liter batch of MRS broth was prepared using the same ingredients, excluding agar. Thirty milliliters of this broth were transferred into a sterile tube and inoculated with an isolated LAB colony from the agar plates. This inoculated tube was incubated at 37°C for 24 hours. Subsequently, 3 liters of MRS broth were transferred into a sterile container and inoculated with 1% of the previously enriched LAB culture. The container was incubated at 37°C for 20 hours.



Figure 16 : cultivating isolated colonie in broth MRS at 37°C for 24h (original,2024)

After incubation, the medium was transferred into sterile 50 ml tubes and centrifuged at 6000 rpm for 15 minutes. The supernatant was discarded, and the LAB pellet was retained and washed three times with sterile distilled water.

At this point, the washed LAB cells needed to be freeze-dried in order for the final product to be in a powder form. After researching freeze dryers in Algeria, we found there are only two available to which we have access,

located at CRAPC (Bousmail/Laghouat). However, it required many days for the request to be accepted, followed by additional time to schedule a specific working date, which will take from 24 to 36 hours of operation.

The following part will show an attempt to make a freeze dryer. Freeze drying is based on a concept called sublimation, which involves extracting water molecules from the product, leaving it dried. Different parameters are used, such as pressure and temperature, as explained by this diagram :

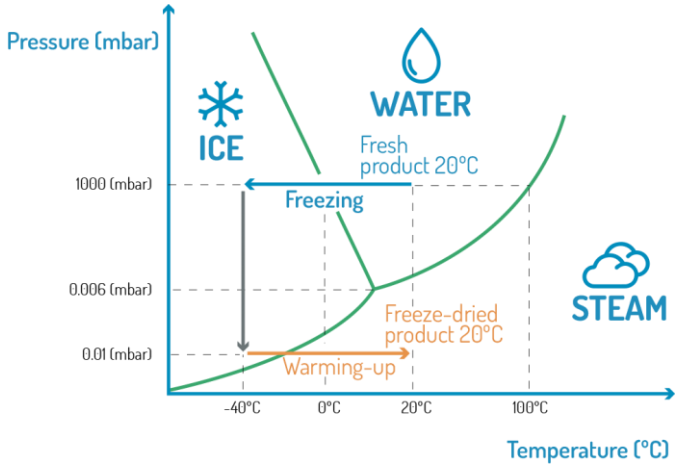


Figure 17 :a representation of condition of water in different pressure condition and temperature.

The dry freezer contains two chambers which need to be at different temperatures, with a gap between them of 15 to 20 degrees Celsius as a minimum, which needs to be maintained throughout the entire process. Other conditions needed are that the product desired to be dried needs to be at least -20 degrees Celsius and heated as soon as the process begins.

Two boxes of agro-alimentary aluminum were chosen, and different holes were made in the cover for the vacuum pump to be attached, and one hole to control the pressure. For the second prototype, thick stainless steel was chosen and covered with thick steel, which contained two holes for the first chamber designed for the vacuum pump, and the second chamber with also two holes, one for the vacuum pump and one for controlling the pressure. A specific adapter was added to control the pressure.



Figure 23 :chamber 1 cover (original,2024)



Figure 20: chamber 2 cover (original,2024)

Process conditions:

The conditions were -20°C for the second chamber, which contains the product desirable to be freeze-dried. The first chamber was submerged in water and frozen at -40°C to maintain the temperature, with the pressure maintained at less than 0.8 bar throughout the entire process, beginning at 0.3 bar. The second chamber was heated with a heating resistor.



Figure 25: chamber 1 at the right temperature

(original,2024)



Figure 24 :chamber 2 at the right temperature (original,2024)

Then , the finale product was tested by adding it to broth MRS and incubated for 24 h at 37 Celsius degree and checked with density meter.

Step 4 : The final step consists of a series of tests with the purpose of choosing the right proportion for the mix which will result in the final product. The specific tests were conducted at a local bakery with the assistance of a professional baker. Initially, standard bread formulations were prepared using the usual ingredients: flour, dry

yeast, sugar, and salt. A specific weight of all-purpose flour was selected for the trials. The proportion of dry yeast was determined based on the baker's recommendation to ensure minimal rising time without imparting an undesirable odor to the bread. The quantities of sugar and salt were also determined based on the baker's experience to optimize the bread's flavor and texture.

The tests then proceeded by replacing sugar with the experimental products made at the beginning of the process (D1, B, G21). Each product was used to replace sugar at 100% proportions, resulting in a total of 4 different bread variations. These breads were then evaluated to determine if the replacements successfully fulfilled their role in the mix, which is being an energy source for the yeast and providing a nice brown color to the bread.

A final test involved adding the lactic acid bacteria (LAB) powder, previously prepared, to the mix. This aimed to achieve better taste, texture, and health benefits in a shorter fermentation time, typically requiring at least 24 hours with conventional starters.



