الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University Mohammed Seddik Benyahia - Jijel
Faculty of Nature and Life Sciences
Department of Applied Microbiology and Food
Sciences

جامعة محمد الصديق بن يحي - جيجل
كلية علوم الطبيعة و الحياة
قسم الميكروبيولوجيا التطبيقية و علوم التغذية

Thesis realized by: **Nabila BENHAMADA**

In Fulfillment of the Requirements for the Degree of Doctor of Science

Branch: Biological Sciences

Option: Food Sciences

The traditional underground stored wheat (*Triticum durum*): nutritional quality and selection of lactic acid bacterial strains with antifungal activity

Examiners committee

Chairman: Prof. Mohammed SIFOUR	University Mohamed Seddik Benyahia-Jijel
Supervisor: Prof. Tayeb IDOUI	University Mohamed Seddik Benyahia-Jijel
Examiners: Prof. Djamel Edine KATI	University Abderrahmane Mira-Béjaia
Prof. Abdelhakim AOUF	University Ferhat Abbas-Setif 01
Dr. Hani BELHADJ	University Ferhat Abbas-Setif 01

Academic year: 2021/2022

Acknowledgments

First of all, I thank **Allah** for giving me the strength to endure as well as the courage to face all the obstacles.

I'd want to express my gratitude to my supervisor, Prof. **Tayeb IDOUI**, who enabled me to complete this work. His instruction and counsel guided me through all stages of my project's writing. He's been an excellent teacher, mentor, and thesis supervisor, providing sound advice and encouragement with just the right amount of wisdom and wit. I'm proud of and glad for the opportunity to work with him.

I'd also like to express my gratitude to my committee members, the chairman, Prof. **Mohammed SIFOUR** and the examiners, Prof. **Djamel Eddine KATI**, Prof. **Abdelhakim AOUF** and Dr. **Hani BELHADJ** for their acceptance to evaluate this work.

I give thanks and pray for my **father**, may God have pity on him, who has always awaited the fruit of his upbringing and care for me. I am grateful to my **mother** for her moral and emotional support throughout my life. I would also like to thank my sisters, brothers and friends for blazing a trail I followed in writing my thesis, without forgetting to thank all the members of my in-laws.

Most importantly, I am grateful to my husband, from the Arabic Literature Department, for his help in my doctoral thesis. Thank you so much, **Kamal**.

A special thanks to my sister, Ms. **Ouahiba BENHAMADA** from the department of Applied Microbiology and Food Sciences, for her assistance to thesis redaction. Big thanks go also to Ms. **Rima MOHDEB** from the Ecological Sciences and Agronomic Sciences Department and Ms. **Ghania BOURZAMA** from the Biochemistry Department, University of Badji-Mokhtar, Annaba.

This research would not have been possible without the funding of the Ministry of Higher Education and Scientific Research.

I am also grateful to the staff of the laboratories of Biology, the Laboratory of Biotechnology, Environment and Health and the Laboratory of Molecular Toxicology at the University of Mohamed Seddik Benyahia, Jijel, and their directors: Ms. **Soumia BOUHALI**, Mr. **Boualem MAYACHE** and Ms. **Houria OULED-HADDAR**. I would also like to thank Ms. **Hadia BOURAOUI.**

My sincere thanks also go to Prof. **Saad IBN-SOUDA KORAICHI**, from the University of Sidi Mohamed Ben Abdellah-Fes, Morocco, who offered me access to the laboratory and research resources. A special thanks to the laboratory team: Ms. **Sarra ER-RAHMANI** and **Safae ER-RAOUAN**. Without forgetting Ms. **Amal EL AABEDY** from the city of innovation.

Finally, I'd like to express my gratitude to everyone who has helped me to finish this thesis, no matter how close or far away.

List of content

List of abbreviations

BP: Broken kernels percentage

CA: Controlled atmosphere

DPPH: 2,2-diphényl-1-picrylhydrazyl

FRAP: Ferric reducing assay power

FWF: Fermented wheat flour

HP: Hydrogen peroxide

HW: Hectoliter weight

LAB: Lactic acid bacteria

MA: Modified atmosphere

MRS: Man, Rogosa and Sharpe

PDA: Potato Dextrose Agar

RP: Reducing Power

TFC: Total flavonoid content

TFWE: Traditionally fermented wheat extract

TKW: Thousand kernel weight

TPC: Total phenolic content

UFWF: Unfermented wheat flour

WJ: Wheat sample from Jijel

WM: Wheat sample from Mila

List of figures

List of tables

Abstract

The aim of this study can be summarized in three points: Firstly, the nutritional quality of traditionally fermented wheat (locally named: Mzeyet) was determined, followed by an assessment of the organoleptic acceptance of the bread produced from composite traditionally fermented wheat flour. Secondly, the impact of natural fermentation on its antioxidant properties was also conducted. Finally, 32 lactic acid bacteria strains were isolated and assessed for their antifungal activity against *Aspergillus niger* and *Aspergillus flavus*.

The results showed that samples of traditionally fermented wheat are more or less rich in proteins (4.59%), crude fibers (1%), fat (11.29%), polyphenols (10.48 mg AGE/g) and flavonoids (7.99 mg QE/g). They also show interesting antioxidant activity. Furthermore, the bread made with a mixture of 10% traditional fermented wheat flour was highly appreciated and had the best sensory qualities. The total phenolic and total flavonoid contents were significantly increased in fermented wheat. Moreover, the antioxidant activity was more effective in fermented wheat. Also, the results showed that the majority of LAB strains presented interesting antifungal activity and the most potent strain, identified as *Lactiplantibacillus plantarum* LS011, had the highest activity against the fungal species. Moreover, the partial characterization of the antifungal compounds suggests that they are organic acids.

Key words: Traditionally fermented wheat, quality, bread, antioxidant activity, lactic acid bacteria, *Lactiplantibacillus plantarum*, *Aspergillus*, antifungal activity, biopreservation.

Résumé

L'objectif de cette étude peut être résumé en trois points : Dans un premier temps, la qualité nutritionnelle du blé fermenté traditionnellement (nom local : Mzeyet) a été déterminée, suivie de l'évaluation de l'acceptabilité organoleptique du pain produit à partir de farine composite de blé fermenté traditionnellement. Dans un second temps, l'impact de la fermentation naturelle sur ses propriétés antioxydantes a été mené. Enfin, 32 souches de bactéries lactiques ont été isolées et évaluées pour leur activité antifongique contre *Aspergillus niger* et *Aspergillus flavus*.

Les résultats ont montré que les échantillons de blé traditionnellement fermenté sont plus ou moins riches en protéines (4,59 %), fibres brutes (1 %), lipides (11,29 %), polyphénols (10,48 mg AGE/g) et flavonoïdes (7,99 mg QE/g). Ils montrent également une activité antioxydante intéressante. De plus, le pain fait avec un mélange de 10 % de farine de blé fermenté traditionnel était très apprécié et présentait les meilleures qualités sensorielles. Les teneurs en phénols totaux et en flavonoïdes totaux étaient significativement augmentées dans le blé fermenté. De plus, l'activité antioxydante était plus efficace. Les résultats ont aussi montré que la majorité des souches de bactéries lactiques présentaient une activité antifongique intéressante et que la souche la plus puissante identifiée comme *Lactiplantibacillus plantarum* LS011 avait l'activité la plus élevée contre les espèces fongiques. En fin, la caractérisation partielle des composés antifongiques suggère qu'il s'agit d'acides organiques.

Mots clés : Blé traditionnellement fermenté, qualité, pain, activité antioxydante, bactéries lactiques, *Lactiplantibacillus plantarum, Aspergillus*, activité antifongique, bioconservation.

ملخص

يمكن تلخيص الهدف من هذه الدراسة في ثلاث نقاط: أولاً، تم تحديد الجودة الغذائية للقمح المخمر تقليديًا (المسمى محليًا: المزيت)، متبوعًا بتقييم القبول الحسي للخبز المنتج من دقيق القمح المركب المخمر تقليديًا. ثانياً، تم إجراء تأثير التخمر الطبيعي على خصائصه المضادة للأكسدة. أخيرًا ، تم عزل 32 سلالة من البكتيريا اللبنية وتقييم نشاطها المضاد للفطريات ضد *niger Aspergillus flavus* و *Aspergillus*.

أظهرت النتائج أن عينات القمح المخمر تقليديًا غنية بالبروتينات (4.59٪) والألياف الخام (1٪) والدهون (11.29٪) والبوليفينول (10.48 مجم AGE / جم) والفلافونويد (7.99 مجم QE / جم). كما أنها أظهرت نشاطًا مثيرًا للاهتمام كمضاد للأكسدة. علاوة على ذلك، فإن الخبز المصنوع من خليط 10٪ من دقيق القمح المخمر التقليدي كان محل تقدير كبير ولديه أفضل الصفات الحسية ، كما زاد محتوى الفينول الكلي والفلافونويد الكلي في القمح المخمر بشكل ملحوظ. علاوة على ذلك ، كان النشاط المضاد للأكسدة أكثر فعالية في القمح المخمر. أظهرت النتائج أيضًا أن غالبية سلالات البكتيريا اللبنية أظهرت نشاطًا مضادًا للفطريات مثيرًا للاهتمام وأن السلالة الأكثر فاعلية التي تم تحديدها على أنها *Lactiplantibacillus plantarum* LS011 كان لها أعلى نشاط ضد الأنواع الفطرية. و في الأخير، يوحي الوصف الجزئي للمركبات المضادة للفطريات بأنها أحماض عضوية.

الكلمات المفتاحية: قمح مخمر تقليديًا ، جودة ، خبز ، نشاط مضاد للأكسدة ، بكتيريا لبنية ، *Lactiplantibacillus plantarum*، *Aspergillus* ، نشاط مضاد للفطريات ، حفظ حيوي.

Introduction

Cereals are considered one of the most important sources of dietary protein, carbohydrates, vitamins, minerals and fiber for people all around the world (**Kohajdovà and Karovicovà, 2007**). Several methods are employed by Algerian farmers to store cereals, but the traditional techniques are still used and give special products. Such techniques are based on the use of underground holes or silos built near the farm at generally high places. These stuffs are called 'Matmours'. This method gives durum wheat a brown color and a very strong acid odor, following the natural fermentation due to native micro-organisms, giving rise to the traditional fermented wheat locally named 'Mzeyet' and in other parts of Algeria it is called 'Elhammoum', which is used as the main ingredient in the preparation of a valued traditional Algerian dish (couscous).

Cereals being a major source of nutrients are still deficient in some basic nutritional components. In this regard, fermentation of cereals comes to extend shelf life, improve palatability, digestibility, nutritive value (**Shekib, 1994; Holzapfel, 2002**) texture, taste and aroma (**Deshpande, 2000; Kohajdovà and Karovicovà, 2007**). Fermented foods are considered prominent constituents of the human diet because of their content of health-promoting compounds (**Şanlier et *al*., 2019**). It has been established that micro-organisms start to modify plant constituents during fermentation (**Tangyu et *al*., 2019**). Such fermentation reduces carbohydrates and non-digestible polysaccharides and oligosaccharides and increases some amino acids as well as vitamins of group B. When cereals are fermented with lactic acid bacteria (LAB), anti-nutrients such as tannin and phytic acid decrease and antioxidant activities increase (**Đorđević et *al.*, 2010**).

Several authors have demonstrated that the spontaneous fermentation of cereals can involve LAB, yeasts and molds (**Kohajdovà and Karovicovà, 2007; ViéraDalodé et *al.*, 2007**). Lactic acid fermentation processes are the oldest and most important economical forms of production and preservation of food for human consumption (**Blandino et *al*., 2003**). This fermentation exerts chemical changes in food accelerated by enzymes of LAB (**Holzapfel, 2002**). LAB have been used in domestic food production for centuries, not only because they were tasty but also because they allowed food to be stored for a long period of time (**Beristain-Bauza et *al*., 2016; Vida Plavec and Berlec, 2020**). They present antagonistic activity towards a significant number

of pathogenic microorganisms **(Djadouni and Kihal, 2012; Cheong et *al*., 2014; Nuraida, 2015).**

Various pathogenic microorganisms, including fungi, which contaminate the nutrients of humans and animals, can be potentially dangerous agents and cause economic losses **(Leyva Salas et *al*., 2017)**. Mycotoxinogenic molds such as *Aspergillus*, *Fusarium* and *Penicillium* play an undeniable role in the deterioration of the marketable quality and hygiene of foodstuffs by synthesizing highly toxic metabolites known as mycotoxins **(Dalié et *al*., 2010; Daou et *al.*, 2021)**. Increased attention has recently been focused on these toxins, since they have been linked to several mycotoxicoses in humans and animals, including gastrointestinal infections, carcinogenicity, mutagenicity and teratogenicity **(Barac, 2019).**

The presence of these microorganisms or their toxins in foods causes serious problems. Therefore, consumers are very concerned about chemical preservatives and processed foods, but they readily accept LAB as a natural way to preserve food and promote their health **(Dopazo et *al*., 2021).** Within this context, the use of biopreservation appears promising to prevent mycotoxinogenic fungal growth.

Through this work, we are interested in:

Firstly, examining the current knowledge of a traditional Algerian fermented food (fermented wheat, Mzeyet or Elhammoum) to provide more information regarding its biochemical characteristics and nutritional quality, as well as to evaluate its potential to be used as a supplement in backing technologies.

Secondaly, clarifying the role of spontaneous fermentation in increasing the antioxidant features of fermented wheat with the perspective of producing a functional ingredient or dietary supplement.

Finally, presenting the ability of LAB isolated from the Algerian traditional fermented wheat and their metabolites to inhibit the growth of *Aspergillus niger* and *Aspergillus flavus*.

Literature review

I. Wheat grain

I.1. Grain anatomy

Wheat grains, also known as caryopsis, are normally oval in shape, though they can range from practically spherical to long, narrow, and flattened shapes depending on the wheat variety. The grain is usually between 5 and 9 mm in length, 35 and 50 mg in weight, and has a crease down one side where it was originally connected to the wheat flower **(Šramkováa et *al*., 2009)**. Wheat grain has been reported to contain 2 - 3 % germ, 13 - 17 % bran and 80-85 % mealy endosperm **(Belderok et *al*., 2000) (Fig. 1).**
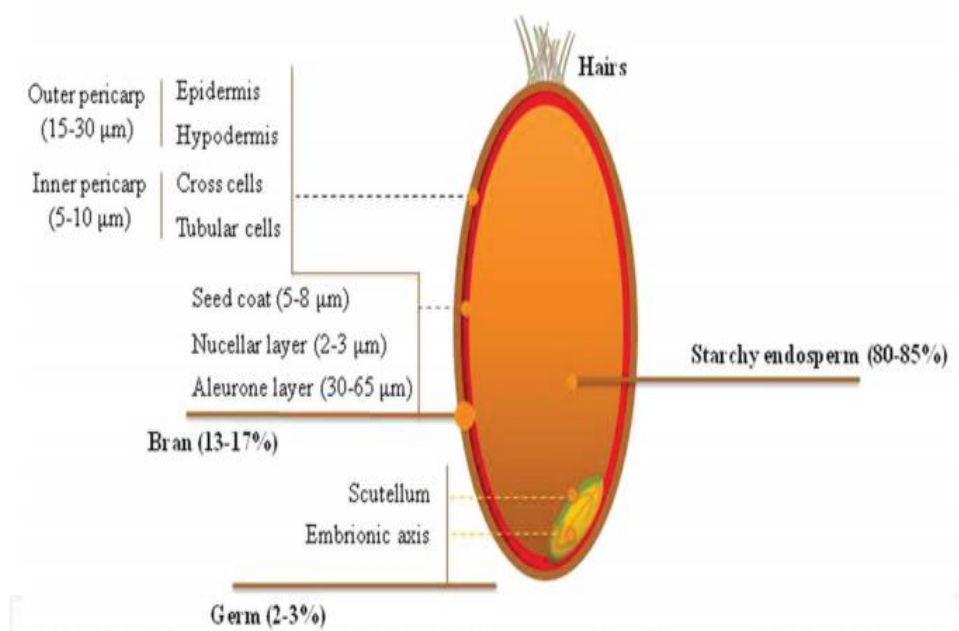
Fig. 1. Wheat grain fractions **(Arshad et *al*., 2017)**

I.1.1. Wheat bran

Wheat bran (WB) is multi-layered and consists of different cell types with different chemical compositions. It is a by-product of the wheat milling process, which is composed of more than 40 % dietary fiber. WB fiber has a complicated chemical composition, but it mostly consists of cellulose and pentosans, which are xylose and arabinose-based polymers that are strongly bonded to proteins. These polymers are present in the cell walls of wheat and in layers of cells such as the aleurone layer. WB is also rich in vitamins, minerals and active compounds, such as alkyl resorcinols, ferulic acid, flavonoids, carotenoids, lignans and sterols. WB has been used for food

and non-food applications **(Šramkováa et *al*., 2009; Apprich et *al*., 2013; Andersson et *al*., 2014; De Brier et *al*., 2015)**; 90 % is used as a livestock feed and only 10 % is used in the food industry **(Hossain et *al*., 2013)**. It is an important source of bioactive compounds, which are related to the human health-protective mechanisms of whole-grain cereals in general. It can be used as a food ingredient or natural antioxidant additive **(Fardet, 2010; Rebolleda et *al*., 2020)**.

I.1.2. Germ

The germ is found at one of the grain's ends. It's rich in protein (26 %) and sugar (17 %), as well as fats (8 - 13 %). In addition, the mineral content is quite high (4.5 %). Wheat germ (WG) is sold separately because it contains a significant amount of vitamin E. It contains half the amount of glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are doubled **(Cornell, 2003; Bin and Ying, 2011)**.

The most nutritious part of the wheat grain is represented by the germ. However, its application is very limited to staple foods. First, because of its high content of unsaturated fatty acids and the high activity of lipase and lipoxygenase, which produce rancidity more rapidly in storage (it has a limited shelf life) **(Sj¨ovall et *al*., 2000; Li et *al*., 2016)**. Second, it contains phytic acid, raffinose, and wheat germ agglutinin, which are well known as anti-nutritional factors **(Zhao et *al*., 2020)**.