Figure 26: testing the product at a locale bakery (original,2024)

Chapter 4
Results and discussion

14 Results and discussion

14.1 Step 1 :dehydration

D1 :

Table 1: comparison between different methods used to make D1 product according on 3 different criteria

	Manipulation Complexity	Process Time	Final product Texture
Method 1	**	72H	thin
Method 2	*	**72H**	**rough**

Manipulation Complexity : very complexed :*** /mediocer :** / easy :*

Product texture : thin=perfect/mediocre= good/ rough= acceptable

Both methodologies used to transform D1 were successful. However, as shown in the table above, there are a few differences that distinguish the best method. Time can be neglected in this case due to the similarity between the two methods. The first method has higher manipulation complexity and results in a finer texture, while the second method has less complexity and results in a rougher texture. The difference in texture can also be neglected since manipulation complexity is far more important. Therefore, we conclude that the second method is better for transforming this product. Other processes have been documented for this product, including sun drying, which provides all the necessary results but requires a much longer process time, making it less effective compared to the methods tested in this study. Additionally, there are less common methods such as freeze drying, which yields superior results across all criteria compared to the methods studied here, but it requires specific equipment.



Figure 27 :the finale product of dehydration D1 (method 2) (original,2024)

B :

Table 2: comparison between different methods used to make product B powder according on 3 different criteria

	Manipulation Complexity	Process Time	Final product Texture
Method 1	**	**30H**	**thin**
Method 2	*	**16H**	**thin**

Manipulation Complexity : very complexed :*** /mediocer :** / easy :*

Product texture : thin=perfect/mediocre= good/ rough= acceptable

Both methods resulted in a very fine product. However, from the table, we can clearly see that the second method has the advantage of requiring less processing time and easier manipulation. The texture can be neglected due to the similarity of the results. The process used in the second method is the most widely used. Additionally, there are two other common methods: cutting the product into very thin slices and dehydrating them, and freeze drying. Although freeze drying is more complex than the methods used in this study, it yields slightly better results.



Figure 28: the finale product of dehydration of product B (method 2) (original,2024)

G21:

Table 3 : comparison between different methodes used to make G21 powder useing 3 different criteria

	Manipulation Complexity	Process Time	Final product Texture
Method 1	*	**72H**	**No results**
Method 2	***	**48H**	unacceptable
Method 3	***	**48H**	unacceptable
Method 4	**	**48H**	**good**

As we observe in the table, 3 out of 4 methods failed. The first method showed no results because the product didn't dehydrate and got burned inside the dehydrator. The second and third methods had an acceptable amount of time and some level of complexity. However, the second method resulted in a texture similar to gum and did not dissolve in water or any other liquid, making it unacceptable for the product's intended purpose. Finally, the last method produced a good product with an acceptable level of both complexity and time.

Among the other methods, freeze drying is the best for this product, offering less complexity, shorter processing time, and better texture.



Figure 29 : G21 after being treated with method 1 (original,2024)



Figure 30: G21 after being treated with method 2 and 3 (original,2024)



Figure 31 : G21 after being treated with method 4 (original,2024)

14.2 Step 2 : harmful bacteria test

B :

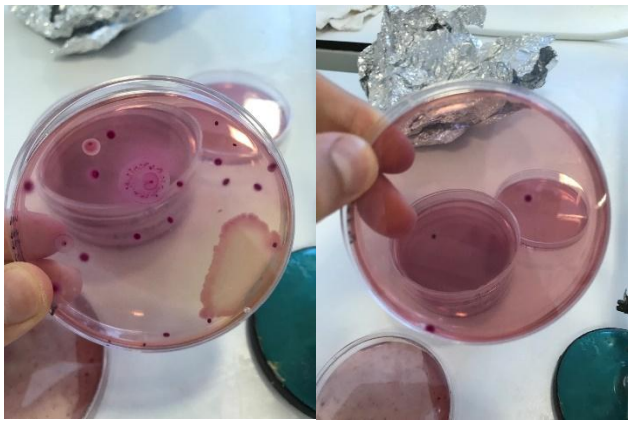


Figure 32:results of different concentration of product B in VRBL medium after 48H incubation at 30°C (original,2024)