I.1.3. Endosperm

The endosperm is surrounded by both the fused pericarp and the seed coat. The aleurone layer of the outer endosperm is made up of a single layer of cubic shaped cells. This aleurone layer is high in proteins and enzymes, both of which are essential for germination. Mealy or starchy endosperm refers to the inner endosperm. The endosperm mainly contains food reserves; it contains most of the energy reserves that nourish the seedling at the time of germination. Aside from carbohydrates, the mealy endosperm contains fats (1.5 %) and proteins (13 %): albumins, glubulins, and the main proteins of the gluten complex, glutenins and gliadins. Mineral (ash) and dietary fiber content is low: at 0.5 % and 1.5 %, respectively **(Belderok et *al*., 2000; Feillet, 2000)**.

I.2. Grain composition and nutritional quality

I.2.1. Proteins

Wheat is an essential source of protein; it contains 8 - 20 % of proteins. Wheat proteins are classified into several groups on the basis of their extrability and solubility in various solvents, genetic background and amino acid composition **(Loponen et *al*., 2004; Šramkováa et *al*., 2009)**. The most well-known classification system classifies the wheat proteins into albumins, globulins, gliadins and glutenins on the basis of solubility **(Osborne, 1924)** **(Table 1).**

Table 1. Classification of wheat proteins on the basis of solubility **(Osborne, 1924)**

Proteins		Soluble in	Location in
Non-gluten protein	Albumins	Water	Embryo (metabolic proteins) and endosperm cells (cytoplasmic proteins)
	Globulins	Dilute salt solution (0.5 M NaCl)	Embryo and aleurone layer (storage proteins) and endosperm cells (Cytoplasmic proteins)
Gluten proteins	Gliadins	70-80 % ethanol	Endosperm (storage proteins)
	Glutenins	Dilute acids or alkali solutions (0.05 M acetic acid)	

The amounts of different proteins vary according to the type of wheat, cultivars and growing conditions **(Johansson et *al*., 2000).** Gliadins and glutenins are the two main wheat storage proteins found in the grain's starchy endosperm cells **(Belderok et *al*., 2000; Shewry et *al*., 2002).** The albumin and globulin groups in wheat grains include structural proteins as well as physiologically active proteins (enzymes). They are concentrated in cereal seed coats, aleurone cells, and germs, with a slightly lower concentration in mealy endosperm. They represent about a quarter of the total grain proteins **(Belderok et *al.,* 2000).**

I.2.2. Carbohydrates

Cereals are the dominant source of carbohydrates in the global diet, providing essential food energy **(Poole et *al*., 2020).** At maturity, the wheat grain consists of 85 % (w/w) carbohydrate, 80

% of which is starch, approximately 7 % low molecular mass mono-, di-, and oligosaccharides and fructans and about 12 % cell wall polysaccharides **(Stone and Morell, 2009).** Carbohydrates can be divided into available (not resistant starch: NRS) and unavailable (dietary fiber: resistant starch: RS) based on their digestion and absorption by humans **(Southgate, 1991)**.

Starch is the major polysaccharide of wheat grain; it is present in its endosperm **(Mallick et *al*., 2013).** At least two types of polymers are distinguishable chemically: amylose and amylopectin **(Šramkováa et *al*., 2009)**. Among starches, the ratio of amylose to amylopectin differs. The level of the two forms in wheat flour is 25 - 28 % and 72 - 75 %, respectively **(Shibanuma et *al*., 1994).**

On the other hand, dietary fiber is an important component present in whole grains and is believed to be (at least partially) responsible for health benefits. Wheat has a total dietary fiber content ranging from 9 to nearly 20 % (dry weight basis), with both insoluble and soluble fractions **(Padayachee et *al*., 2017; Gartaula et *al*., 2018)**. Arabinoxylan and β-D-glucan are the major types of dietary fiber components in the cell walls of starchy endosperm cells in wheat. Small amounts of cellulose and glucomannans may also be present in these cell walls. Wheat endosperm typically has a low cellulose concentration (5%) **(Evers et *al*., 1999; Prasadi and Joye, 2020).**

I.2.3. Lipids

Lipids are only found in trace amounts in cereals (2 %), but they have a substantial impact on the quality and texture of foods due to their capacity to form inclusion complexes with proteins and starch due to their amphipathic nature **(Ruibal-Mendieta et *al*., 2004)**. In addition, the bran, carbohydrates, and proteins of the endosperm are connected with considerable levels of germ lipids (11 %). The majority of associated lipids are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serin, as well as lysophosphatidyl derivates. The principal sterols were identified as β-sitosterol, campesterol and C28 and C29 saturated sterols **(Cornell, 2003).**

I.2.4. Antioxidants

Cereals, fruits and vegetables are major sources of antioxidants, containing hundreds of β-carotene, vitamins and trace elements **(Miller et *al*., 2000)**. Wheat grains and fractions present significant levels of antioxidant activity as well as a variety of phytochemicals such as phenolic acids (ferulic and vanillic acids), carotenoids, and tocopherol. The beneficial phytochemicals are

mostly present in the aleurone fraction of wheat bran **(Yu, 2007; Singh et *al*., 2012; Li et *al*., 2015**; **Arshad et *al*., 2017; Dhillon et *al*., 2020)**. These antioxidants vary according to growth location **(Yu et *al*., 2003; Cheng et *al*., 2006)**.

Wheat antioxidants have strong interaction with free radicals and convert the radicals to less reactive components, showing radical scavenging capacities. Besides their capacity to interact with reactive oxygen species (ROS), wheat antioxidants may have other biological activities. They may have potential in chemoprevention of breast and prostate cancers, osteoporosis, and cardiovascular diseases **(Mazur and Adlercreutz, 1998; Begum et *al*., 2004).**

II. Wheat storage

II.1. Main storage methods

Since prehistoric times, many storage systems and procedures have been devised with the aim of extending the use of seasonal food resources beyond the period when they are available **(Pena-Chocarro et *al*., 2015).** In Africa, there are three main storage techniques with differing architectures: traditional / local storage, which includes local cribs and rhombus, platforms, open fields, roofs and fire places; improved / semi modern grain storage techniques, which include ventilated cribs, improved rhombus and brick bins; and modern centralized storage at the commercial level involving silos and warehouses. The first two storage techniques predominate since farming is mostly done by subsistence farmers **(Adesuyi et *al*., 1980; Udoh et *al*., 2000).** Whether traditional or modern, there are five main storage methods for cereals. During the grain storage period, each has numerous advantages and disadvantages **(Pekmez, 2016):**

II.1.1. Underground storage

Underground storage for long-term conservation has become a critical component of farming communities' cereal surplus handling. Grain is said to be preserved for years in underground pits. The pits keep the grain cool and are airtight in some cases. The grain on top and around the edges, on the other hand, is frequently mouldy **(Bhardwaj, 2014).**

II.1.1.1. Underground storage in Algeria

The storage in underground pits still exists in Algeria, these pits are called locally 'Matmours' **(Fig. 2)**. In the past, the storage was used just to store wheat and ensure consumption throughout the year, but currently the purpose of this storage is to obtain a traditional product called 'Mzeyet' to use to prepare black couscous in the North-Eastern regions of Algeria. The concerned regions

are: Jijel, Mila, Setif and Constantine. The 'Mzeyet' obtained is dark brown in color and has an accentuated acid odor due to spontaneous fermentation through the native microflora of wheat, which is provided especially by LAB.
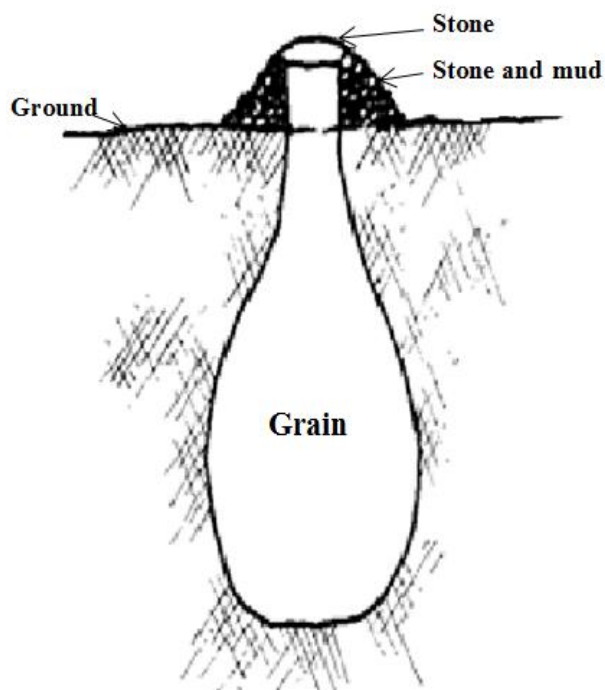
Fig. 2. Sectional view of an underground silo (matmour) **(Lindblad and Druben, 1980).**

II.1.1.2. Structure of underground pits

The underground pits had various capacities and could contain more than 1000 kg. The silos could be placed outside or inside the houses. In general, the mouth was round, large enough to allow one person to go through, and bell-shaped in cross section. The top of the pit is sealed by using a flat stone and mud or cow dung to ensure an air-tight structure and to prevent the entry of water and pests. If pits are dug into soil, the soil must be compact and hard to avoid as far as possible the infiltration of water **(Pena-Chocarro et *al*., 2015).**

This storage method in undergrowth silos 'Matmours' **(Fig. 2)** still exists in Algeria. In the past, it was used just to store wheat and ensure consumption throughout the year, but currently the purpose of this storage is to obtain a traditional product called 'Mzeyet' to use to prepare black couscous in the North-Eastern regions of Algeria. The concerned regions are: Jijel, Mila, Setif

and Constantine. The 'Mzeyet' obtained is dark brown in color and has an accentuated acid odor due to spontaneous fermentation through the native microflora of wheat, which is provided especially by lactic acid bacteria.

II.1.1.3. Spontaneous fermentation in underground pits

Fermentation is an exothermic metabolic process which involves the consumption of food nutrients through the activities of microorganisms (either native or deliberately introduced) that serve as fermenters. Spontaneous fermentation of cereals in underground pits involves LAB, yeasts and fungi. The fermentation with filamentous fungi, LAB, and yeast may be considered as a potential process to increase the release of phenolic compounds contributing to the production of food products with an added value. In fact, during growth of microorganisms due to the action of cellulolytic, pectinolytic and ligninolytic enzymes, the profile of phenolic compounds is changed. These enzymes, produced by microorganisms, are involved in release of phenolic components by softening the kernel structure and breaking down of cell wall matrix **(Sandhu et al., 2017).**

During fermentation of cereals, several authors reported an increase in protein and amino acids (AAs) with the authors attributing this to the increase activities of hydrolytic enzymes, the degradation of complex proteins to AAs through proteolysis as well as the production of additional AAs during fermentation. It was suggested that the degradation of storage protein and synthesis of new protein could have caused this increase **(Adebiyi et al., 2017) (Fig. 3).**

II.1.2. Bulk storage

Grain can be stored and preserved in both vertical and horizontal warehouses. The surface of bulk stacked cereals must be adequately leveled in this method (wheat, barley, rye, oat, corn, chickpea and lentil). On a unit area basis, more grain can be stored. It also makes grain sample control simple, reduces personnel costs, and saves time **(Pekmez, 2016).** The bulk storage system is being improved further by installing a pest-monitoring system (acoustic detection) and automation for aeration, grain cooling, and pest-control measures **(Rajendran, 2003).**
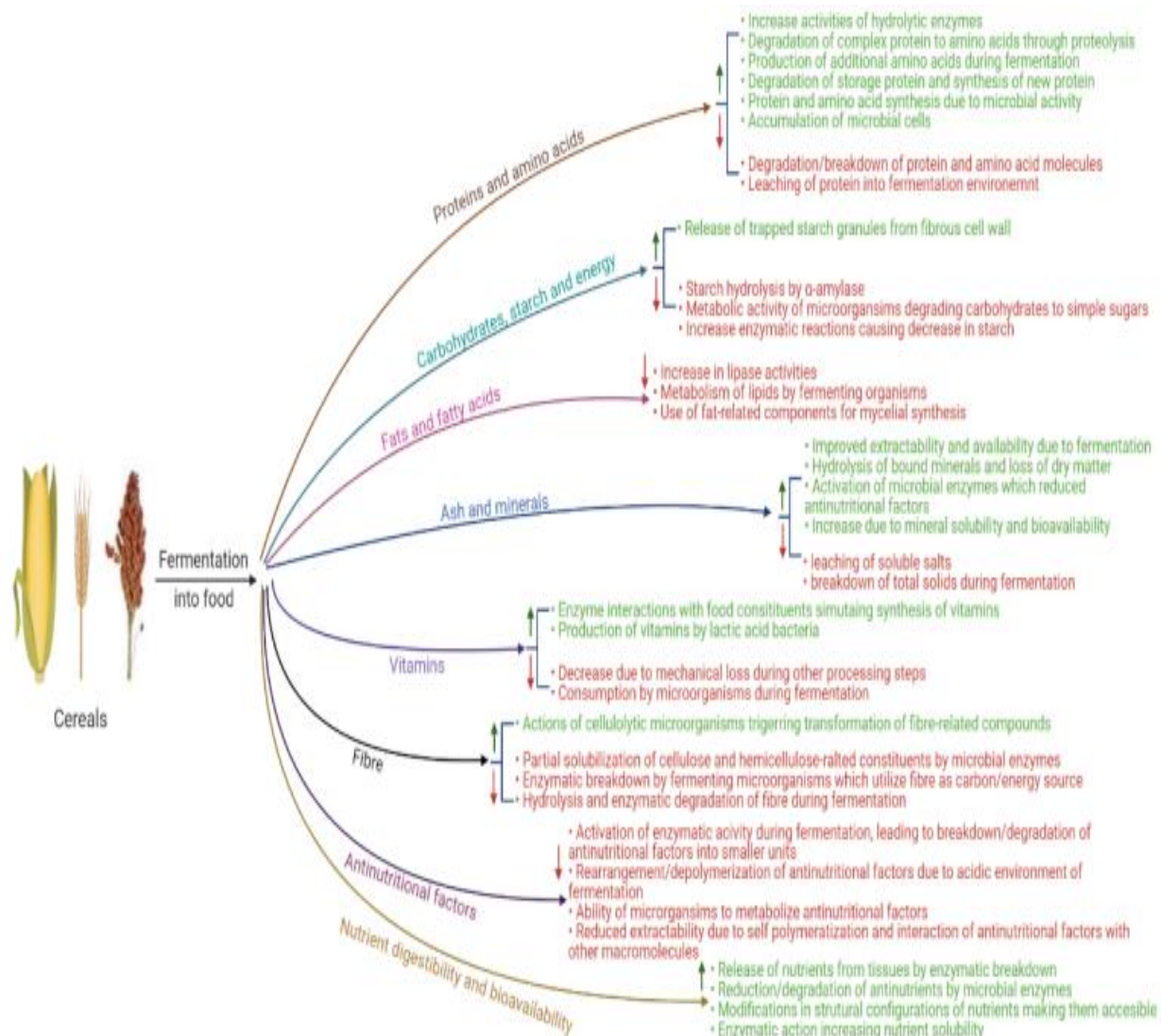
Fig. 3. A summarized mechanisms of nutrient modifications in fermented cereals (**Adebo et *al*., 2022**).

II.1.3. Storage in bag

This is the most commonly used method of grain storage in several countries in any of a variety of buildings, e.g. with or without plastered walls, and with an earth, stone, or cement floor, and a corrugated iron or thatched roof, stone, local brick, corrugated iron, or mud and wattle. Bag storage increases operating expenses, increases pest losses, and increases spillage. Furthermore, if the flooring is not properly constructed, water infiltration occurs. This raises the humidity in the

warehouse, allowing *Cryptolestes* spp. to multiply; it also destroys the bags' bottom layer **(Semple, 1992; Rajendran, 2003).**

II.1.4. Storage in warehouse

A warehouse is built for the storage and physical protection of grains or bagged grains. It may also include materials and equipment required for the packaging and handling of bagged grain, and chemicals to control storage pests. Some factors such as topography, soil characteristics, accessibility, orientation and proximity to human dwellings should be considered when locating the warehouse. In this technique, the cereal and cereal products could be stored in bulk and also in sacks **(Pekmez, 2016).**

II.1.5. Storage in silo

Silos are an efficient method of storing grain. Bulk grain is space-saving and can be processed mechanically, reducing packaging and processing costs. Recycling grain in silos helps through aeration to reduce potential increases in grain temperature. This is essential in silo management. For bulk grain storage, there are many types of silos in varied sizes. Silos are made of concrete, bricks, or sheet metal that is fastened together **(Nwaigwe, 2019).**

II.1.6. Advanced storage methods

In many industrialized countries, grain storage methods have evolved with advances on the primary systems, such as aeration, refrigerated storage, modified atmospheric storage, and hermetic storage systems **(Said and Pradhan, 2014).**

II.1.6.1. Storage by aeration of grain

Aeration is a widely used technique for the preservation of stored grain. It is defined as the forced circulation of grain-quality surrounding air, or air that has been properly conditioned, through a mass of grain in order to improve grain storability. Aeration is an appropriate method of lowering grain temperature, and it is accomplished by the use of fans and mechanical aeration. This system is designed for low-humidity environments. Commercially, forced aeration plays an important and effective role in preserving grain **(Navarro and Noyes, 2001).**

II.1.6.2. Refrigerated storage

The decrease of the temperature below 18 °C in subtropical climates when ambient temperatures are too high to reduce insect activity is a principal objective of refrigerated aeration. Cooled ambient air is passed over the bulk grains using the current aeration system. This storage

technique, combined with the air drying method, can provide information on the feasibility of aeration for safe commercial storage in tropical climates **(Navarro and Noyes, 2001; Navarro and Navarro, 2016)**.

II.1.6.3. Modified atmosphere technology

It is a new gaseous application technology, modified atmospheres, that has successfully replaced fumigants **(Navarro and Navarro, 2016)**. Modified atmospheres (MA) and controlled atmospheres (CA) offer alternatives to the use of chemical residues to control pests that attack stored grains, oilseeds, processed products and some packaged foods. CA systems also prevent fungal growth and maintain product quality **(Said and Pradhan, 2014)**.

II.1.6.4. Hermetic storage

In this method, the generation of an oxygen-depleted and carbon dioxide-enriched interstitial atmosphere is a result of the respiration of the aerobic organisms living in the commodity. By reducing O_2 and increasing CO_2 concentrations through respiratory metabolism, insects and other aerobic organisms in the grain, as well as the grain itself, can create the changed atmosphere. Low O_2 and high CO_2 environment kills insect and mite pests and prevents aerobic fungi from growing, which reduces losses due to the activity of these last **(Weinberg et *al*., 2008; Navarro and Navarro, 2016)**.

II.2. Losses during storage

II.2.1. Insects

Insects are the first invaders and one of the main threats to the maintenance of grain quality during storage. They consume, contaminate, and disseminate microorganisms **(Rajandran, 2003)**. It is critical to monitor and diagnose stored-grain insect infestations early in order to take timely and effective pest control measures to protect stored grains **(Cai et *al.*, 2022)**. Insect infestation-related losses of stored commodities are usually solely measured in terms of weight loss. In fact, insect infestation has an impact on nutritional value, with some nutritional components being more severely altered than others **(Stathers et *al*., 2020)**. Over 100 insect species contaminate stored grain. The majority of them are beetles, some of them are moths, and the rest are primitive insects known as psocids **(Rajandran, 2003)**.

II.2.2. Mites

At all stages of processing and during its storage, mites are very important pests of wheat and cereals in general. They are very difficult to observe with the naked eye and can lead to serious economic losses if proper management has not been taken into account. The mites are the cause of both the qualitative and quantitative losses. They preferably feed on germ and demolish its contents; they also consume the other parts of the grain, but to a lesser extent (**Mahmood et** *al.***, 2012; Mahmood et** *al.***, 2013**).

II.2.3. Rodents

Rats and mice may cause considerable damage to crops in the field and products in storage. This can occur in various ways (**Hayma, 2003**):

- ✓ consumption of part of the product;
- ✓ contamination of part of the product with their excrement;
- ✓ damage to buildings, storage containers and packing material;
- ✓ they are also carriers of diseases which are harmful to humans**.**

II.2.4. Micro-organisms

Field and storage micro-organisms including fungi, yeasts, and bacteria have been implicated in the deterioration of grains during storage. Yeasts predominate in sealed silos when oxygen levels are low and grain moisture is high. Bacteria grow in grains at a water activity exceeding 0.9 % and subsequently cause grain degradation. The sources of contamination by both field and storage fungi are soil and decaying debris, but they may also be present in harvesting and grain-handling equipment. Important field fungi that infect the grain in the standing crop or on the farm before threshing are *Alternaria*, *Cladosporium*, *Fusarium*, and *Drechsclora* spp. They infect the grains that have more than 20 % moisture. The damaging effects of field fungi are stopped as the grain moisture comes down during storage; the field fungi either die or remain as dormant mycelium in the grain (**Rajandran, 2003; Bala, 2016**).

II.3. Effects of storage conditions on wheat quality

II.3.1. Moisture

In stored grains of uniform moisture content, the moisture will move if a temperature gradient exists in the bulk as a result of temperature variations surrounding the grain bin. If the grain's moisture content remains constant, the equilibrium relative humidity of the air surrounding the

grain rises as the temperature rises. As a result, with stored grains with homogeneous moisture content, a relative humidity gradient exists parallel to the temperature gradient **(Bala, 2016).**

II.3.2. Temperature

Temperature is a crucial factor limiting the distribution and abundance of insects, mites and fungi contaminating and destroying stored grain **(Bala, 2016).** The temperature of wheat increases during storage. Insect infestation is the reason behind the increase in temperature in wheat grains. Insects not only consume grains as a source of growth (energy), but also respirate and emit heat into the environment **(Sawant et *al*., 2012) (Fig. 4).**
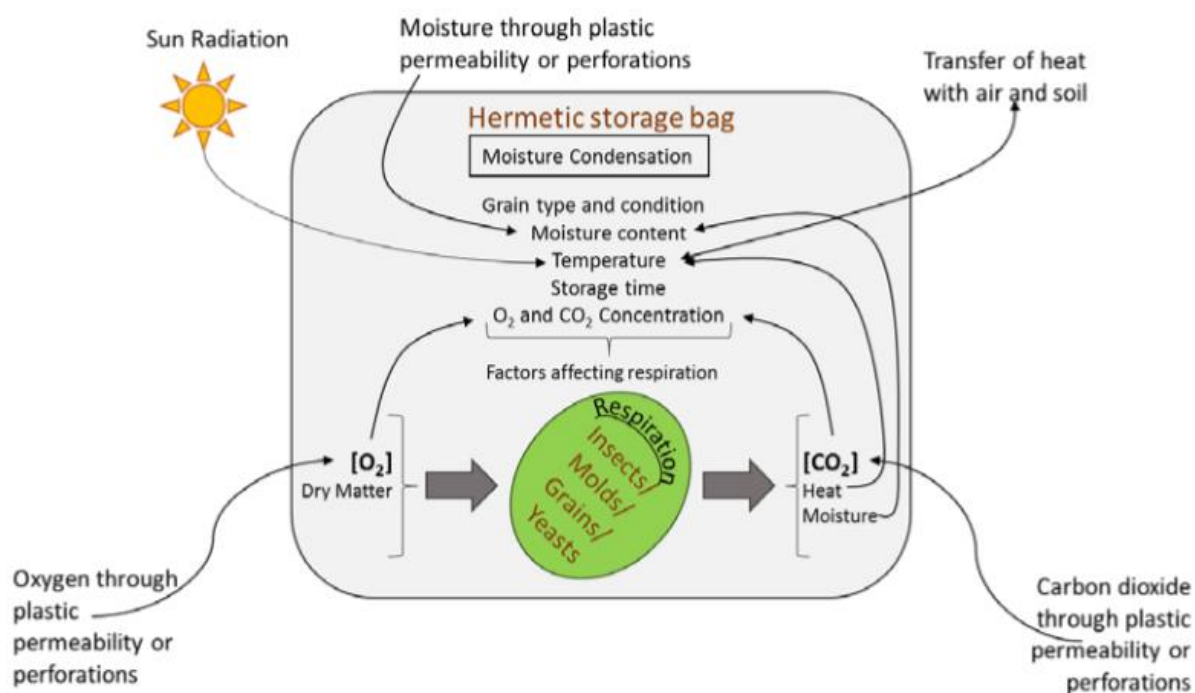
Fig. 4. Factors affecting the grain and microorganism respiration in the hermetic storage **(Rodriguez et *al*., 2008).**

II.3.3. Biochemical changes

During storage, protein and lipid content change, which is related to respiration (**Rehman and Shah, 1999).** The protein content of the wheat during storage decreases. This decrease is attributed to the proteolytic activity of the stored wheat which increased by higher temperature and humidity conditions. The endopeptidases cleave peptide bonds within the chain whereas the exopeptidases cleave single amino acids from either the carbon or nitrogen end of the molecule

(**Bakker-Arkema et** *al.***, 2007**). Otherwise, molds can use the nutrients in the wheat including proteins for their growth and survival which might have caused the decrease in protein content of the wheat during storage (**Mhiko, 2012**).

III. Wheat microflora

III.1. Microbial contamination of grains

During crop growth, harvesting, and postharvest drying and storage, cereal grains are subject to microbial contamination. Air, dust, water, dirt, insects, birds, and rodent feces, as well as contaminated equipment and improper handling, are all causes of contamination (**Magan and Aldred, 2007; Los et** *al.***, 2018**) (**Fig. 5**).

Generally, the microflora of cereals and cereal products is varied and includes molds, yeasts, bacteria (psychrotrophic, mesophilic, and thermophilic/thermoduric), LAB, spore forming bacteria (*Bacillus spp.*), bacterial pathogens, coliforms, and Enterococci. Bacterial pathogens that contaminate cereal grains and cereal products and cause problems include *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. Coliforms and enterococci also occur as indicators of unsanitary handling and processing conditions and possible fecal contamination (**Bullerman and Bianchini, 2009**).

Fungal contamination is one of the major causes of food spoilage. It not only brings about great economic losses but also represents a high risk for human and animal health through the synthesis of mycotoxins (**MacDonald et** *al.***, 2004; Tutelyan, 2004**).

Various species of fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* are the crucial contamination agents of cereal grains (**Erdenetsogt et** *al.***, 2019**). Toxicogenic *Alternaria* and *Fusarium* species are often classified as field fungi, while *Aspergillus* and *Penicillium* species are considered storage fungi (**Logrieco et** *al.***, 2003**).

III.1.1. Field microflora

The grains are infected by microorganisms known as field microflora depending on the time of harvest and the conditions under which the crops were cultivated. The contamination of the kernels is predominated by bacteria, with yeast being the second most abundant component. However, the number of filamentous fungi increases during the latter stage of ripening (**Noots et** *al.***, 1999; Nierop, 2006**).
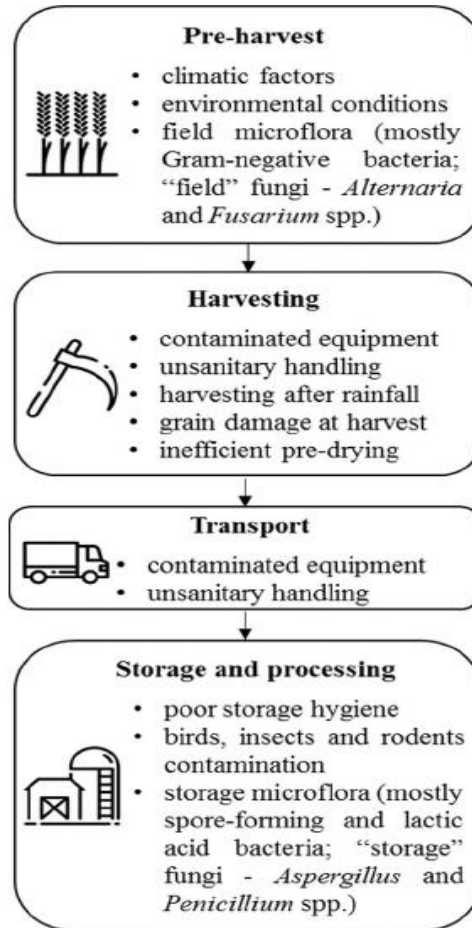
Fig. 5. Sources and factors of microbial contamination during cereal grain processing (**Los et *al*., 2018**)

Arguably, bacterial species contaminating grain are generally non-pathogenic compared with contamination by bacterial pathogens such as *Salomonella, Escherichia coli* and *Bacillus cereus*. *Salmonella* and *E. coli* are enteric bacteria, and their presence on grain is usually an indication of fecal contamination from birds or rodents. This can happen during harvesting, but it's more likely to happen as a result of inadequate hygiene in road or rail trucks or poor pest management during storage. Contamination levels with enteric pathogens are relatively low (**Hocking, 2003**).

Fungi that grow on crops have traditionally been split into two categories: field and storage fungi (**Pitt and Hocking, 2009**). Field fungi are fungi that invade seeds developing on the plants in the field or after the seeds have matured and the plants are either still standing or cut and swathed, awaiting threshing. Field fungi consist primarily of species of *Alternaria*, *Cladosporium*,

Fusarium, and *Helminthosporium*, which infest grain in the field at high water activity and high relative humidities, reaching 90 % to 100 % **(Bullerman and Bianchini, 2009).** Colonization of the grain by *Alternaria* spp. leads to a black fungus discoloration, observable both on the surface of the kernels and under the pericarp; such contamination is favored by high rainfall just before harvest **(Kosiak et *al*., 2004).**

III.1.2. Storage microflora

Though modern methods are used during harvesting and proper storage practices are maintained to minimize contamination and infestation by microorganisms and pests, these conditions are not always met **(Los et *al*., 2018).**

Generally, wheat grain stored under ideal conditions has water activity below the minimum needed for microbial growth. These storage conditions are unfavorable for the growth of bacteria that is why bacteria are not significantly involved in the spoilage of dry grain. However, pathogenic and spoilage microorganisms may survive in a dormant state and be transferred to processed products where they become a problem **(Bullerman and Bianchini, 2009; Just´e et *al*., 2011)**. Enteric pathogens, such as *Salmonella* spp., may be among these microflora if fecal contamination has occurred anywhere from preharvest through milling. Other spore-forming bacterial pathogens, such as *Bacillus cereus* and *Clostridium botulinum*, may be present as well due to soil contamination. Nonpathogenic bacteria can present spoilage problems in milled products and foods made from milled products **(Manthey et *al*., 2004)**.

Yeast and mold are among the common spoilage microorganisms found on wheat grain. In this case, the spoilage occurs usually due to inefficient drying, which favors microbial growth and may result in increased mycotoxin levels **(Magan and Aldred, 2007**; **Harris et *al*., 2013)**. At low relative humidity (65 % to 90 %) and lower moisture contents (14 % to 16 %), stored-grains can be invaded by *Eurotium*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, and *Wallemia* **(Bullerman and Bianchini, 2009)**. At colder temperatures, *Penicillium* species predominate, but *Aspergillus* and *Eurotium* species are more prevalent at ambient temperatures (20 to 25 °C). **(Magan and Aldred, 2007)**.

The following elements influence fungal development in storage **(Atanda et *al*., 2011):**
- ✓ nutrient composition of the grain;
- ✓ moisture and temperature conditions;

✓ competition or the presence of stored product insects are examples of biotic factors.

III.2. Biocontrol of molds by LAB

Biological control of mold has received tremendous attention in recent years. It has been reported that a wide range of microorganisms including bacteria, yeast, and mold, have the ability to control mycotoxigenic mold growth **(Topcu et *al*., 2010).**

III.2.1. Mold development and mycotoxin formation in stored grain

Xerophilic molds that start the deterioration process in stored grains at low water activity can usually not be detected on freshly harvsted grains, as they occur in very low numbers. As molds grow and become established, their metabolic activities create microenvironments with elevated temperatures and moisture content. In a process known as fungal succession, when the moisture content rises, conditions become more favorable for other, less xerophilic molds **(Wicklow, 1995).** *Alternaria* and *Fusarium* do not compete well at low water activity (aw), hence mycotoxins in dried or stored grain are unlikely. Storage fungi (*Aspergillus* and *Penicillium*), on the other hand, form mycotoxins in stored grains but not before harvest **(Hocking, 2003; Kumar et *al*., 2020)**.

III.2.1.1. Mycotoxigenic fungi

Crops may be invaded by mixtures of mycotoxigenic fungi, resulting in multi-mycotoxin contamination **(Perrone et *al*., 2020)**. Some mycotoxins are produced by multiple species of fungi, and some fungi can secrete multiple mycotoxins. Furthermore, an infected substrate may contain several mycotoxins **(Zain, 2011)**. According to the literature, *Aspergillus*, *Fusarium* and *Penicillium* are the most mycotoxin-producing genera **(Pitt and Hocking, 2009; Ismaiel and Papenbrock, 2015; Alshannaq and Yu, 2017) (Table 2).**

The principal molds contaminating wheat in the field or during storage and belonging to *Fusarium, Alternaria, Aspergillus* and *Penicillium* genera are detailed as follows:

✓ ***Fusarium* genus:** a filamentous fungus of the *Ascomycota* phylum. Among 20 species, 14 are significant to crop producers because of the diseases they cause **(Early, 2009; Munkvold, 2017).** The genus produces a wide range of secondary metabolites (mycotoxins), well-known are fumonisins, zearalenone, deoxynivalenol and additional trichothecenes **(Thrane, 2014; Munkvold, 2017)**. The most significant economic consequences are linked to deoxynivalenol (DON) and its derivatives **(Munkvold, 2017; Stepien, 2020).**

Table 2. Main producers of common mycotoxins and toxic effects

Mycotoxins	Producing fungi	Effetcs	Sources
Aflatoxins	*Aspergillus flavus A parasiticus, A nomius*	Carcinogenecity, genotoxicity and immunotoxicity	**(Kumar, 2018; Benkerroum, 2020)**
Ochratoxins	*Aspergillus ochraceus, A. carbonarius, A. niger Penicillium verrucosum*	Nephrotoxicity, neurotoxicity, teratogenecity, immunotoxicity and carcinogenecity	**(El Khouri and Atoui, 2010; Heusner and Bingle, 2015)**
Deoxynevalenol	*Fusarium graminearum, F. culmorum*	Gastrointestinal toxicity, reproduction toxicity, immunodepression and immunostimulation	**(Berthiller et *al.,* 2011; Ji et *al.,* 2019)**
Zearalenone	*Fusarium graminearum, F. culmorum, F. cerealis, F. equiseti, F. crookwellense and F. semitectum*	Reproduction toxicity	**(Gil-Serna, 2014; Mahato et *al.,* 2021).**
Fumonisins	*Fusarium verticilliodes, F. proliferatum, F. nygamai, F. napiforme and F. globosum*	Hepatotoxicity, nephrotoxicity and carcinogenicity	**(Glenn, 2007: Ahangarkani et *al.,* 2014)**
Patulin	*Penicillium expansum, Aspergillus clavatus and Byssochlamys nivea*	Genotoxicity, neurotoxicity, immunotoxicity, carcinogenicity and teratogenicity	**(Liu et *al.,* 2003; Errampalli, 2014)**

✓ *Alternaria* **genus:** It comprises a group of fungi in the family *Pleosporaceae* (*Pleosporales*, *Dothideomycetes*, *Ascomycota*) **(Lawrence et *al.,* 2016)**. It is a widespread fungus genus with various species that damage agricultural products such as cereal grains, fruits, and vegetables before and after harvest **(Patriarca and Pinto, 2018)**. In the last few decades, more than 300 *Alternaria* metabolites have been discovered, some of which exhibit phytotoxic, antibiotic, antifungal, and antiprotozoal activity **(Dalinova et *al.,* 2020)**. *Alternaria* spp. produces

toxic metabolites that can accumulate in plant foods. They are divided into three structural groups: dibenzopyrone derivatives such as alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT); perylene derivatives such as altertoxins (ATX-I, ATX-II, and ATX III); and tetramic acid derivatives such as tenuazonic acid (TA) **(Patriarca and Pinto, 2018)**.