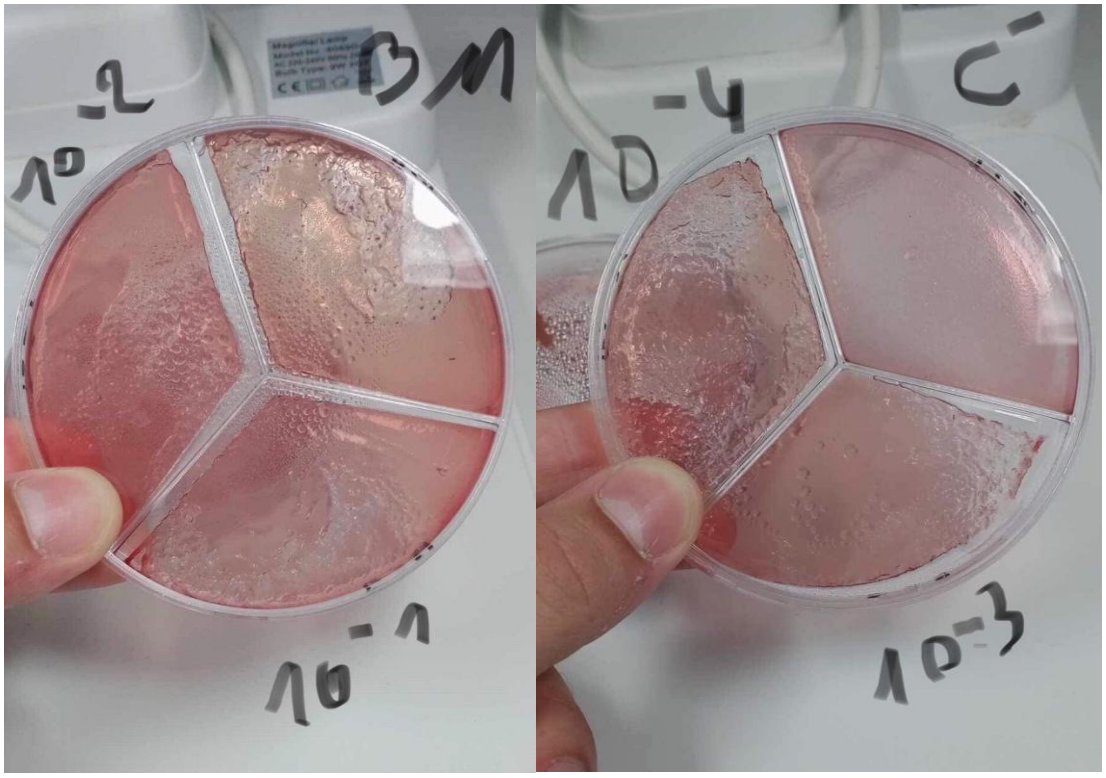


Figure 33: results of different concentration of product B in VRBL medium after 48H incubation at 44°C (original,2024)

G21 :

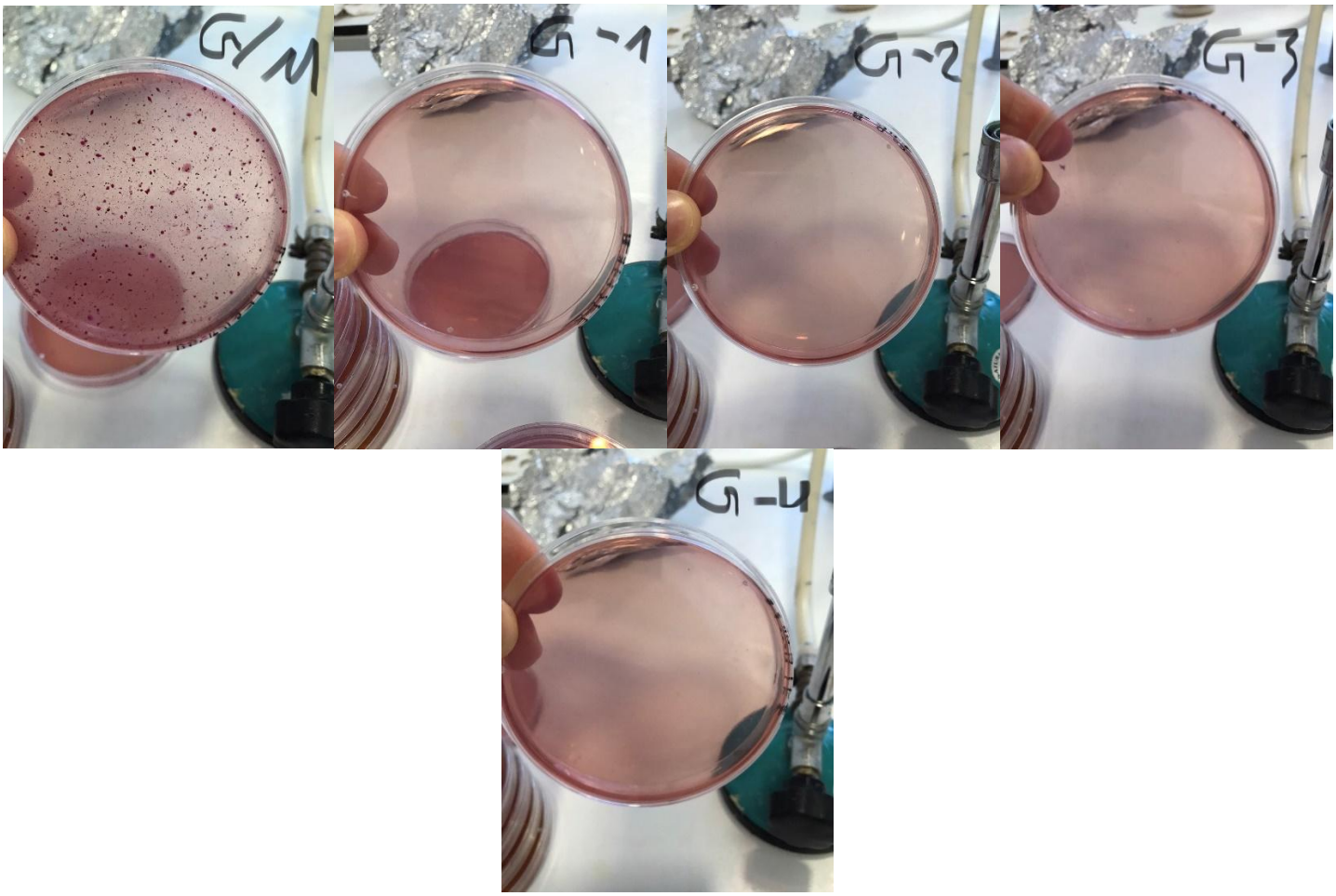


Figure 34: results of different concentration of product G21 in VRBL medium after 24H incubation at 30°C (original,2024)