✓   *Aspergillus* **genus:** It is a filamentous fungus with about 250 species. It belongs to the order *Eurotiales*, class *Eurotiomycetes*, and phylum *Ascomycota* **(Kotta-Loizou, 2021)**. Members of the genus *Aspergillus* can be found in a wide range of ecosystems and environmental and climatic zones **(Abdel-Azeem et *al.*, 2019)**. The primary mycotoxins generated by *Aspergillus* species include aflatoxins, sterigmatocystin, ochratoxins, fumonisins, patulin, gliotoxin, and cyclopiazonic acid **(Varga et *al.*, 2015).** However, several new novel secondary metabolites are expected to be discovered in the near future from this genus **(Siddiquee, 2018)**.

✓   *Penicillium* **genus:** This filamentous fungus belongs to the phylum *Ascomycota*. It is a large genus with over 400 described species **(Yin et *al.*, 2017)**. *Penicillium* is a diverse genus that plays an important role in the decomposition of organic matter. It causes economic losses in the food industry **(Visagie et *al.*, 2014)**. The majority of *Penicillium* species are capable of producing mycotoxins: cyclopiazonic acid (*Penicillium chrysogenum*), penicillic acid (*Penicillium cyclopium*), patulin or clavacin (*Penicillium expansum*, *Penicillium griseofulvum*), citrinin (*Penicillium expansum*), ochratoxin A (*Penicillium verrucosum*) **(Pitt, 2000; Frivasd et *al.*, 2004)**.

III.2.1.2. Mycotoxin occurrence in wheat

Mycotoxins are harmful secondary metabolites produced by fungi that contaminate a wide range of agricultural commodities before and after harvest. It is believed that their purpose is thought to be for fungal defense or competition **(Magan and Aldred, 2007; Janik et *al.*, 2020; Daou et *al.*, 2021).** Mycotoxins can infect a variety of foods and cause cancer, genotoxicity, teratogenicity, nephrotoxicity, and hepatotoxicity. Contamination of foodstuffs with mycotoxins causes diseases all around the world **(Agriopoulou et *al.*, 2020)**.

The contamination of mycotoxins is greatly influenced by regional climatic conditions. Although data on the occurrence of *Fusarium* mycotoxins in durum wheat is quite limited, these latter are the main mycotoxins present in wheat; they pose a worrying problem for human and animal health **(Cheli et *al.*, 2013).** According to previous studies, the most contaminating mycotoxin in wheat and wheat products has been shown to be deoxynivalenol (DON) **(Visconti and Pascale,**

2010; Cheli et *al*., 2017; Palumbo et *al*., 2020; Zhao et *al*., 2021; Kova'c et *al*., 2022). According to **Righetti et *al*. (2021)**, the contamination of wheat by DON and zearalenone (ZEN) indicates *Fusarium* presence in the soil.

III.2.2. Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive organisms that are non-spore-forming, non-respiring but aerotolerant, cocci or rods, catalase-negative, and fastidious organisms with a high tolerance for low pH **(Schillinger et *al*., 2006; Tamang, 2014).** They produce lactic acid as one of the key fermentation products by utilizing carbohydrates during fermentation and also make organic substances that contribute to the flavor, texture, and aroma, resulting in unique organoleptic characteristics (**Bintsis, 2018**; **Ayivi et *al*., 2020**).

LAB are classified based on cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns **(Quinto et *al*., 2014)**. *Lactobacillus, Leuconostoc, Pediococcus,* and *Streptococcus* are the four genera that make up the core group **(Bintsis, 2018).** Following taxonomic revisions, several new genera have been proposed, and the remaining group contains the following: *Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Lactococcus, Oenococcus, Tetragenococcus, Vagococcus,* and *Weissella* **(Khalid, 2011)**. Recently, scientists have reclassified the genus Lactobacillus into 25 genera, including the emended genus *Lactobacillus*, which includes host-adapted organisms that have been referred to as the *Lactobacillus delbrueckii* group, *Paralactobacillus* and 23 novel genera **(Zheng et *al*., 2020).**

LAB are a type of probiotic bacteria that are found in abundance in the human and mammalian gut microbiota and provide considerable health benefits to the host **(Li et *al*., 2020).** LAB are also therapeutically useful as an antidote for many foodborne related diseases. The impact of LAB is therefore critical in promoting a healthy microbiota and increased immunity against diseases and infections **(Ayivi et *al*., 2020)**. Because LAB are capable of delivering antigens to the mucosal and systemic immune systems and generating specific antibody responses in serum and secretions, they may be used as a live vaccine prophylaxis against COVID-19 due to their contribution to improving the immune system **(Ibrahim et *al*., 2020)**.

III.2.2.1. Lactic acid bacteria in bio-preservation

LAB have been used in domestic food production for centuries, not only because they were tasty but also because they allowed food to be stored for a long period of time. Several members of this group had considerable antagonistic activity (**Matevosyan et *al*., 2019**).

Food fermentation has been widely practiced using LAB which are able to preserve food and prevent spoilage due to the end products of fermentation, such as alcohols and organic acids. These LAB are attracting the attention of consumers, who increasingly tend to use natural ingredients as preservatives instead of chemicals (**Sieuwerts, 2016; Asioli et *al*., 2017**).

The use of LAB in food preservation has attracted an increasing interest from researchers. This is known as bio-preservation, which is a natural way to use regulated microbiota as an alternative for extending shelf life and food preservation. As a result, one of the many properties gained from LAB in the context of food safety/spoilage is bio-preservation (**Ayivi et *al*., 2020**). Several studies have demonstrated the ability of various LAB to inhibit pathogenic microorganisms and degrade mycotoxins, as well as the probiotic and antimicrobial activities of cell-free extracts of LAB isolates from various sources (**Todorov, 2009; Shehata et *al.*, 2019; Divyashree et *al*., 2021**).

III.2.2.2. Antifungal effects of LAB metabolites

Antifungal activities of LAB have been reported in several studies (**Dalié et *al*., 2010; Sevgi and Tsveteslava, 2015; Bazukyan et *al*., 2018; Djaaboub et *al*., 2018; Salas et *al*., 2018; Matevosyan et *al*., 2019; Zebboudj et *al*., 2020; De Simone et *al*., 2021; Xu et *al*., 2021**). Furthermore, LAB strains can reduce fungal mycotoxins either by generating anti-mycotoxinogenic metabolites or by absorbing them (**Dalié et *al*., 2010**).

During fermentation, LAB induces acidification due to the production of organic acids (lactic acid particularly) and fatty acids. These organic acids are known as the most common antifungal components (**Dalié et *al*., 2010; Falguni et *al*., 2010; De Simone et *al*., 2021**). The other most important organic acids are: formic, propionic, butyric, phenyllactic and hydroxyl-phenyllactic acids (**Lavermicocca et *al*., 2003**; **Prema et *al*., 2010**; **Guimarães et *al*., 2018**).

Due to their lipophilic nature, organic acids readily diffuse across the fungal cell membrane and accumulate in the cytoplasm. Numerous studies have confirmed that organic acids are major metabolites of LAB that significantly affect fungi growth by inhibiting mycelial growth, and that

the antifungal activity of LAB is generally associated with the production of organic acids **(Lavermicocca et *al*., 2003; Magnusson et *al*., 2003; Prema et *al*., 2010; Belguesmia et *al*., 2014; Mieszkin et *al.,* 2017; Guimarães et *al*., 2018; Sadiq et *al*., 2019)**. The sensitivity of most fungi to metabolites, such as lactic and acetic acids, complicates studies on the effect of LAB on fungus **(Bonestroo et *al*., 1993).**

Many other antifungal compounds including hydrogen peroxide, diacetyl, lactones, alcohols, cyclic peptides, bacteriocins and reuterin have been reported to be produced by LAB **(Magnusson and schnurer, 2001; Strom et *al*., 2002; Rouse et *al*., 2008; Crowley et *al.,* 2013; Aunsbjerg et *al*., 2014).**

Bacteriocins are antimicrobial peptides produced by some microorganisms, including LAB, as primary metabolites from ribosomes. They are predominantly active against closely related organisms, mostly Gram-positive bacteria, to gain a competitive edge for nutrients in the environment **(Parada et *al.,* 2007; Zacharof and Lovitt, 2012).** In addition, LAB producing bacteriocins are good candidates as dairy starter cultures that play an important role in food application processes **(Perez et *al*., 2014).** These compounds are generally only active against closely related bacterial species and there is no evidence that bacteriocins have any effect on the growth of yeast or molds **(Magnusson et *al.,* 2003).** However, according to **Adebayo and Aderiye (2010)**; bacteriocins produced by some strains, like *Lactobacillus casei* and *Lactobacillus brevis,* showed an interesting inhibitory effect against *Penicillium citrinum.*

23

Article 1. A review submitted to *Carpathian Journal of Food Science and Technology*

WHEAT STORAGE AND LOSSES: AN OVERVIEW OF TRADITIONAL STORAGE IN ALGERIA

Nabila BENHAMADA[1*], Ouahiba BENHAMADA[1] and Tayeb IDOUI[1]

[1] *Laboratory of Biotechnology, Environment and Health. University of Jijel, 18000, Algeria*

*Corresponding author: * n.benhamada@yahoo.fr*

ABSTRACT

Wheat storage ensures the consumption of the grains throughout the year since harvest is guaranteed once a year. Although there are numerous methods for storing wheat, traditional techniques were always used in Algeria. During storage, nutritional quality is enhanced, whereas wheat grains are infested with pests and diverse microbial populations that can cause grain alteration with important losses. Preharvest, harvest, transportation, storage, and processing are all potential sources of microbial contamination. Fungi are the most contaminating flora; they present a risk of mycotoxin excretion. Whereas, lactic acid bacteria represent the fermentation flora that can control fungal development and mycotoxin secretion. This paper deliberates on different storage methods, including Algerian traditional storage and incurred losses.

Key words: Wheat; Storage; Matmour; Losses; Microflora.

Experimental part

I. Traditionally fermented wheat: nutritional quality and sensory evaluation of bread produced from composite fermented wheat flour

The aim of this part of the study is to examine the current knowledge of a traditional Algerian fermented food (fermented wheat, Mzeyet or Elhammoum), to provide more information regarding its biochemical and nutritional quality, as well as to evaluate its use as a supplement in bread technology.

I.1. Materials and methods

I.1.1. Fermented wheat samples origins

Throughout this study, we have used eight samples of traditionally fermented wheat in underground silos ('matmours'). The samples were collected from two regions situated in Eastern Algeria, namely: Jijel and Mila **(Fig. 6)**. They were collected over a period of three years due to the low production of this product by some farmers for their own private consumption and not for trade.

Fig. 6. Sample's collection sites. Circles indicate different sampling areas; Boudria Beni Yadjis in Jijel and Minar Zarza and Bouhatem in Mila (source: https://www.cartograf.fr/les-pays-algerie.php).

The eight samples were obtained after being subjected to traditional spontaneous fermentation in Matmours (**Fig. 7**) for approximately 10 months in rural areas. The appearance of some samples is shown in **Fig. 8**.

Fig. 7. Appearance of a 'Matmour' **(original photos)**. This 'matmour' represents one of the fermented wheat sample collection sites, located in the commune of Boudria BeniYadjis, Wilaya of Jijel, Algeria. This underground silo can expand to about 5000 kg.

Fig. 8. Some samples of traditionally fermented wheat (**original photos**). A: WM3; B: WM4; C: WJ2.

I.1.2. Physical characteristics of traditionally fermented wheat kernels

I.1.2.1. Thousand kernel weight (TKW)

The weight in grams of a 1000 wheat grain sample is measured as thousand kernel weight (TKW) or thousand grain weight (TGW).

According to **Godon and Loisel (1997),** the thousand kernel weight was determined by weighing a test portion of the wheat samples, then removing the impurities, followed by determination of the exact weight of the whole grains and finally counting the number N of these grains.

The thousand kernel weight is determined by the formula number (1).

$$(1)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\text{TKW (g)} = \frac{P \cdot 1000}{N}$$

Where:

P: whole grains weight (g);

N: counted grains number.

I.1.2.2. Hectoliter weight (HW)

Test weight or specific weight (hectoliter weight) is a parameter used in grading wheat, and it is used as an index of the strength, roundness, maturity and absence of damage of the grain. This physical parameter was determined according to the AACC method 55-10 (**AACC, 2000**), where the weight of grains was measured, then it was converted to weight per unit volume (kg / hl).

I.1.2.3. Broken kernels percentage

To determine the broken kernel percentage, the number of broken kernels was counted in relation to a test sample of 100 representative seeds of each wheat sample to be analyzed (**Gacem et al., 2011**).

The broken kernels (BK) percentage is determined by the formula number (2).

$$(2)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\text{BK (\%)} = \frac{BK}{100}$$

I.1.2.4. Impurities level

The impurities level was determined according to **Mauzé et al. (1972)**. Briefly; a test portion of traditionally fermented wheat was weighed, the impurities were eliminated and then weighed again.

The impurities level is determined by the formula number (3).

$$(3)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\text{I (\%)} = \frac{i1}{i} \cdot 100$$

Where:

i: impurities weight (g);

i_1: test portion weight (g).

I.1.3. Biochemical characteristics of traditionally fermented wheat

I.1.3.1. Moisture content

The moisture determination was done according to the method 44-15A from **AACC (1983).** Briefly; a test portion of traditionally fermented wheat was dried at 105 °C until the weight of the sample was stabilized. The moisture was determined using the formula number (4).

(4)………………………………..…..$H\ (\%) = \frac{(p1-p)}{p1} . 100$

Where:

p_1: sample weight before drying (g);

p: sample weight after drying (g).

I.1.3.2. Ash content

Ash (or mineral matter: MM) is the inorganic residue remaining after incineration. To determine it, 5 g of the sample was heated to 550 °C in a muffle furnace for 5 hours **(AFNOR, 1972)**. The rate in this matter is given by the formula number (5).

(5)………………………………………….**Ash** $(\%) = \frac{Ri}{P} . \frac{100}{(100-H)}$

Where:

Ri: residue weight after incineration (g);

P: test portion weight (g);

H: humidity (%).

I.1.3.3. pH evaluation

To determine the pH, a solution composed of 5 g of ground wheat and 45 ml of distilled water is prepared, then the pH is measured after letting it stand for 1 hour using a pH meter **(Gacem et al., 2011)**.

I.1.3.4. Greasy acidity

To determine the greasy acidity, 5 g of ground wheat was added to 30 ml of 95 % ethanol. The mixture was centrifuged for 5 min at 6000 rpm. After centrifugation, 20 ml of liquid was titrated in the presence of 5 drops of phthalene phenol with 0.05 N NaOH solution until the color changed to pink. At the same time, a control test was carried out **(AFNOR, 1982).** The greasy acidity is determined according to the formula number (6).

$$(6)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\mathbf{GA}\ (\%) = \mathbf{7.35} \cdot \frac{(v1-v0)}{m} \cdot \frac{100}{(100-H)}$$

Where:

v1: volume of NaOH for the sample titration (ml);

v0: volume of NaOH for the control titration (ml);

m: sample portion weight (g);

H: moisture content (%).

I.1.3.5. Protein content

Total proteins were measured according to the protocol described by **Lecoq (1965)**. Briefly, 5 g of each fermented wheat sample was put in a mineralization flask with a clamp of catalyst (copper potassium sulphate), and 15 ml of pure sulfuric acid, then the mixture was gradually heated: first a cold attack for 15 min until the appearance of white vapor of sulfur trioxide, then the heating is made more energetic; hot attack for 5 h. When the solution becomes clear, it is cooled and made up to 100 ml with distilled water (the distillation was carried out in a semi-automatic distiller where 20 ml of 35 % sodium hydroxide solution and 25 % of boric acid are added to a 250 ml flask). The liberated ammonia was collected in a boric acid solution containing the color indicator (mixture of methyl blue and methyl red). The excess ammonia is then dosed with 0.05 N sulfuric acid.

The total nitrogen content is determined by the formula number (7).

$$(7)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\mathbf{N}\ (\%) = \frac{\left[\frac{V}{V'} \cdot (N-N') \cdot 0,05 \cdot 1,4\right]}{P}$$

Where:

V: mineralized solution volume (ml);

V': volume of sodium hydroxide solution added (ml);

N: quantity of sulfuric acid used in titration (ml);

N': quantity of sulfuric acid used in titration of the blank (ml):

P: weight of the test portion (g).

The protein content was calculated by multiplying the total nitrogen rate (N %) by the coefficient 6.25.

I.1.3.6. Starch content

Starch content was determined using the procedure of **Lecoq (1965).** In short: 0.1 g of ground wheat was mixed with 5 ml of potassium hydroxide (1 N). The mixture was well homogenized, then neutralized with 5 ml of hydrochloric acid (1 N). The mixture was boiled in a water bath for 15 min. After this treatment, the volume is adjusted to 10 ml, and then centrifuged for 5 min at 6000 rpm. 0.05 ml of the supernatant was taken and mixed with 4.85 ml of distilled water and 0.1 ml of iodine potassium iodide (I_2KI) reagent. Finally, after incubation for 10 min, the absorbance was read at 720 nm. The calibration curve was made using pure starch.