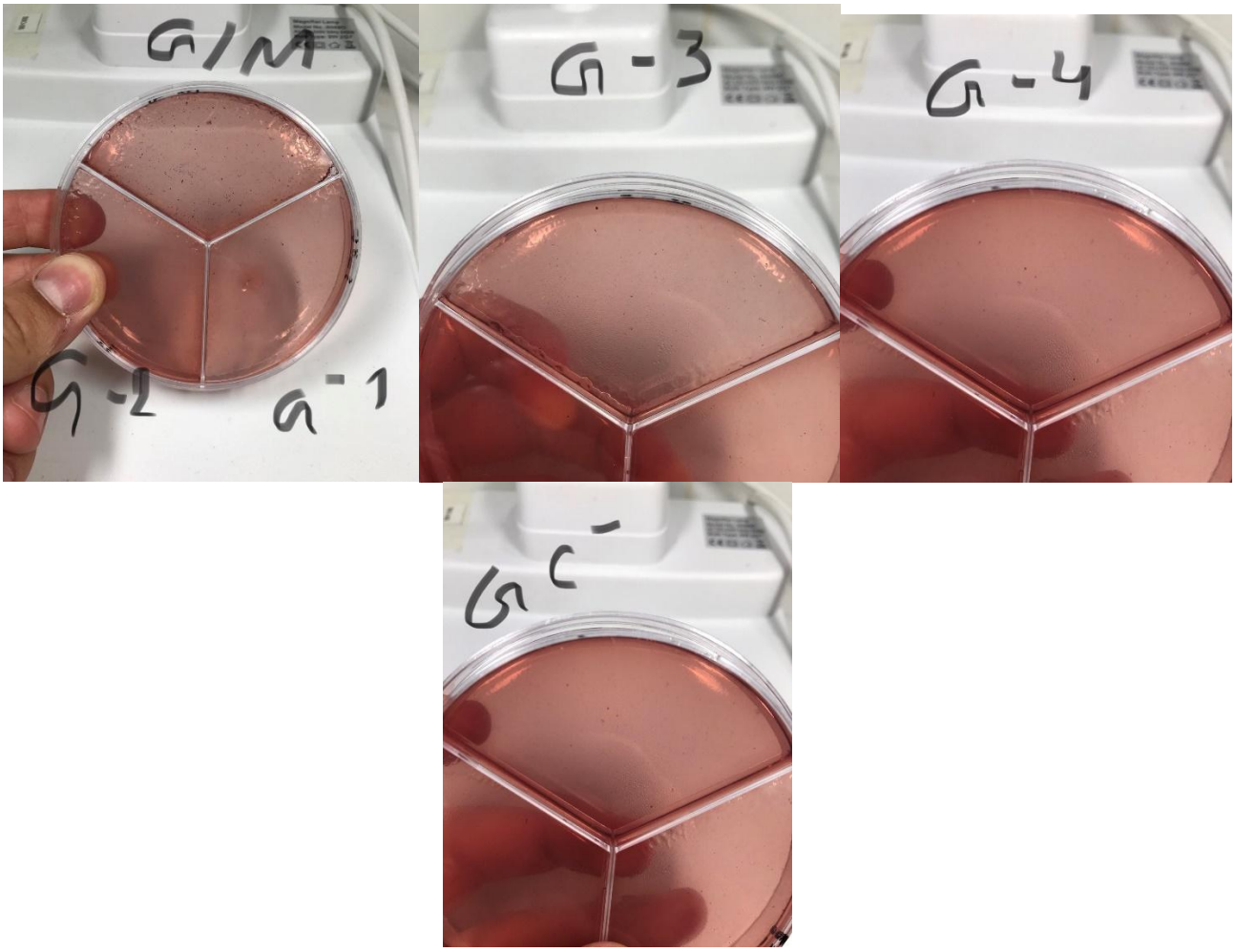


Figure 35: results of different concentration of product G21 in VRBL medium after 24H incubation at 44°C (original,2024)

D1 :