I.1.3.7. Crude fiber content

Crude fiber content was measured using the **AOAC** procedure **(1995)**. 1 g of each ground wheat sample was mixed with 50 ml of 0.3 N sulfuric acid, and then the mixture was boiled for 30 min, with stirring every 5 min, ensuring that the material didn't adhere to the walls of the flask. 25 ml of NaOH (1.5 N) and 0.5 g of ethylene diamine tetra-acetic acid (EDTA) were added and heating was continued for 5 min. The obtained mixture was filtered in a filter crucible, washed respectively with 25 ml of sulfuric acid (0.3N), with 3 volumes of distilled water (50 ml for each), with 25 ml of ethanol and finally with 25 ml of acetone. At the end, the crucible was heated at 130 °C for 2 hours and then passed in an oven at 400 °C for 2 hours. The crude fiber content (cellulose content) in g is determined by the formula number (8).

$$(8)\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\textbf{Cel}\ (\%) = \frac{(P-P')-P''}{Pt}\ .\ \textbf{100}$$

Where:

P: weight of the crucible after filtration (g);

P': weight of the crucible after incineration (g);

P": weight of the crucible without sample before incineration (g);

Pt: weight of the test sample (g).

I.1.3.8. Fat content

The total fat has been determined according to the **Serna-Saldivar (2012)** procedure. Briefly: 20 g of ground wheat was prepared in petroleum ether (250 ml), then transferred to the Soxhlet apparatus. The mixture (soxhlet cartridge) was heated for 5 hours until the material was drained of fat. The contents of the flask were cooled to a temperature of 70 to 80 °C. The fat content is calculated according to the formula number (9).

(9)………………………………………**F** (%) $= \frac{(P2-P1)}{P3} . \mathbf{100}$

Where:

P1: empty flask weight (g);

P2: weight of empty flask with extracted oil (g);

P3: test portion weight (g).

I.1.3.9. Carbohydrates content

Carbohydrates were determined by difference as described by **Srivastava et *al*. (2002),** using the formula number (9).

(9). **Carbohydrates** $(\%) = \mathbf{100} - [\boldsymbol{proteins}\ (\%) + \boldsymbol{fat}\ (\%) + \boldsymbol{ash}\ (\%) + \boldsymbol{moisture}\ (\%)]$

I.1.3.10. Fatty acids composition

For the determination of fatty acid composition, we followed the method described by **Ollivier et *al*. (2006)**. First, the preparation of the methyl esters was carried out: in a tube containing 20 mg of the fat extracted by Soxhlet, 0.5 ml of heptane was added. After stirring, 0.2 ml of 2 N

methanolic sodium hydroxide solution was added, and the resulting mixture was placed in a thermostat bath at 60 °C for 1 minute.

After stirring for 10 seconds, 0.2 ml of 2 mol / l hydrochloric acid (HCl) was added. Following stirring, the contents were transferred to a glass tube to be vaporized in a ventilated environment. This vaporized quantity was taken up in 50 μl of heptane. After standing, the clear upper phase containing the methyl esters was recovered and 0.5 μl of each sample was injected into gas phase chromatography. The injection conditions are as follows:

- ✓ stationary phase: SE30 dimethyl polysiloxane;
- ✓ mobile phase: Helium;
- ✓ solvent: Heptane;
- ✓ detector: FID;
- ✓ column: Capillary type SE3 30 apolar with a diameter of 0.25 μm and 25 m in length;
- ✓ column temperature: 180-200 °C.

I.1.3.11. Mineral content

Clear ashes of ground wheat were taken up in 1 ml of concentrated hydrochloric acid (HCl) and then washed with lukewarm water. The solution was filtered in a 100 ml graduated flask and then completed with distilled water up to the mark. This solution was determined by atomic absorption spectrophotometry of the elements: Mg, Fe, Cu and Zn by flame mission **(AFNOR, 1972).**

I.1.4. Antioxidant activity of methanolic wheat extract

I.1.4.1. Preparation of methanolic extract

A methanolic extract of fermented wheat was prepared using the method described by **Bruneton (1993)**. After grinding the sample, a portion of the powder (one volume) was mixed with three volumes of 70 % methanol and allowed to macerate for 48 hours. The mixture was then filtered and concentrated using a rotary evaporator at a temperature of 45 °C. This extract was used for determination of total phenolic and flavonoid contents and antioxidant activity.

I.1.4.2. Total polyphenolic content

Total phenolic content (TPC) was investigated using the Folin-Ciocalteau assay **(Othman et *al*., 2007)**. Briefly: 0.2 ml of methanolic extract was mixed with 1.5 ml of Folin Ciocalteau reagent. After 5 min, 1.5 ml of 7 % sodium carbonate solution was added and the mixture was incubated

for 90 min. Then the absorbance was measured at 750 nm. The total phenolic content was expressed as mg gallic acid equivalents per g of dry weight (mg GAE / g).

I.1.4.3. Total flavonoid content

To determine the total flavonoid content (TFC), aluminium chloride complex forming assay was used according to **Djeridane et al. (2006)**. In this test, 1.5 ml of methanolic extract was added to 1.5 ml of aluminum chloride solution (2 %). The mixture was allowed to stand in darkness for 30 min. The absorbance of this reaction mixture was recorded at 430 nm and the results are expressed as mg quercetin equivalents per g of dry weight (mg QE/g).

I.1.4.4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant activity of traditionally fermented wheat extracts (TFWE) was measured as scavenging free radical potential in ethanolic solution of DPPH, as described by **Brand-Williams et al. (1995)**. 100 µl of TFWE was added to 3 ml of 0.025 g / l DPPH ethanolic solution freshly prepared. After incubation for 30 min at room temperature and in darkness, the absorbance was recorded at 517 nm and the antiradical activity was calculated as a percentage of DPPH discoloration compared to the control using the formula number (10).

(10)………………………………...**Inhibition** (%) $= \frac{(A-B)}{A} \cdot \mathbf{100}$

Where:

A: absorbance of pure DPPH in oxidized form;

B: absorbance of the sample.

I.1.5. Bread preparation and analysis

I.1.5.1. Flour blends and backing process

Three flour blends were prepared by mixing wheat flour with traditionally fermented wheat flour (TFWF) in the proportions of 90 / 10, 80 / 20 and 70 / 30 (w / w), while 100 % unfermented wheat flour (UFWF) was used as a control. The four flour samples were stored at room temperature for bread production.

Bread was backed from the flour samples using the usual dough method in a commercial bakery, located in Jijel, Algeria. The formulations of the breads with TFWF were developed by

modification of the traditional formula, using different levels of TFWF addition (0 %, 10 %, 20 % and 30 %) in substitution for part of the UFWF **(Table 3).**

To obtain bread, the ingredients (UFWF, TFWF, fresh yeast, salt, flour improver and water) were mixed manually in the laboratory and kneaded. After kneading, the dough was divided into 250 g dough pieces and these were balled and allowed to rise at room temperature for 20 min. The dough pieces were then shaped before being placed for 40 min in a fermentation chamber (humidity - temperature: 75 % - 35 °C). The baking takes place at 210 °C for 40 min in a plate oven equipped with a steam injection system. Once baked, the breads were allowed to cool down for 30 min, and kept at room temperature for further assessment **(Balla et *al*., 1999).**

Table 3. Bread making ingredients

Ingredients	Formulation			
	UFWF (g)	10 % TFWF (g)	20 % TFWF (g)	30 % TFWF (g)
UFWF	250	225	200	175
TFWF	00	25	50	75
Yeast	12.5	12.5	12.5	12.5
Salt	4.25	4.25	4.25	4.25
Flour improver	0.1	0.1	0.1	0.1
Water	-	-	-	-

UFWF: unfermented wheat flour, TFWF: traditionally fermented wheat flour; -: variable amounts

I.1.5.2. Gas retention and sensory evaluation of bread

The properties of gas retention during fermentation were evaluated by manually measuring the dough height for 60 min.

The bread samples were subjected to sensory evaluation about 1 h after baking by a jury composed of 6 tasters that were very familiar with bread. The sensory characteristics of the breads were evaluated according to the following criteria: loaf color, crust, crumb texture, aroma,

taste and overall acceptability of the bread sample. The panelists rated the acceptability of the product on a 01-09 point hedonic scale **(Ijah et *al*., 2014).**

I.1.6. Statistical analysis

All data analysis was performed using SPSS software version 22.0 for Windows. The data obtained from the analysis are expressed as the mean ± standard deviation (SD). Statistical differences were analyzed by one way analysis of variance (ANOVA) at $p < 0.05$. Correlation analysis between some parameters was performed using Pearson correlation at $p < 0.05$. The correlation matrices were assessed by STATISTICA 10.

I.2. Results and discussion

I.2.1. Physical characteristics of traditionally fermented wheat

The results of the physical characteristics of traditionally fermented wheat are shown in **Fig. 9.**

I.2.1.1. Thousand kernel weight (TKW)

The thousand kernel weight (TKW) of grains indicates the grain quality, e.g. longer, round and sound grains generally have a higher thousand kernel weight **(Amir et *al*., 2020).**

The results of thousand kernel weights among different traditionally fermented wheat samples are significantly different ($p < 0.001$***). They have been presented in **Fig. 9**, which shows that TKW ranged from 35.18 ± 0.4 to 42.21 ± 0.16 g. TKW is significantly higher in sample WM2 and lower in sample WJ1. All samples present an average of 39.13 g which belongs to the interval 35.01- 49.95 g reported by **Khan et *al*. (2015).** According to **Amir et *al*. (2020)**, the results for TKW ranged from 36.00 to 49.3 g in different wheat varieties under study. **Iqbal et *al*. (2015)** reported values of 38.94 and 45.50 g in two varieties of wheat. These results agree with ours.

Variations in TKW values can be affected by water availability and water soluble carbohydrates (WSC). In fact, a water deficit during the grain setting period can cause grain weight decrease by reducing TKW. This hypothesis was confirmed by **Farhat et *al*. (2021)**.

According to **McIntyre et *al*. (2012)** and **Sanchez-Bragado et *al*. (2014)**, WSC stored in the stems and leaf sheaths contribute to grain growth as the major carbon resource. **Zhang et *al*. (2022)** reported that TKW was affected primarily through WSC, and its cumulative contribution rate was comparatively lower.

I.2.1.2. Hectoliter weight (HW)

Hectoliter weight (HW), or test weight, is the principal quantitative parameter used by receiving units as an indicator of the wheat grain quality and confers a rough signal of the size and shape of wheat grain **(Okuyama et *al*., 2020; Martin et *al*., 2022).**
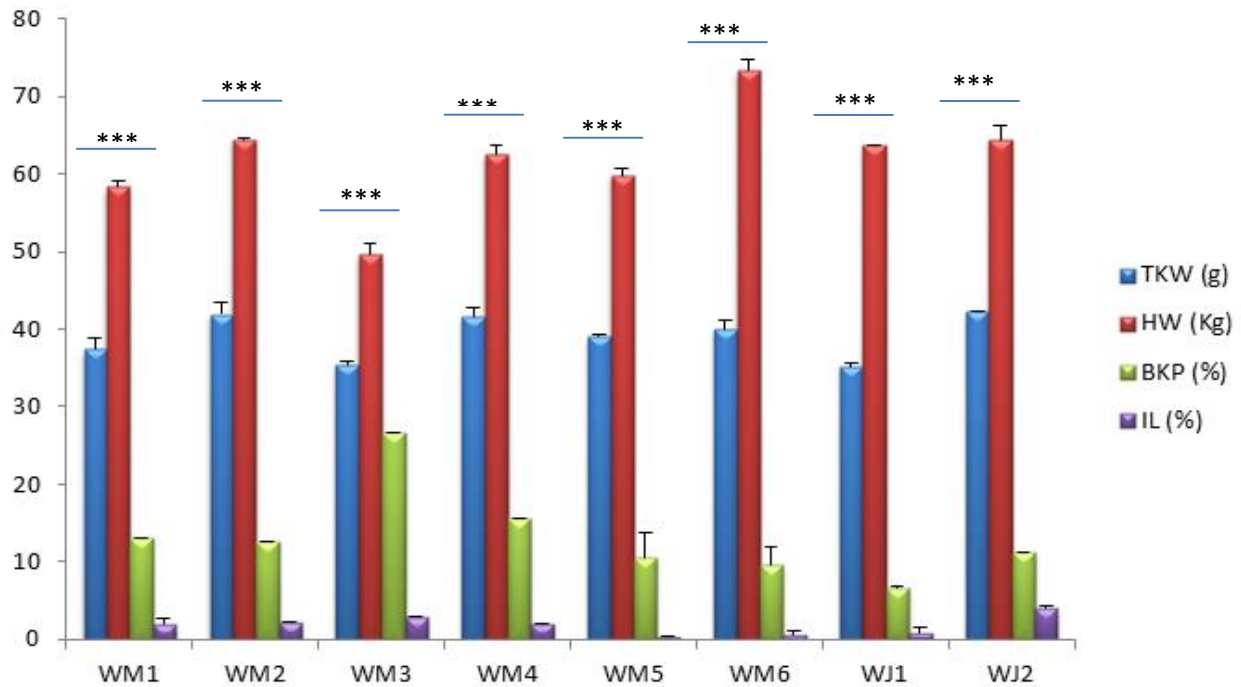
Fig. 9. Physical characteristics of traditionally fermented wheat.TKW: thousand kernel weight, HW: hectoliter weight, BKP: broken kernel percentage, IL: impurities level. ANOVA test was used (n = 3), *** indicates significant difference at $P < 0.001$ (inter and intra-groups).

The results of HW are shown in **Fig. 9.** High HW is registered in sample WM6 (73.31 Kg/hl) followed by WM2, WJ2, WJ1 and WM4 with 64.73, 64.43, 63.77 and 62.6 Kg/hl respectively (*p* < 0.001***). The lowest value of HW is noted in sample WM3 with only 49.6 Kg/hl. The result noted in sample WM6 (73.31 kg/hl) is close to those found by **Martin et *al*. (2022)**, who measured HW in winter wheat grain (*Triticum aestivum*) using three different equipment (A, B and C), they noted the following results: 77.15, 75.08 and 74.69 Kg/hl respectively. Two values, 80.50 and 80 Kg/hl were registered in two varieties of wheat grain (*Triticum aestivum*) by **Iqbal et *al*. (2015)**; they seem to be very high in comparison with ours.

Genetic differences and stress factors such as nutrient deficiencies, high temperatures during grain filling, plant lodging, insect damage, or adverse weather events like frost and hail affect test weight negatively. As reported by **Donelson et *al*. (2002)**, any damage caused by weathering, shriveled or immature grains, as well as rain-induced field sprouting tends to reduce test weight.

In comparison with studies cited above, we noticed that almost all samples presented low values of HW. These results are completely normal and this can be explained as follows: Wheat grains stored for a period more or less long in underground silos undergo fermentation, most often of the lactic type **(Gourchala et *al*., 2014).** During the fermentation process, the dry matter is degraded. In fact, starch which is considered a source of fermentable sugars, is the most degraded substrate due to high amylase activity **(Bekhouche et *al.*, 2014; Ganzle, 2014).** Proteins may also be degraded by certain endogenous grain proteases **(Kamal Eldin, 2012).** All these events are responsible for the decrease in specific weight.

The hectoliter weight has also been positively correlated with the thousand kernel weight **(Fig. 10).** The correlation is significant at $p < 0.05$ ($r = 0.544*$, $p = 0.006**$). These findings of correlation were confirmed by **Iqbal et *al*. (2016)**. However, **Joshi et *al*. (2018)** reported that the correlation was greatly influenced by the environment.
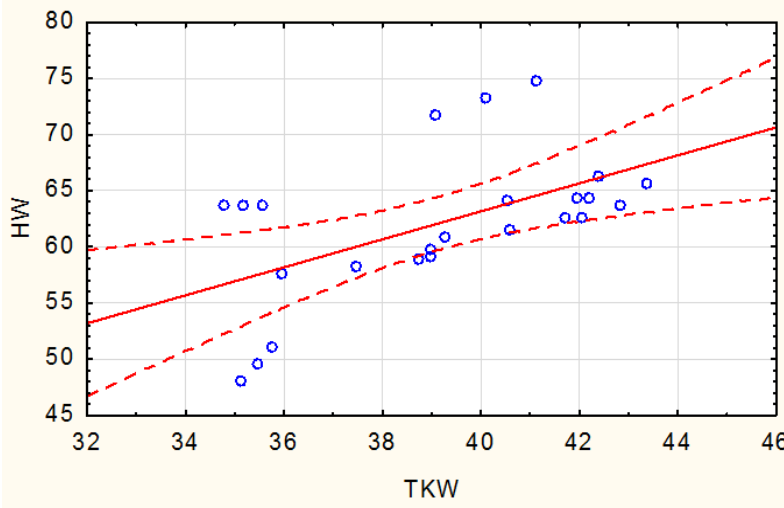
Fig. 10. Correlation between thousand kernel weight (TKW) and hectoliter weight (HW) using Pearson's r correlation ($r = 0.544*$, $p = 0.006**$), Correlation matrices were assessed by STATISTICA 10.

I.2.1.3. Broken kernel percentage (BKP)

Grain kernel damage during harvest and handling causes physical and physiological changes to grain, which reduces the grain quality and leads to significant weight loss. Impact is one of the most common damage mechanisms, causing external and internal cracks or even kernel fragmentation **(Chen et *al*., 2020).**

The results of broken kernel percentage (BKP) in the eight samples of traditionally fermented wheat vary significantly ($p < 0.001$***). The highest value is registered in the sample coded WM3 (26.66± 0.01 %) followed by WM4, WM1, WM2, WJ2, WM5 and WM6 with the values of 15.66 ± 0.01, 13 ± 0.1, 12.66 ± 0.06, 11.33 ± 0.01, 10.67 ± 3.2 and 9.67 ± 2.3 % respectively. The lowest value is registered in the sample coded WJ1 (6.66 ± 0.00 %).

Our results disagree with those reported in a previous study of **Gacem et *al*. (2011)**. The authors studied the physicochemical characteristics of local stored wheat at the Algerian Interprofessional Office of Cereals (AICO) in parallel with imported wheat. They registered the values 5.34 % and 4.32 % in the two samples respectively. The results predict that our samples have been exposed to pressing during the storage, and microbial damage responsible of grain breakage.