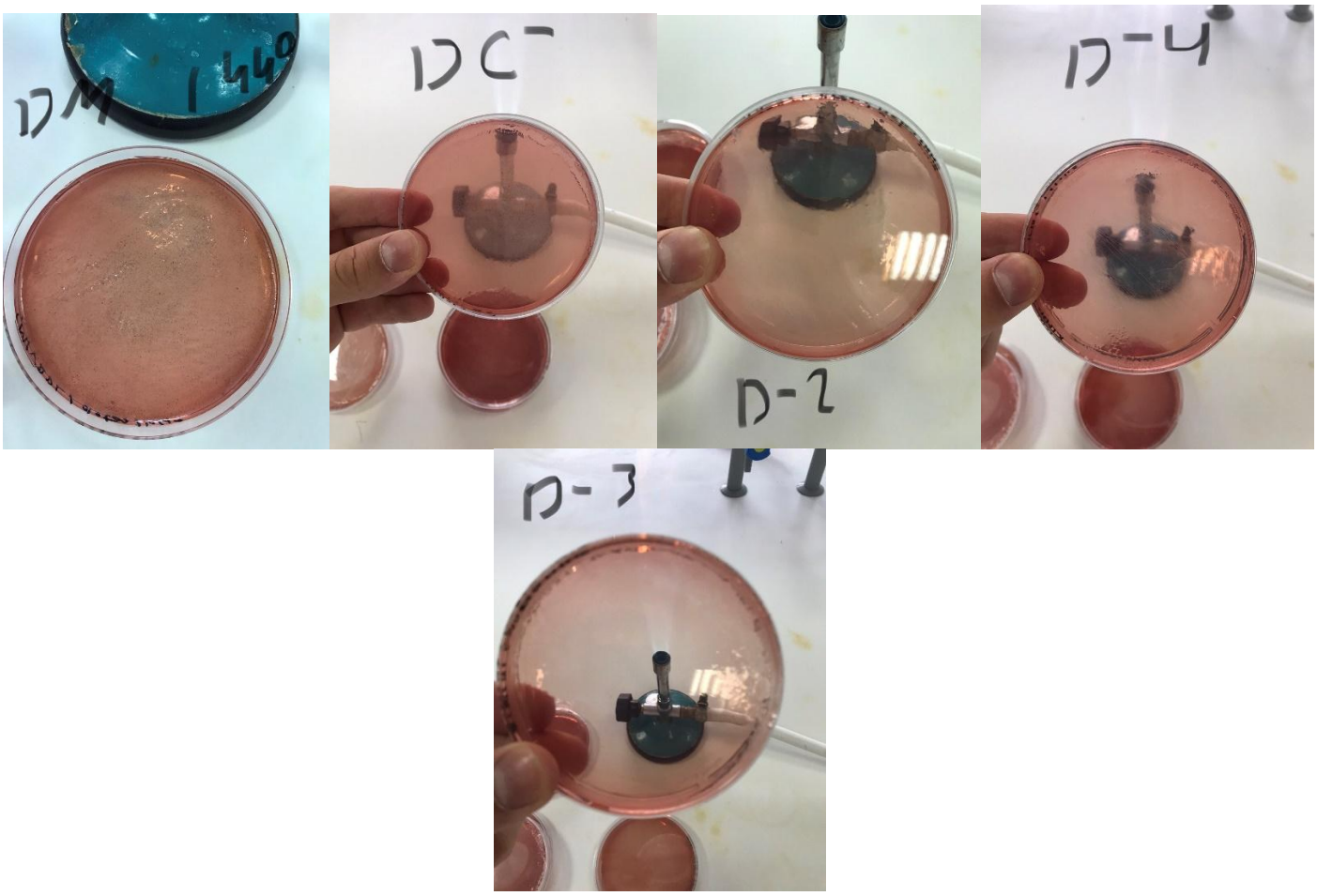


Figure 36: results of different concentration of product D1 in VRBL medium after 24H incubation at 44°C (original,2024)

The test for harmful bacteria was negative in all products under both conditions (30 degrees and 44 degrees Celsius), as shown in the pictures. There were no colony-forming units (CFU) on the plates, except for product B at 30 degrees Celsius, which showed a number of colonies after 48 hours of incubation. This indicates that the test needs to be repeated and reviewed at a 24-hour incubation period to confirm if the results are acceptable. Another issue that arose in our work was the loss of photographic data for product D1 at 30 degrees Celsius. While the initial test showed no colonies and was acceptable, the experiment needs to be repeated to ensure accuracy and completeness before publication.

14.3 Step 3 : cultivation and dry freezing

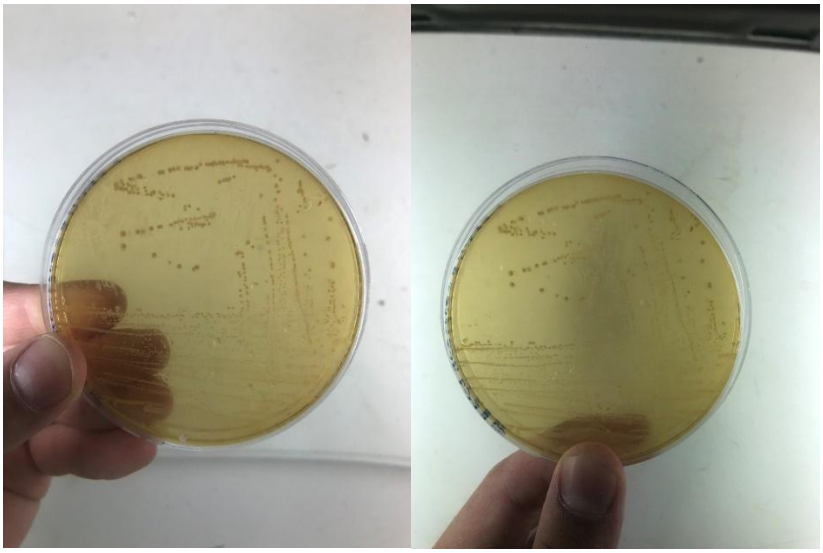


Figure 37 : lactic acide bacteria isolated in MRS medium (original,2024)

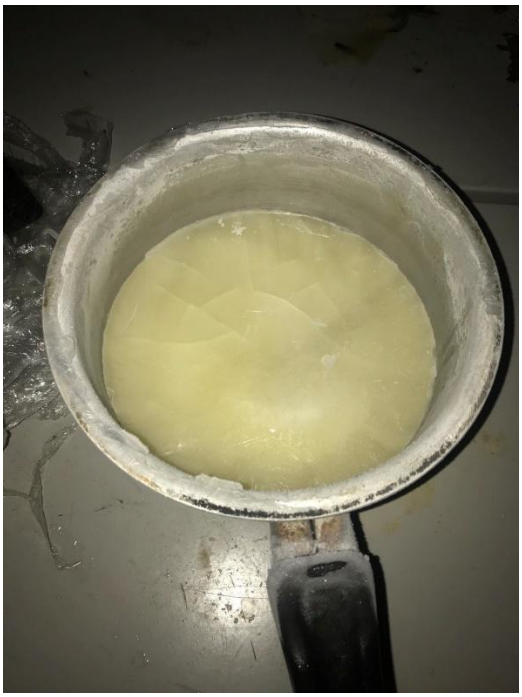


Figure 38 : cultivated and washed LAB preserved at -40°C (original,2024)



Figure 39 :lactic acid bacteria after freeze drying process (original,2024)

The results show the successful isolation of lactic acid bacteria after inoculation in MRS medium, followed by successful preservation at -20 degrees Celsius. After cultivation in MRS broth and cell washing, we obtained freeze-dried LAB, which was a perfectly satisfying final product, indicating further success in both the construction of the freeze-drying machine and the lyophilization process.

conclusion and perspective

15 conclusion and perspective :

Bread, often considered one of the oldest processed foods, has a rich and extensive history dating back to prehistoric times. Over millennia, early bread-making practices evolved with the understanding of fermentation and the development of gluten proteins in wheat, eventually leading to the sophisticated bread varieties crafted by bakers around the world today.

As part of the preparation of this dissertation, we achieved the main part of our desired goals, and our final product met the expected outcomes. By using our product, we effectively eliminated one of the most harmful additives commonly used by bakers and individuals in bread-making: sugar. This substitution is significant as high sugar consumption is linked to an increased risk of type 2 diabetes (Lau et al., 2021) Our natural processed product successfully replaces sugar, addressing this health concern.

Additionally, our incorporation of lactic acid bacteria addresses the issue of bread losing its nutritional value over time. Traditional bread-making requires at least 24 hours in optimal conditions and considerable expertise to produce a nutrient-rich starter. In contrast, our product achieves the necessary nutritional requirements within 2 to 3 hours, simplifying the process and ensuring consistent results. This enhancement leads to improved dough consistency, enhanced texture, increased volume, extended shelf life, and a boost in flavor.

Despite these successes, several perspectives warrant further investigation. Future research should explore additional natural products that can replace sugar and further enhance the nutritional profile of bread. Another important perspective is the incorporation of different strains of lactic acid bacteria in the mix. Studying their various properties and interactions could lead to even greater improvements in bread quality and health benefits.

Bibliographic References

16 Bibliographic References

An A-Z of breads from around the world [WWW Document], n.d. URL https://www.bhf.org.uk/informationsupport/heart-matters-magazine/nutrition/cooking-skills/dough/a-to-z-of-breads (accessed 6.19.24).

Arendt, E.K., Ryan, L.A.M., Dal Bello, F., 2007. Impact of sourdough on the texture of bread. Food Microbiology, 3rd International Symposium on Sourdough 24, 165–174. https://doi.org/10.1016/j.fm.2006.07.011

Arranz-Otaegui, A., Gonzalez Carretero, L., Ramsey, M.N., Fuller, D.Q., Richter, T., 2018. Archaeobotanical evidence reveals the origins of bread 14,400 years ago in northeastern Jordan. Proceedings of the National Academy of Sciences 115, 7925–7930. https://doi.org/10.1073/pnas.1801071115

Bai, F.-Y., Han, D.-Y., Duan, S.-F., Wang, Q.-M., 2022. The Ecology and Evolution of the Baker's Yeast Saccharomyces cerevisiae. Genes 13, 230. https://doi.org/10.3390/genes13020230

Bhatia, S.C., 2016. Food biotechnology. Woodhead publishing India, New Delhi.