I.2.1.4. Impurities level

Impurities are damaged wheat kernels and all inorganic and organic matter, other than wheat grains, as well as: dust, stones, pieces, metal and grains of other cereals ….etc. Impurity occurs due to poor post-harvest practice; wheat with high impurity will decrease flour yield and machine ability **(Awulachew, 2020).**

The impurities level in the samples varies significantly ($p < 0.001$***) between 0.36 and 2.88 %, as shown in **Fig. 9.** The figure clearly shows that the sample WJ2 presents the highest impurity level (2.88 %). Our samples appear pure compared to the standards fixed by **Awulachew (2020)**; in fact, he reported an interval of 6-15 %.

Among the impurities present in our samples (fermented wheat grains) are stones, foreign seeds, sticks …Their origin can be attributed to harvesting, transportation, or underground storage. Alternatively, impurities may be added to agricultural foods during transport, storage, or processing **(Demeke et *al*., 2006).**

There is a positive and significant correlation between broken kernel percentage and impurities level at $p < 0.05$ ($r = 0.458$*, $p = 0.024$*) **(Fig. 11).**
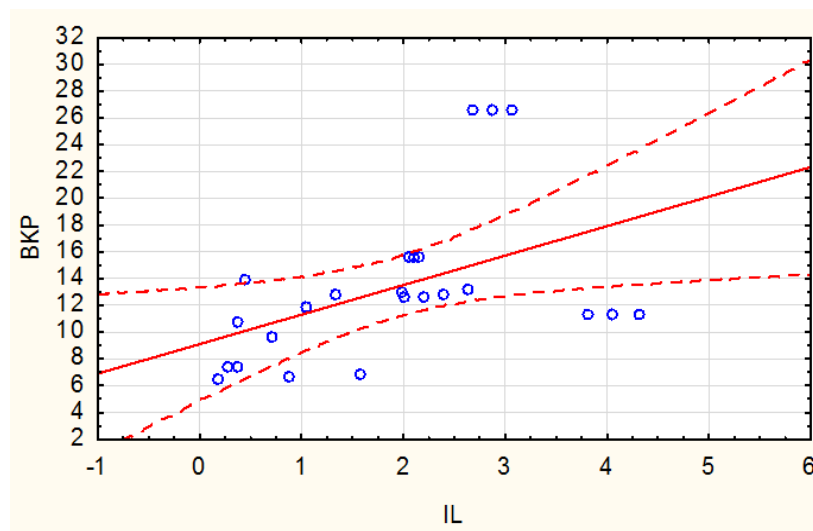
Fig. 11. Correlation between broken kernel weight (BKW) and impurities level (IL) using Pearson's *r* correlation, ($r = 0.458^*$, $p = 0.024^*$), Correlation matrices were assessed by STATISTICA 10.

I.2.2. Biochemical analysis of traditionally fermented wheat

The results of biochemical analysis of traditionally fermented wheat are presented in **Table 4**.

I.2.2.1. Moisture content

The moisture content of the traditionally fermented wheat samples varies significantly ($P < 0.001^{***}$) between 6.93 and 11.94 % with an average of 9.16 ± 1.73 %. The sample coded WM6 has greater moisture content than the other samples. The low moisture content of WJ1 indicates a good stable shelf life for storage stability. The findings in the current study are in accordance with those reported by **Khan et al. (2015),** who registered a range from 8.32 to 11.63 % in different wheat varieties. According to them, genotypic variability is a main factor influencing moisture content.

On the other hand, it was reported that moisture content didn't exceed 10.57 % in wheat grains of different varieties. However, **Amjad et al. (2010)** found that the moisture content in Punjab wheat varieties ranged from 12.92 to 13.32 %.

Our results are acceptable because moisture content higher than 12 % may enhance microbial growth, therefore, low levels of moisture content are favorable and increase the shelf life of the

samples **(Ojo et *al*., 2017; Odey and Lee, 2019**). **Raza et *al*. (2010)** reported that high moisture content enhances proteolytic and lipolytic activities, leading to a loss of nutrients.

Table 4. Biochemical characteristic of traditionally fermented wheat samples

Means ± SD	WM1	WM2	WM3	WM4	WM5	WM6	WJ1	WJ2
Moisture (%) (***)	7.6 ± 0.00	9.5 ± 0.00	8.66 ± 0.57	10.66 ± 0.57	7.72 ± 0.33	11.94 ± 0.36	6.93 ± 0.00	10.33 ± 1.15
Asch content (%)	1.50± 0.00	1.58± 0.00	0.83± 0.00	1.39± 0.00	2.45± 0.00	1.82± 0.00	1.67± 0.00	1.67± 0.00
pH (***)	5.61 ± 0.30	5.75 ± 0.02	4.57 ± 0.02	3.94 ± 0.09	6.89 ± 0.17	6.33 ± 0.26	4.93 ± 0.09	4.04 ± 0.03
Greasy acidity (%) (***)	10.86 ± 3.74	10.43 ± 3.21	9.18 ± 0.62	7.44 ± 0.00	2.19 ± 0.27	6.46 ± 0.69	8.39 ± 3.24	12.19 ± 1.00
Total sugars (%) (***)	80.52 ± 0.57	49.08 ± 0.29	55.10 ± 0.72	79.81 ± 0.73	87.70 ± 0.65	81.83 ± 0.65	79.13 ± 0.02	65.86 ± 0.67
Starch (%) (***)	38.60 ± 0.07	13.20 ± 0.60	10.00 ± 0.04	11.50 ± 0.12	39.00 ± 1.00	56.00 ± 8.00	31.20 ± 0.07	13.70 ± 0.07
Crude fiber (%) (***)	1.00 ± 0.00	2.01 ± 0.00	0.26 ± 0.02	0.51 ± 0.02	1.37 ± 0.20	2.30 ± 0.30	0.29 ± 0.00	0.29 ± 0.03
Total proteins (%)	0.17 ± 0.00	5.46 ± 0.00	18.81 ± 0.00	1.96 ± 0.00	1.42 ± 0.00	1.31 ± 0.00	5.84 ± 0.00	1.75 ± 0.00
Total fat content (%)	ND	ND	ND	6.13 ± 0.00	0.75 ± 0.00	1.30 ± 0.00	5.30 ± 0.00	ND

WM1, WM2, WM3, WM4, WM5, WM6, WJ1 and WJ2: traditionally fermented wheat samples, ND: not determined, values are means ± SD, differences were evaluated by one way analysis of variance (ANOVA), *** indicates significant difference at $p < 0.001$.

I.2.2.2. Ash content

Ash content can serve as an important indicator of the wheat flour's quality and use (**Czaja et al., 2020**). Referring to **Table 4**, the ash content in samples varies between 0.83 and 2.45 %. The lowest value is noted in WM3 and the highest value is registered in WM5. Apart from the value recorded in the sample WM3 (0.83 %), the other values in the seven other samples (1.50 %, 1.58 %, 1.39 %, 2.45 %, 1.82 %, 1.67 % and 1.67 % for WM1, WM2, WM4, WM5, WJ1 and WJ2 respectively) are close to each other and close to those registered by **Ciudad-Mulero et al. (2021)**. In fact, they noted a range from 1.4 to 2.2 % in *Triticum turgidum* L. and 1.6 to 2.1 % in *Triticum aestivum* L.

According to **Czaja et al. (2020)**, forty-nine samples of wheat flour were characterized by ash content in the 0.5 - 2.5 % range in which our results belong. Similar findings by scientists and researchers have been reported; **Khan et al. (2015)** reported the ash content ranged between 1.52 - 2.01 %, **Iqbal et al. (2015)** registered the values of 1.50 % and 1.55 % in two varieties of wheat. They also reported that the effect of wheat varieties was not significant on the ash content of whole wheat flour of both wheat varieties. However, **Buriro et al. (2012)** suggest that wheat variety and growing atmospheric conditions are decisive factors controlling ash content.

I.2.2.3. pH evaluation

As shown in **Table 4**, the eight samples under study present significant differences in pH values ($p < 0.001***$). These values vary from 3.94 to 6.89 with an average of 5.25. The most acidic sample is WM4 (3.94 ± 0.09), while, the sample WM5 has a pH close to neutral (6.89 ± 0.17).

Similar results were reported in an earlier study conducted by **Gourchala et al. (2014)**, in which they compared naturally fermented wheat samples to unfermented ones; they registered a value of 5.63 ± 0.014 in fermented wheat against 6.28 ± 0.021 in the control sample. They noted a reduction of about 10.35 % in pH values. The same results were also found by **Mokhtari et al. (2020),** where they reported that fermented wheat has a lower pH value (5.23 ± 0.005) compared to unfermented one (6.31 ± 0.02).

However, our values are higher than those reported by **Doukani et al. (2013),** where a value of 4.45 was recorded as being the most acidic. The lowest pH value (3.94) makes the WM4 sample very acidic. This can be explained by a long fermentation time in Matmour, which leads to a large bacterial activity responsible for the accumulation of lactic and other organic acids,

subsequently causing a decrease in pH values **(Kohajdovà and Karovicovà, 2007).**

I.2.2.4. Greasy acidity

In the same table, greasy acidity values range from 2.19 ± 0.27 to 12.19 ± 1 % ($p < 0.001***$). The lowest value was recorded in sample WM5 and the highest one in sample WJ2. The registered values of greasy acidity in the samples seem to be higher compared to those obtained by **Doukani et *al.* (2013)**; according to them, the fermented wheat had a greasy acidity of about 1.51 %. The increase in greasy acidity level in the eight samples may be explained by grain storage conditions, which lead to hydrolysis of triglycerides by endogenous and exogenous lipases and, thereafter, the accumulation of free fatty acids **(Feillet, 2000).**

It has been established that lipase during the storage period splits grain triglycerides into glycerol and free fatty acids, which in cereals are mainly represented by three unsaturated fatty acids: oleic, linoleic and linolenic acid with one, two, or three double bonds, respectively. These changes reliably reflect the indicator of the fat acidity value **(Kaliyan et *al.*, 2005; Zhang et *al.*, 2014).**

Otherwise, **Kechkin et *al.* (2020)** reported that during the storage of wheat grain samples at different temperatures and relative air humidity for 8 months, the temperature under the experimental conditions does not significantly affect the increase of the fat acidity value in the samples. The relative air humidity in the grain mass plays a more significant role in the increase in the fat acidity value index.

Indeed, we note a significant negative correlation between greasy acidity values and the recorded pH values ($r = - 0,611**$, $p = 0.002**$) **(Fig. 12).**

I.2.2.5. Sugar content

Sugar contents in the eight samples vary significantly ($p < 0.001***$). It ranges from 49.08 ± 0.29 to 87.70 ± 0.65%. We notice that sample WM5 has the highest carbohydrate content (87.70 ± 0.65 %) followed by samples WM6, WM1, WM4 and WJ1. However, low values are registered in samples WM2, WM3 and WJ2 with 49.08 ± 0.29 %, 55.10 ± 0.72 % and 65.86 ± 67 % respectively **(Table 4)**.

The low content of sugar in some TFW samples is due to excessive fermentation in underground silos. The same results were found in previous studies **(Deshpande, 2000; Doukani *et al.*, 2013).** In a study by **Gourchala et *al.* (2014)**, it has been demonstrated that total sugar content in

fermented wheat is lower (48.67 ± 0.05 g / 100g) compared to unfermented wheat (81.08 ± 1.12 g / 100g).
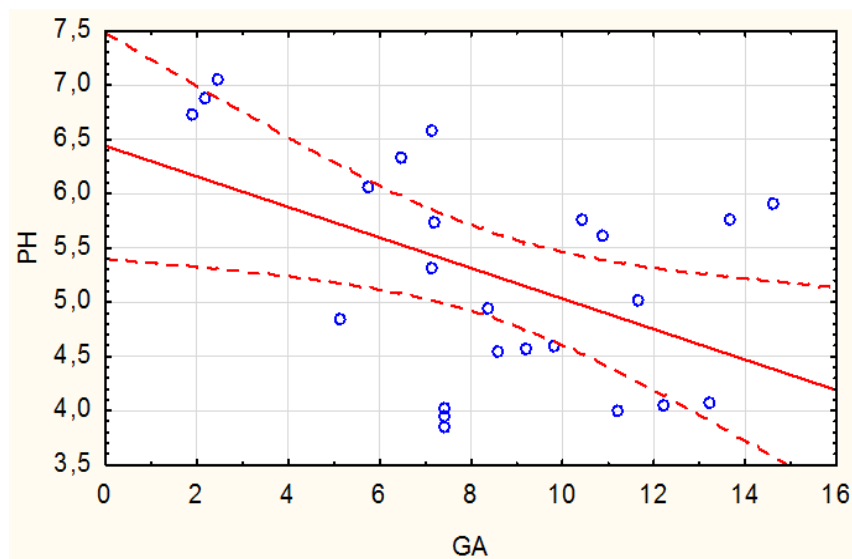
Fig. 12. Relationship between pH and greasy acidity (GA) in traditionally fermented wheat samples using Pearson's *r* correlation (*r* = - 0,611**, *p* = 0.002**), Correlation matrices were assessed by STATISTICA 10.

Otherwise, low values of total sugar content in some TFW samples are related to several factors, such as storage time and water infiltration. Wheat seeds contain amylolytic enzymes which release maltodextrins, maltose and glucose. These endogenous enzymatic activities play an important role in starch degradation, which is considered a source of fermentable sugars (**Ganzle, 2014).** Thus, through the natural underground storage system, fermented wheat undergoes a fermentation process in a biotope rich in natural nutrients; carbohydrates are used as a fermentable substrate by wheat microflora during fermentation, which decreases sugar content. For this reason, fermented wheat is suitable for diabetics.

I.2.2.6. Starch content

Starch represents a major part of the carbohydrates in the mature wheat kernel (**Matz, 1991; Bushuk and Rasper, 2012**). As shown in **Table 4**; starch contents of TFW samples range from 10 ± 0.04 to 56 ± 8 % (26.68 % in average) ($p < 0.001$***). These values are low compared to

those found by **Gourchala et *al.* (2014),** who recorded a value of 48.17 %. According to **Matz (1991)**, starch content in unfermented wheat varied from 60 to 68 %. During fermentation, starch is the most degraded substrate **(Bekhouche et *al.,* 2014),** and this is due to high amylase activity **(Ganzle, 2014),** which explains its low content in TFW samples.

On the other hand, we note that for sample WM4, starch content is low (11.50 ± 0.12 %) even if the total sugar content is high (79.81± 0.73 %). This result proves that the starch is more exposed to degradation.

I.2.2.7. Protein content

The protein content is an important criterion for assessing the quality of wheat. As shown in **Table 4**, values of total protein content vary from 0.17 to 18.81 %. WM1 protein content (0.17 %) seems to be very far from the limit suggested by **Matz (1991)** and **Feillet (2000),** which required a protein content of 7 to 18 % and 10 to 15 %, respectively.

The low protein content in the eight samples can be linked to protein degradation during the fermentative process, which favors the action of certain endogenous cereal proteases **(Kamal Eldin, 2012).** On the other hand, the production of free amino acids resulting from the hydrolysis of cereal proteins during fermentation **(Thiele et *al.,* 2004)** improves cereal nutritional quality **(Blandino et *al.,* 2003)** by increasing the content of essential amino acids such as lysine, methionine and tryptophan **(Adams, 1990).**

Otherwise, the high level of protein content in the WM3 sample (18.81 %) can be explained by bacterial synthesis of new protein products during spontaneous natural fermentation **(Kohajdovà and Karovicovà, 2007).**

Apart from WM3, which presents high protein content, the other samples present an average of about 4.59 % which is very far from the values recorded by **Mokhtari et *al*. (2020)** (12.76 ± 0.05 %)**. Kohajdovà and Karovicovà (2007)** reported that the hydrolysis of proteins during fermentation is also linked to a significant increase in nutritional value by increasing the bioavailability of nutritional compounds, vitamins, bio-elements, and other biologically active substances.

On the other hand, the increase in protein levels in some samples didn't mean that the sample didn't undergo fermentation, because several authors reported an increase **(El-Hag et *al*., 2002)** while others observed a decrease **(Osman, 2011).**

It appears that most of these effects are relative to changes due to loss of dry matter as a result of microorganisms hydrolyzing and metabolizing carbohydrates and fats as a source of energy **(Nkhata et *al*., 2018).**

I.2.2.8. Crude fiber content

The results given in **Table 4** indicate that TFW samples have a crude fiber content ranging from 0.26 and 2.3 % with an average of 0.71 % ($p < 0.001^{***}$), which is low compared to the limits given by **Matz (1991)** and **Feillet (2000)**, ranging from 1.5 to 2 % and 2 to 4 % respectively. However, these results are in agreement with those obtained by **Doukani et *al*. (2013),** who noted a decrease in crude fiber content in fermented wheat grains compared to the control sample. The decrease can be explained as cellulose hydrolysis due to microbial activity during grain fermentation **(Jesperson, 2003).**

Otherwise, the mechanism of the decrease in fiber in fermented cereal was attributed to the partial solubilization of cellulose and hemicellulose types of materials by microbial enzymes **(Alo et *al*., 2012).** Other authors reported that the reduction in fiber of some fermented cereals was ascribed to an enzymatic breakdown by LAB used in fermentation, which utilized the fiber as a carbon source **(Ogodo et *al*., 2017).**

I.2.2.9. Total fat content

The results reveal that total fat content values vary between 0.75 and 6.13 % with an average of 3.37 % **(Table 4).** The values appear to be higher than those found by **Doukani et *al*. (2013)** (1.08 %). In fact, **Matz (1991)** and **Feillet (2000)** suggested a fat content of 1.5 to 2 % and 2 to 3 % in unfermented wheat grains, respectively. In a previous study, **Mokhtari et *al*. (2020)** registered an increase in fat content in fermented wheat (1.37 ± 0.07 %) compared to unfermented wheat (1.08 ± 0.05 %), but no significant difference between the two samples was noted. The increase in fat content in some samples could be explained by the degradation of the lipids associated with starch granules during starch degradation.

WM5 and WM6 fat contents (0.75 and 1.3 %) are lower than the values pointed to above; this may be the result of hydrolysis of triglycerides and liberation of fatty acids during storage fermentation.