Bond, S., 2023. 37 Types Of Bread From A To Z (With Photos!) [WWW Document]. Live Eat Learn. URL https://www.liveeatlearn.com/types-of-bread/ (accessed 6.19.24).

Borsuk, Y., Bourré, L., McMillin, K., Sopiwnyk, E., Jones, S., Dyck, A., Malcolmson, L., 2021. Impact of Ferment Processing Parameters on the Quality of White Pan Bread. Applied Sciences 11, 10203. https://doi.org/10.3390/app112110203

Bread Rises And Falls With Gluten [WWW Document], 2015. . AIP. URL https://ww2.aip.org/inside-science/bread-rises-and-falls-with-gluten (accessed 6.16.24).

Cabrera-Chávez, F., Calderón De La Barca, A.M., 2010. Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients. Journal of Cereal Science 52, 337–341. https://doi.org/10.1016/j.jcs.2010.06.020

Calvert, M.D., Madden, A.A., Nichols, L.M., Haddad, N.M., Lahne, J., Dunn, R.R., McKenney, E.A., 2021. A review of sourdough starters: ecology, practices, and sensory quality with applications for baking and recommendations for future research. PeerJ 9, e11389. https://doi.org/10.7717/peerj.11389

Cavalieri, D., McGovern, P.E., Hartl, D.L., Mortimer, R., Polsinelli, M., 2003. Evidence for S. cerevisiae Fermentation in Ancient Wine. Journal of Molecular Evolution 57, S226–S232. https://doi.org/10.1007/s00239-003-0031-2

Chemistry of Food Browning: The Maillard Reaction [WWW Document], n.d. . ThoughtCo. URL https://www.thoughtco.com/maillard-reaction-and-why-foods-brown-604048 (accessed 6.16.24).

Corsetti, A., Settanni, L., Chaves López, C., Felis, G.E., Mastrangelo, M., Suzzi, G., 2007. A taxonomic survey of lactic acid bacteria isolated from wheat (Triticum durum) kernels and non-conventional flours. Systematic and Applied Microbiology 30, 561–571. https://doi.org/10.1016/j.syapm.2007.07.001

Crossland, I., 2015. Sourdough and digestibility. Sustainable Food Trust. URL https://sustainablefoodtrust.org/news-views/sourdough-and-digestibility/ (accessed 6.2.24).

De Angelis, M., Mariotti, L., Rossi, J., Servili, M., Fox, P.F., Rollán, G., Gobbetti, M., 2002. Arginine Catabolism by Sourdough Lactic Acid Bacteria: Purification and Characterization of the Arginine Deiminase Pathway Enzymes from Lactobacillus sanfranciscensis CB1. Applied and Environmental Microbiology 68, 6193–6201. https://doi.org/10.1128/AEM.68.12.6193-6201.2002

De Angelis, M., Minervini, F., Siragusa, S., Rizzello, C.G., Gobbetti, M., 2019. Wholemeal wheat flours drive the microbiome and functional features of wheat sourdoughs. International Journal of Food Microbiology 302, 35–46. https://doi.org/10.1016/j.ijfoodmicro.2018.08.009

De Vero, L., Iosca, G., Gullo, M., Pulvirenti, A., 2021. Functional and Healthy Features of Conventional and Non-Conventional Sourdoughs. Applied Sciences 11, 3694. https://doi.org/10.3390/app11083694

De Vuyst, L., Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions. Trends in Food Science & Technology, Second International Symposium on Sourdough - From Fundamentals to Applications 16, 43–56. https://doi.org/10.1016/j.tifs.2004.02.012

Fardet, A., Leenhardt, F., Lioger, D., Scalbert, A., Rémésy, C., 2006. Parameters controlling the glycaemic response to breads. Nutr. Res. Rev. 19, 18–25. https://doi.org/10.1079/NRR2006118

Gänzle, M.G., Qiao, N., Bechtner, J., 2023. The quest for the perfect loaf of sourdough bread continues: Novel developments for selection of sourdough starter cultures. International Journal of Food Microbiology 407, 110421. https://doi.org/10.1016/j.ijfoodmicro.2023.110421

Garcia-Vaquero, M., Pastor, K., Orhun, G.E., McElhatton, A., Rocha, J.M.F. (Eds.), 2023. Traditional European Breads: An Illustrative Compendium of Ancestral Knowledge and Cultural Heritage. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-031-23352-4

Garcia-Vaquero, M., Pastor, K., Orhun, G.E., McElhatton, A., Rocha, J.M.F. (Eds.), 2023. Traditional European Breads: An Illustrative Compendium of Ancestral Knowledge and Cultural Heritage. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-031-23352-4

Gluten: A Benefit or Harm to the Body?, 2017. . The Nutrition Source. URL https://nutritionsource.hsph.harvard.edu/gluten/ (accessed 6.16.24).

Hansen, A., Schieberle, P., 2005. Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. Trends in Food Science & Technology, Second International Symposium on Sourdough - From Fundamentals to Applications 16, 85–94. https://doi.org/10.1016/j.tifs.2004.03.007

Hernández-Parada, N., González-Ríos, O., Suárez-Quiroz, M.L., Hernández-Estrada, Z.J., Figueroa-Hernández, C.Y., Figueroa-Cárdenas, J.D.D., Rayas-Duarte, P., Figueroa-Espinoza, M.C., 2022. Exploiting the Native Microorganisms from Different Food Matrices to Formulate Starter Cultures for Sourdough Bread Production. Microorganisms 11, 109. https://doi.org/10.3390/microorganisms11010109

Lau, S.W., Chong, A.Q., Chin, N.L., Talib, R.A., Basha, R.K., 2021. Sourdough Microbiome Comparison and Benefits. Microorganisms 9, 1355. https://doi.org/10.3390/microorganisms9071355

Lhomme, E., Lattanzi, A., Dousset, X., Minervini, F., De Angelis, M., Lacaze, G., Onno, B., Gobbetti, M., 2015. Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. International Journal of Food Microbiology 215, 161–170. https://doi.org/10.1016/j.ijfoodmicro.2015.09.015

Luminaire, L., 2023. Bread: A Symbol of Unity and Cultural Significance – Confident Corn [WWW Document]. URL https://confident-corn.com/latest/bread-significance (accessed 6.12.24).

McGovern, P.E., Glusker, D.L., Exner, L.J., Voigt, M.M., 1996. Neolithic resinated wine. Nature 381, 480–481. https://doi.org/10.1038/381480a0

McGovern, P.E., Zhang, J., Tang, J., Zhang, Z., Hall, G.R., Moreau, R.A., Nuñez, A., Butrym, E.D., Richards, M.P., Wang, Chen-shan, Cheng, G., Zhao, Z., Wang, Changsui, 2004. Fermented beverages of pre- and proto-historic China. Proceedings of the National Academy of Sciences 101, 17593–17598. https://doi.org/10.1073/pnas.0407921102

Plessas, S., 2021. Innovations in Sourdough Bread Making. Fermentation 7, 29. https://doi.org/10.3390/fermentation7010029

Practical Strategies to Help Reduce Added Sugars Consumption to Support Glycemic and Weight Management Goals | Clinical Diabetes | American Diabetes Association [WWW Document], n.d. URL https://diabetesjournals.org/clinical/article/39/1/45/32080/Practical-Strategies-to-Help-Reduce-Added-Sugars (accessed 6.14.24).

Rosell, C.M., 2011. The Science of Doughs and Bread Quality, in: Flour and Breads and Their Fortification in Health and Disease Prevention. Elsevier, pp. 3–14. https://doi.org/10.1016/B978-0-12-380886-8.10001-7

Seal, C.J., Brownlee, I.A., 2015. Whole-grain foods and chronic disease: evidence from epidemiological and intervention studies. Proc. Nutr. Soc. 74, 313–319. https://doi.org/10.1017/S0029665115002104

Slavin, J., 2004. Whole grains and human health. Nutr. Res. Rev. 17, 99–110. https://doi.org/10.1079/NRR200374

Struyf, N., Van Der Maelen, E., Hemdane, S., Verspreet, J., Verstrepen, K.J., Courtin, C.M., 2017. Bread Dough and Baker's Yeast: An Uplifting Synergy. Comp Rev Food Sci Food Safe 16, 850–867. https://doi.org/10.1111/1541-4337.12282

Symnaczik, S., 2022.

The Science of Sugar & How it Plays a Vital Role in Baking | Imperial Sugar [WWW Document], 2016. URL https://www.imperialsugar.com/blog/the-science-of-sugar-how-it-plays-a-vital-role-in-baking (accessed 6.14.24).

Therdthai, N., Zhou, W., 2014. Manufacture, in: Zhou, W., Hui, Y.H., De Leyn, I., Pagani, M.A., Rosell, C.M., Selman, J.D., Therdthai, N. (Eds.), Bakery Products Science and Technology. Wiley, pp. 473–488. https://doi.org/10.1002/9781118792001.ch27

Types of Bread [WWW Document], n.d. . WebstaurantStore. URL https://www.webstaurantstore.com/article/801/bread-types.html (accessed 6.19.24).

Wang, Q., Liu, W., Liti, G., Wang, S., Bai, F., 2012. Surprisingly diverged populations of *S accharomyces cerevisiae* in natural environments remote from human activity. Molecular Ecology 21, 5404–5417. https://doi.org/10.1111/j.1365-294X.2012.05732.x

Xu, J., Bietz, J.A., Carriere, C.J., 2007. Viscoelastic properties of wheat gliadin and glutenin suspensions. Food Chemistry 101, 1025–1030. https://doi.org/10.1016/j.foodchem.2006.02.057

Ye, L., Zheng, W., Li, X., Han, W., Shen, J., Lin, Q., Hou, L., Liao, L., Zeng, X., 2023. The Role of Gluten in Food Products and Dietary Restriction: Exploring the Potential for Restoring Immune Tolerance. Foods 12, 4179. https://doi.org/10.3390/foods12224179