According to **Onoja and Obizoba (2009)** and **Igbabul et *al*. (2014)**, the mechanisms involved in the fat content increase might be linked to the increased activity of lipolytic enzymes during the

fermentation, the extensive breakdown of large molecules of fat into simple fatty acids, the fat from dead microflora and/or the assumption that fermenting microflora did not use the fat as a source of energy.

Furthermore, most studies on fermented cereals reported a reduction in fat content (6-34 %). This reduction has been associated with the metabolism of lipids by the fermenting organisms and the leaching of soluble inorganic salts **(Adebo et _al_., 2022).** The two events of increase or decrease in fat content could occur under very specific conditions, which are related to the loss of dry matter **(Nkhata et _al._, 2018).**

I.2.3. Fatty acid composition of traditionally fermented wheat

Table 5 shows the fatty acid profiles of three fermented wheat samples (WM1, WM2 and WJ1, respectively). In the WM1 sample, the only detected fatty acid is C18:1 (oleic acid). It is also detected in the two other samples. In sample WM2, in addition to oleic acid (C18:1), we recorded the apparition of two other peaks, presenting C18:0 (stearic acid) and C16:0 (palmitic acid). Concerning the sample WJ1, we detected the following fatty acids: C18:1 (oleic acid), C18:2 (linoleic acid) and C16:0 (palmitic acid).

Table 5. Fatty acid composition of traditionally fermented wheat

Fatty acids	**WM1**	**WM2**	**WJ1**
Palmitic C16:0	nd	d	d
Stearic C18:0	nd	d	nd
Oleic C18:1	d	d	d
Linoleic C18:2	nd	nd	d

WM1, WM2 and WJ1: traditionally fermented wheat samples. d: detected, nd: not detected

According to **Lafiandra et _al_. (2012)**, durum wheat (_Triticum durum_) kernels contain about 2.4 - 3.8 % dry basis of lipids. The fatty acids of wheat lipids are mostly unsaturated (C18:2, C18:1, C18:3 and C16:1). **Narducci et _al_. (2019)** detected six main fatty acids in all samples of durum

wheat in order of decreasing amounts: linoleic (C18:2) > palmitic (C16:0) ≈ oleic (C18:1) > linolenic (C18:3) > stearic (C18:0) > palmitoleic (C16:1). According to them, the major fat components were saturated and unsaturated C16 and C18 and particularly C16:0, C18:1 and C18:2, which together represented around 90 % of the total. These findings are close to ours. Indeed, C16:0, C18:1 and C18:2 presented 85.71 % of the total fatty acids of traditionally fermented wheat.

In almost all published works, it is reported that only C16:0, C18:0, C18:1, C18:2 and C18:3 are the most important fatty acids in durum wheat. **Zarroug et *al*. (2015)** and **Guven and Kara (2016)** detected C18:2, C18:1 and C16:0 as predominant fatty acid types in durum wheat germ oil. For **Gonzalez-Thuillier et *al*. (2015),** the most abundant fatty acids are C16:0 and C18:2. Again, C18:2 was detected as predominant fatty acid in wheat germ oil in the study of **Relina et *al*. (2020).** In soft wheat (*Triticum aestivum*), **Nasiroleslami et *al*. (2021)** detected linolenic acid (C18:3) as the main fatty acid (30.9 - 45.6 %).

There are publications reporting the detection of other fatty acids as well as C14, C17, C20, C22 and C24 in kernels or in germ oil **(Beleggia et *al*. 2013; Zarroug et *al*. 2015; Guven and Kara 2016; Relina et *al*., 2020).** While these fatty acids were not found in our samples, this finding agrees with that of **Osuna et *al*. (2014),** who found only C16:0, C18:0, C18:1, C18:2, C18:3, and C20:1 in wheat flour.

Fatty acid levels and types in wheat kernels depend on a set of factors, some of which are genetic, such as species and variety, while others depend on the environment. Climate conditions are also an important factor; it is known that increased unsaturated fatty acid content is associated with cold tolerance and is considered as a general biological pattern **(Armanino et *al.*, 2002; Relina et *al.*, 2020).**

I.2.4. Mineral content

For human health, minerals are essential micronutrients, and wheat stands out for being an important source of these components, including microelements such as Fe, Cu, Mn, and Zn; and macroelements, such as Mg, Ca, Na, and K **(Ciudad-Mulero et *al.*, 2021).**

The mineral composition (microelements: Fe, Zn, Cu, and Mn) was evaluated in the different samples of traditionally fermented wheat using atomic absorption spectrophotometry by flame mission. The mineral concentration varies widely among the different samples **(Fig. 13).**

Iron (Fe) is an essential trace metal for almost all organisms, including humans. It plays an important role in the synthesis of hemoglobin. Moreover, it is a structural component of myoglobin as well as a cofactor of several enzymes **(Garcia-Oliveira et *al.*, 2018; Peng et *al.*, 2021)**. The results of the determination of iron in the wheat samples indicate that the two samples coded WM2 and WJ1 are rich in this microelement (4.2616 and 3.6213 ppm, respectively), followed by WM4 (2.015 ppm) and WM1 (1.0214 ppm). The sample coded WJ2 presents the lowest value at only 0.205 ppm.
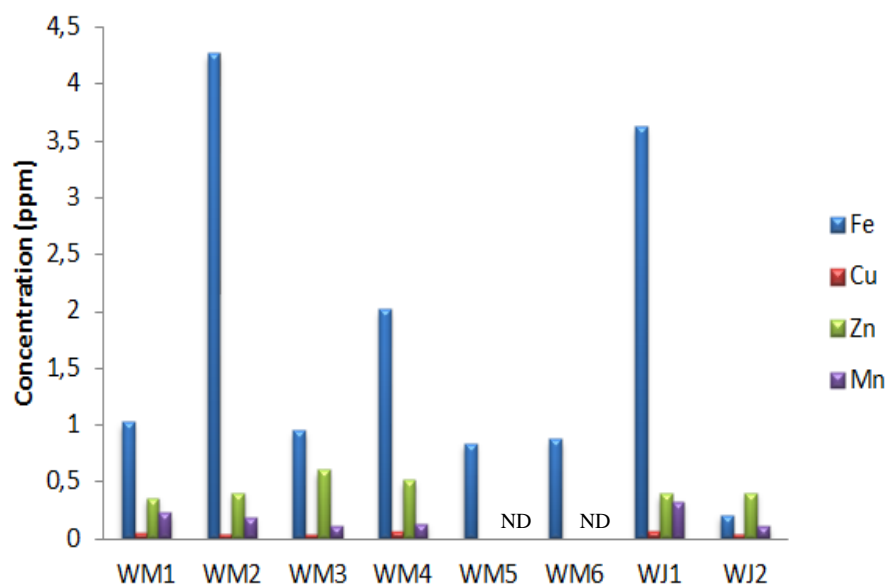
Fig. 13. Mineral content in different traditionally fermented wheat samples (WM1, WM2, WM3, WM4, WM5, WM6, WJ1 and WJ2), ND: not determined.

Zinc (Zn) plays many essential roles in all biological systems. It has important enzymatic, regulatory, and structural functions (**Garcia-Oliveira et *al.*, 2018; Carocho et *al.*, 2020; Stanton et *al.*, 2022).**

After iron, it is clear that the six wheat samples are rich in zinc. WM3 and WM4 present interesting values of 0.6 and 0.511 ppm, respectively. The other samples present close values, with an average of 0.384 ± 0.02 ppm. Regarding these results, Fe and Zn were the main microelements. Our results are in line with those reported by **Ciudad-Mulero et *al.* (2021)** in

white flour, **Cubadda et *al*. (2009)** in durum wheat, and **De Brier et *al*. (2015)** and **Vignola et *al*. (2016)** in bread wheat.

Pranoto et *al*. (2013) reported that the increase of iron and zinc in some fermented foods is associated with a decrease in the amount of phytates. This may also be linked to the loss of dry matter during fermentation due to the degradation of carbohydrates and proteins by microbes **(Day and Morawicki, 2018).**

Copper (Cu) is an indispensable trace metal element in the human body, which is mainly absorbed in the stomach and small intestine and excreted into the bile. It is essential for life processes like energy metabolism, reactive oxygen species detoxification, iron uptake, and signaling in eukaryotic organisms **(Ruiz et *al*., 2021; Wang et *al*., 2021).**

In our samples we have registered close values in Cu content. The two samples, WM4 and WJ1, present the highest values (0.0626 and 0.061 ppm, respectively). The other samples present an average of 0.041 ppm. Cu content is low in the eight samples in comparison with other studies. In fact, **Benayad et *al*. (2021)** registered a value of 1.10 ± 0.22 mg/100g in whole durum wheat flour. **Ciudad-Mulero et *al*. (2021)** found high levels of Cu in whole durum wheat flour, ranging from 0.6 0.0 to 2.4 0.1 mg/100g. The low content of copper in the samples may be related to the stress which induces changes in grain micronutrient accumulation **(De Santis et *al*., 2021).**

Manganese (Mn) is the twelfth most abundant element in the earth's crust and is widely distributed throughout the surface of the planet, naturally occurring in rocks, soil, water, and food. It shows similar bio-geochemical behavior to iron (Fe) in the environment **(Fuchida et *al*., 2022; Studer et *al*., 2022)**. It is an essential element acting as a co-factor of superoxide dismutase, and it is potentially beneficial for cardiometabolic health by reducing oxidative stress **(Wong et *al*., 2022).** Regarding Mn content in traditionally fermented wheat samples, the values range between 0.103 and 0.323 ppm. These values seem to be low in comparison with previous studies. **Ciudad-Mulero et *al*. (2021)** have shown an interval of 1.2 ± 0.0 to 2.7 ± 0.1 mg/100g in whole grain wheat. Also, **Stepien et *al*. (2019)** registered a mean of about 2.84 ± 0.47 mg/100g in winter wheat (*Triticum aestivum* L.) grain.

According to **Ciudad-Mulero et *al*. (2021),** genotype and environmental factors affect the mineral content of wheat. Minerals are concentrated in wheat bran. This fact emphasizes the

significance of wheat flour consumption, particularly whole grain flour and bran fraction, as a mineral source with proven health benefits.

I.2.5. Antioxidant activity of methanolic wheat extract

Antioxidant activity was assessed by total and flavonoid content determination, and DPPH radical scavenging capacity in methanolic extracts of traditionally fermented wheat.

I.2.5.1. Total polyphenolic and flavonoid content

Cereals contain a number of phytochemicals, including phenolics, flavonoids, anthocyanins, etc, which play an important role in health benefits because of their high antioxidant capacity. Because cereal phytochemicals significantly reduced oxidative stress damage, consumption of whole grain is thought to have significant health benefits in preventing chronic diseases such as cardiovascular disease, diabetes, and cancer **(Hung, 2016).**

The results presented in **Table 6** show that the total phenolic content (TPC) of the samples varies significantly ($p < 0.001$***). TPC in traditionally fermented wheat extracts (TFWEs) ranges from 9.37 ± 0.09 to 11.97 ± 0.03 mg GAE / g, WM6 wheat extract shows the best TPC (11.97 ± 0.03 mg EAG / g) whereas, the lowest value is recorded in the sample WM2.

Table 6. Total phenolic and flavonoid content in traditionally fermented wheat samples

Means ± SD	WM1	WM2	WM3	WM4	WM5	WM6	WJ1	WJ2
Total phenolic content (mg GAE/g) (***)	10.36 ±0.11	9.37 ±0.09	9.81 ±0.14	10.61 ±0.08	11.21 ±0.12	11.97 ±0.03	9.72 ±0.10	10.79 ±0.16
Total flavonoid content (mg QE/g) (***)	8.36 ±1.13	8.25 ±0.28	9.22 ±0.17	9.24 ±0.07	3.77 ±0.00	7.51 ±0.00	9.11 ±0.41	8.51 ±0.42

WM1, WM2, WM3, WM4, WM5, WM6, WJ1 and WJ2 traditionally fermented wheat samples. Values are means ± SD, differences were evaluated by one way analysis of variance (ANOVA), n = 3, *** indicates significant difference at $p < 0.001$.

Comparing our results by those obtained in a recent study by **Zhang et *al*. (2021),** in which they noted an average TPC of about 2.6 mg GAE / g in four cultivars of unfermented wheat, we conclude that fermentation in underground silos significantly increases the TPC in fermented

wheat samples. Indeed, **Gourchala et *al.* (2014)** quantified total polyphenols in Elhammoum 'durum wheat fermented product', and they noted a significant increase in the total polyphenol content in the fermented wheat (23.75 mg GAE / g) compared to the unfermented wheat sample (18.32 mg GAE / g). The same findings were reported in the study of **Zhang et *al.* (2012),** where they recorded an increase in TPC after fermentation of soft wheat (*Triticum aestivum* Linn) using *Cordyceps militaris.*

On the other hand, some studies have found that total polyphenols in some cereals decrease during fermentation **(El Hag et *al*., 2002; Zhang et *al*., 2021).** According to **Zhang et *al*. (2021),** the reason for the decrease of TPC in four wheat cultivars after storage in paper milling bags under aerated conditions or plastic bags under vacuum conditions for eight weeks, is linked to storage time rather than storage conditions in all cultivars. As reported in the study by **Srivastava et *al*. (2007),** the decrease in TPC is caused by oxidation. Indeed, phenolic compounds are not totally stable and are easily degraded during storage after harvest.

The same table shows total flavonoid content (TFC) in the eight samples of TFWEs. There is a significant difference between the samples ($p < 0.001***$). The sample coded WM4 has the highest value (9.24 ± 0.07 mg QE /g). High values are also recorded in the samples WM3 and WJ1. The other results are intermediates in WJ2, WM1, WM2 and WM6. The lowest value is registered in WM5 with only 3.77 ± 0.00 mg QE / g. The TFC recorded in our samples seems to be very low compared to those reported in previous studies. **Zhang et *al.* (2012)** and **Sandhu et al. (2016)** proved that fermentation using fungus species promotes the increase of total polyphenols and flavonoids. **Dordevic et *al.* (2010)** found that fermentation using LAB and yeast can enhance polyphenol and flavonoid content.

Many different factors influence the content of bioactive compounds, such as stress conditions, both biotic and abiotic **(Di Loreto et *al*., 2018)**. **Gotti et *al*. (2018)** found that ancient varieties of common wheat show a higher level of these compounds.

I.2.5.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity
Fig. 14 shows the DPPH antioxidant activity of TFWE samples. The decrease in the absorbance of DPPH radicals at 517 nm induced by antioxidants determines their reduction capacity. During the radical scavenging assay, DPPH radical without extracts of traditionally fermented wheat was stable over time. However, in the presence of several concentrations of TFWE, the DPPH radical

is reduced to non-radical DPPH-H. This reduction depends on the used concentrations. It was found that the DPPH scavenging effect of TFWE increased with the increase in their concentration.

The highest DPPH antioxidant capacity is attributed to the WM3 fermented wheat sample (87.97 %) followed by WJ1 (86.55 %). At 75 % dilution, WJ1 also records the highest activity (85.77 %). The lowest activity is observed in the WM2 sample at all dilutions.

DPPH antioxidant activity is effective in traditionally fermented wheat; this can be explained by the enhancement of antioxidants like polyphenols and flavonoids by fermentation. According to **Zhang et *al.* (2012),** DPPH antioxidant activity was more effective in wheat fermented using *Cordyceps militaris* than in unfermented wheat.
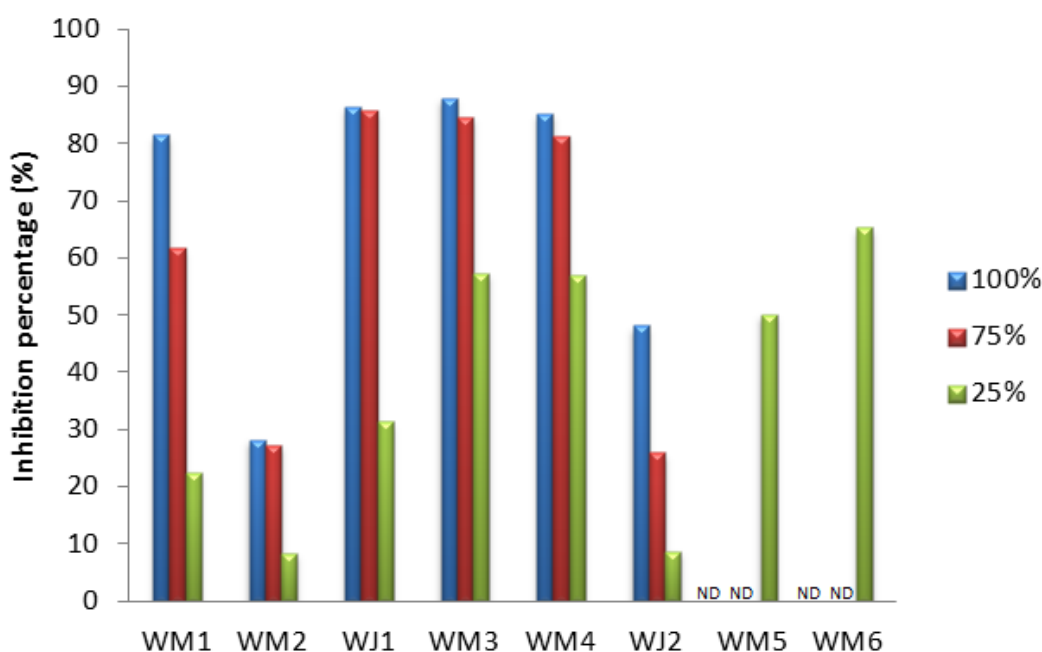
Fig. 14. DPPH scavenging activity of different dilutions of traditionally fermented wheat extracts. WM1, WM2, WM3, WM4, WM5, WM6, WJ1 and WJ2: traditionally fermented wheat samples, ND: not determined.

Otherwise, **Zhang et *al*. (2021)** reported losses in antioxidant activity of about 30 % from the DPPH assay during storage of four wheat cultivars. Indeed, when we compared our findings to those of **Horvat et *al*. (2020),** who reported an inhibition percentage of wheat grain ranging from 13–15%, we noticed that TFWE antioxidant activity increased during fermentation. The same observation is drawn from the results obtained by **Mpofu et *al*. (2006)** and **Sandhu et *al*. (2016).**

Variable mechanisms of the antioxidant capacity of cereals in general and wheat in particular have been documented. For example, it was reported that fermentation of wheat using filamentous fungi enhanced DPPH scavenging capacity by the release of bioactive compounds such as peptides and oligosaccharides **(Dey and Kuhad, 2014).** It has also been reported that micro-organisms used in fermentation contribute to the degradation of cell walls and subsequently the synthesis of bioactive molecules with antioxidant activity **(Adebo and Medina-Meza, 2020).**

I.2.5.3. Correlation between TFC/TPC and DPPH radical scavenging activity

A positive correlation is noted between flavonoid content and antioxidant activity ($r = 0.856*$, $p = 0.03*$) (**Table 7, Fig. 15**). Whereas, there is no significant relationship between TPC and DPPH radical scavenging activity in traditionally fermented wheat ($r = 0.208$), this finding disagrees with that found by **Horvat et *al*. (2020),** who reported a significant positive correlation ($r = 0.598$) between TPC and DPPH radical scavenging activity in unfermented wheat.

It should be noted that antioxidant activity is not necessarily linked to the content of bioactive compounds but to the nature of these compounds. In fact, **Zilic et *al*. (2011)** reported that phenolic profiles and carotenoids influence the antioxidant properties of grain. Otherwise, **Zavala-Lopez and García-Lara (2017)** found that other phytochemicals rather than TPC may play an important role in scavenging activities in some cereals. In the same way, **Antognoni et *al*. (2019)** reported that fermentation by *Lactobacillus plantarum* (29DAN, 83DAN) increased the carotenoid content in the wheat dough. This finding can explain the non-significant correlation between TPC and DPPH in our results.

Otherwise, the relationship between bioactive compounds and antioxidant capacity is extraction solvent dependent. In fact, **Lyu et *al*. (2022)** reported that the correlation between DPPH and TPC in hop strobili was statically significant in ethanol extracts, whereas, no significant correlation was observed in water extracts.

Table 7. Correlation between TFC/TPC and DPPH

Relationship	*r*	*P*
Between TFC and DPPH scavenging activity	0.856	0.03*
Between TPC and DPPH scavenging activity	0.208	0.517^{ns}

TFC: total flavonoid content, TPC: total phenolic content, * $p < 0.05$, ns: not significant.
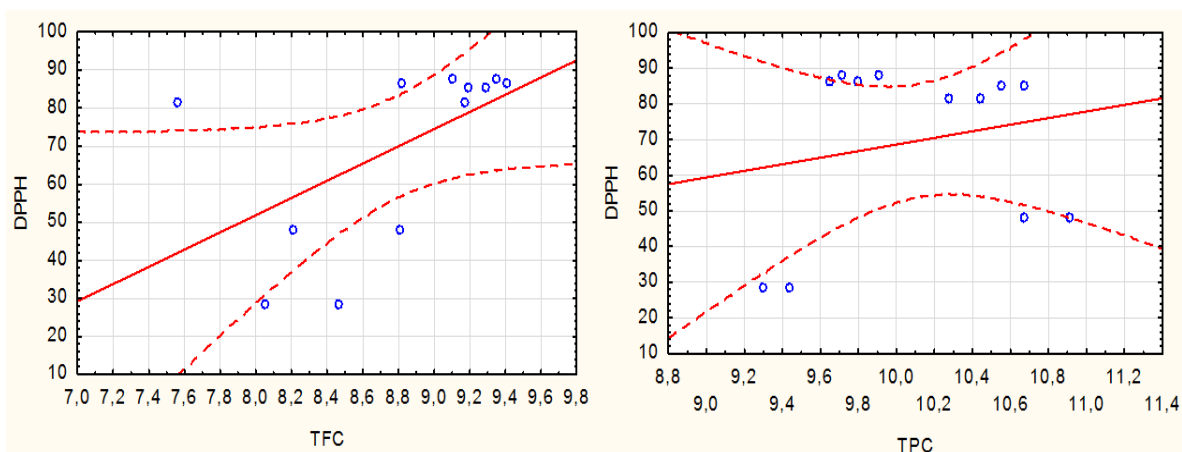
Fig. 15. Correlation between TFC/TPC and DPPH. Pearson's *r* correlation of SPSS version 22 was used. Correlation matrices were assessed by STATISTICA 10.

I.2.6. Bread analysis

I.2.6.1. Gas retention

The breads obtained are presented in **Fig. 16**. **Table 8** shows the measurement of dough height at 0, 20, 40 and 60 min. The dough height increases as time fermentation increases.

At 20 min, the sample with 10 % traditionally fermented wheat (TFW) has the highest value of dough height, while the bread obtained from 30 % TFW has the lowest value. The same results are noted at 40 and 60 min. It seems that the bread from 10 % and 20 % TFW has better gas retention than that from unfermented wheat (100 % UFW and 30 % TFW). The decrease in gas retention as fermented wheat flour proportions increase is probably due to a decrease in the

elastic property of the flour, which depends on the gluten content. Such proteins could undergo enzymatic degradation during the fermentation of the grains in the Matmour.

Fig. 16. Aspects of produced breads **(original photos).** A: UFW (100 %), B: TFW (10 %), C: TFW (20 %), D: TFW (30 %) and E: aspect of breads during fermentation

Table 8. Physical properties of bread samples (dough height) (cm)

Fermentation time (min)	UFW (100%) ***	UFW / TFW (90:10%) ns	UFW / TFW (80:20%) **	UFW / TFW (70:30%) ***
00	2 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00
20	2.3 ± 0.02	3.15 ± 0.21	3.05 ± 0.63	2 ± 0.00
40	2.6 ± 0.00	3.85 ± 1.06	3.5 ± 0.28	2.9 ± 0.14
60	3.2 ± 0.00	4.25 ± 1.06	3.95 ± 0.21	3.1 ± 0.14

UFW: unfermented wheat, TFW: traditional fermented wheat. Values are mean $\pm$ SD, differences were evaluated by one-way analysis of variance (ANOVA), *** indicates significant difference at $p < 0.001$, ** indicates significant difference at $p < 0.01$, ns: not significant.

The results obtained in our study are in agreement with those found by **Okafor et *al.* (2012)** for wheat mushroom powder bread, and **Oloyede et *al.* (2013)** for fermented unripe plantain flour, in regards to the measurement of the loaf volume of bread samples. Comparing the results for TFW dough gas retention to those of UFW dough, the highest values in TFW could be explained by the fact that the TFW flour is rich in simple products (such as fermentable sugars) used during the fermentative process of *Saccharomyces cerevisiae*.

I.2.6.2. Sensory evaluation of breads

As shown in **Fig. 17,** which represents the mean sensory scores of experimental and control bread samples manufactured with different proportions of UFW and TFW, the bread from 10 % TFW is the most appreciated with a score of 7.03 ± 0.21 compared with breads from other formulations (20 % and 30 % TFW), whereas, the bread obtained from 30 % TFW mixture is the least appreciated with a score of 4.83 ± 0.00 ($p < 0.001$***).

According to **Garcia-Gomez et *al.* (2022)**, during sensory evaluation of Galician breads, loaves with different shapes (equal ingredients) showed different sensory profiles. Indeed, texture, freshness and taste were the most correlated to global acceptance. Furthermore, and according to our results, it is possible that the most brownish color of the crumb (**Fig. 16**), which is dependent on the incorporation percentage of TFW flour, is the cause of the decrease in the acceptance of the products by consumers, who prefer bread with a lighter color **(Gomes et *al.*, 2016).** This result is confirmed by the negative correlation between the incorporation percentage of TFW flour and the sensory score of bread samples ($r = - 0.991$**, $p < 0.001$***).

Although the bread from 10 % TFW had a score close to that of the bread from 100 % UFW, it is less acceptable than the latter. This result is similar to that found by **Ameh et *al.* (2013),** who noticed that the 100 % wheat bread was organoleptically more acceptable than the wheat bread supplemented with rice bran.

Udofia et *al.* (2013) studied the sensory evaluation of wheat-cassava-soybean composite flour bread. They reported that all samples of highly supplemented bread samples failed to meet the important parameters of pure bread; the failure hindered general acceptability and preference of the familiar bread sample. The same result was obtained in our study for bread with the same proportion (70:30 %, UFW:TFW).
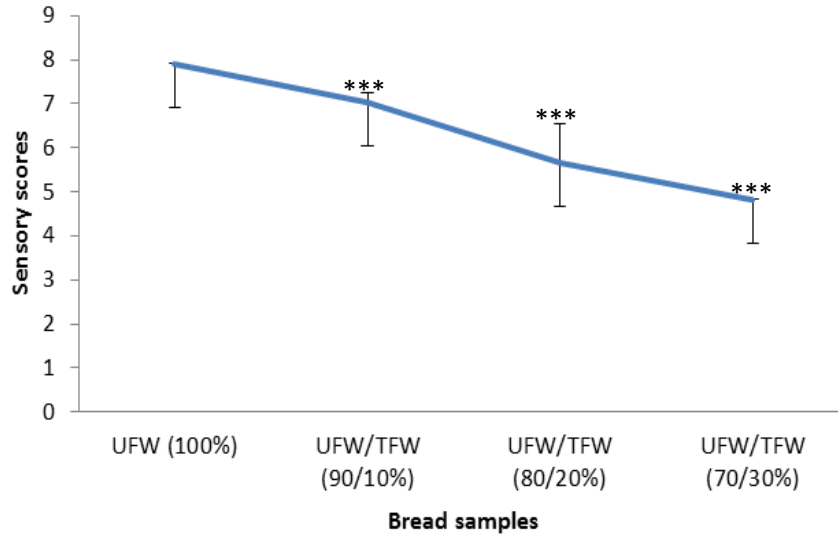
Fig. 17. Sensory scores of bread samples. UFW: unfermented wheat, TFW: traditionally fermented wheat. ANOVA test was used (n = 3), *** indicates significant difference at $P < 0.001$.

Several studies talk about the relationship between the percentage of additional flours to wheat flour and the sensory acceptance of bread. **Shongwe et al. (2022)** concluded that the sensory evaluation revealed that it is possible to include peanut flour up to 20 % for acceptable bread. **Oloyede et al. (2013)** also reported low overall acceptability for bread produced from 30 % fermented unripe plaintain flour substitution. According to our results, breads of good sensory qualities could be produced from up to 10 % fermented wheat flour substitution in unfermented wheat flour. It is the same conclusion obtained by **Olaoye et al. (2006)** when they used soy flour substitution in wheat flour.

Article 2. Traditional fermented wheat: nutritional quality and sensory evaluation of bread produced from composite fermented wheat flour

II. Antioxidant properties of *Triticum durum* obtained by traditional spontaneous fermentation in underground silos

To the best of our knowledge, no studies have already considered the use of natural fermentation (in underground silos) to enhance the functional properties of durum wheat. This part of study is aimed to clarifying the role of spontaneous fermentation in increasing the antioxidant features of fermented wheat, with the perspective of producing a functional ingredient or dietary supplement.

II.1. Materials and methods

II.1.1. Samples

Three different samples of traditionally fermented wheat (TFW) were collected from Mila, a region situated in Eastern Algeria. The samples were obtained after being subjected to traditional spontaneous fermentation in underground silos (called Matmours) for approximately 10 months in rural areas. Unfermented wheat was purchased from a market in Jijel, Algeria.

II.1.2. Extract preparation

The wheat grain powder was extracted in distilled water (1/2) (W/V) for 10 min at room temperature. The extract was filtered using Whatman N°1 filter paper **(Talbi et *al*., 2015).** The filtrate of aqueous extract was freshly used for further assessment in *in vitro* assays.

II.1.3. Phenolic compounds content

The total phenolic content (TPC) was investigated using the Folin-Ciocalteau assay **(Othman et *al*., 2007).** Briefly: 0.2 ml of the traditionally fermented wheat extract (TFWE) was mixed with 1.5 ml of Folin-Ciocalteau reagent. After 5 min, 1.5 ml of 7 % sodium carbonate solution was added and the mixture was incubated for 90 min, then the absorbance was measured at 750 nm. The TPC was expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g).

II.1.4. Flavonoid content

To determine the total flavonoid content (TFC), aluminum chloride complex forming assay was used according to **Djeridane et *al*. (2006).** 1.5 ml of each sample was mixed with 1.5 ml of 2 % aluminum chloride solution. The mixture was allowed to stand in darkness for 30 min and absorbance was recorded at 430 nm. The results are expressed as mg quercetin equivalents per g of dry weight (mg QE/g).

II.1.5. Antioxidant activity of water extracts

II.1.5.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the fermented wheat extracts was measured as scavenging free radical potential in a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by **Mansouri et *al*. (2005).** 100 μl of TFWE were added to 1300 μl of 0.004 % DPPH methanolic solution freshly prepared. After incubation for 30 min at room temperature and in darkness, the absorbance was recorded at 517 nm and the antiradical activity was calculated as percentage of DPPH discoloration compared to the control using the formula number (11).

(11)…………………………………...**Inhibition** (%) $= \frac{(A-B)}{A} . \mathbf{100}$

Where:

A: absorbance of pure DPPH in oxidized form;

B: absorbance of the sample.

II.1.5.2. Ferric reducing antioxidant power (FRAP) assay

The ability of the extracts to reduce ferric iron (Fe^{+3}) into ferrous iron (Fe^{+2}) was assessed by the method described by **Costa et *al*. (2010)**: 2.5 ml of the wheat extract was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After the addition of 2.5 ml of 10 % trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1 % ferric chloride, and the absorbance was measured at 700 nm. The results are expressed as absorbance values. The increased absorbance of the reaction mixture indicates an increase in reducing power.

II.1.5.3. Hydrogen peroxide scavenging activity

The ability of fermented wheat extracts to scavenge hydrogen peroxide was determined according to the method of **Ruch et *al*. (1989)**. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The extracts in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without

hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both fermented wheat extracts and the standard compound were calculated:

$$(12)\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\quad \text{Scavenged } H_2O_2\ (\%) = \frac{Ac - As}{Ac} \cdot 100$$

Where:

A_C: absorbance of the control

A_S: absorbance of TFWE samples.

II.1.6. Statistical analysis

All data analysis was performed using SPSS software version 22.0 for Windows. The data obtained from the analysis is expressed as the mean ± standard deviation (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) at $p < 0.05$ and Tukey's posthoc test. Correlation analysis between some parameters was performed using Pearson correlation at $p < 0.05$. The correlation matrices were assessed by STATISTICA 10.

II.2. Results and discussion

II.2.1. Phenolic contents

Cereal bioactive compounds, especially polyphenols, are known to possess a wide range of disease preventive properties that are attributed to their antioxidant and anti-inflammatory activity **(Ed Nignpense et al. 2021).** Phenolic compounds play a role in free radical scavenging capacities. They are one of the most effective bioactive constituents that contribute to antioxidant activity **(Boo, 2019; Pawlowska et al., 2019; Zhou et al., 2019).** Most of these bioactive compounds are found in the outer layer of the wheat grain (aleurone, bran and germ), thus forming the constituents of whole wheat flour, and providing more antioxidant benefits than refined flour **(Di Silvestro et al., 2012).**

In this study, we were interested in evaluating the level of bioactive compounds in aqueous extract of traditionally fermented wheat. According to **Fig. 18.A**, the total phenolic compounds (TPC) of the samples vary significantly ($p < 0.001$ ***). The highest TPC is obtained in traditionally fermented wheat extract (TFWE) from the sample WM2 (1.12 ± 0.007 mg GAE / g).

It is clear that TFWEs present increased values compared to the control. These results are in perfect agreement with those found by **Gourchala et *al*. (2014),** where an increase of TPC of fermented wheat was recorded compared with unfermented wheat**.** The same results were obtained by **Zhang et *al*. (2012).** Previous research has linked free phenolic content to β-glucosidase and amylase activities in fermented food substrates **(Stojiljkovi´c et *al*., 2016; Xiang et *al*., 2019).** These endogenous enzymatic activities play an important role in starch degradation, which is considered a source of fermentable sugars, leading to an increase in the total phenolic content **(Gänzle, 2014). Salar et *al*. (2012)** reported an increase in TPC during the fermentation of whole grains of maize, through the activities of β-glucosidase, which is capable of hydrolyzing phenolic glycosides to release free phenolics.

The increase in phenolic compounds level during fermentation of cereals in general, and wheat in particular, has been widely discussed in several publications **(Yin et *al.*, 2018; Chen et *al*., 2020; Spaggiari et *al*., 2020; Kasote et *al*., 2021).** Otherwise, **Adebo et *al*. (2018)** and **Adebo and Medina-Meza (2020)** reported that the fermentation process influences the whole cereal grain polyphenolics, leading to changes in inherent levels and/or the formation of subsequent monomers or polymers. They also reported higher bioactive compounds (catechin, gallic acid, and quercetin) after fermentation in a study on ting from fermented whole grain sorghum. On the other hand; they registered a decrease in levels of total flavonoid content (TFC), total tannin content (TNC) and total phenolic content (TPC). Decreases in levels of TPC, TFC, and TNC could be explained by degradation and hydrolysis of the phenolic compounds. However increase in catechin, gallic acid, and quercetin was attributed to the release of these bioactive compounds after fermentation with *Lactobacillus* strains **(Adebo and Medina-Meza, 2020).**

II.2.2. Flavonoid contents

The same figure (**Fig. 18.B**) shows total flavonoid content (TFC) in traditionally fermented wheat extracts (TFWEs). We have registered a very significant increase in TFC for the three samples compared with the control sample ($p < 0.001$ ***). The best result is recorded in the sample coded WM2 (0.30 ± 0.007 mg QE g^{-1}). Our findings are in agreement with those obtained in previous studies. In fact, it is well known that the use of microorganisms in cereal fermentation, whatever fungus species or LAB, promotes the increase of phenolic and flavonoid content **(Đorđević et *al*., 2010; Zhang et *al*., 2012; Sandhu et *al*., 2016).**
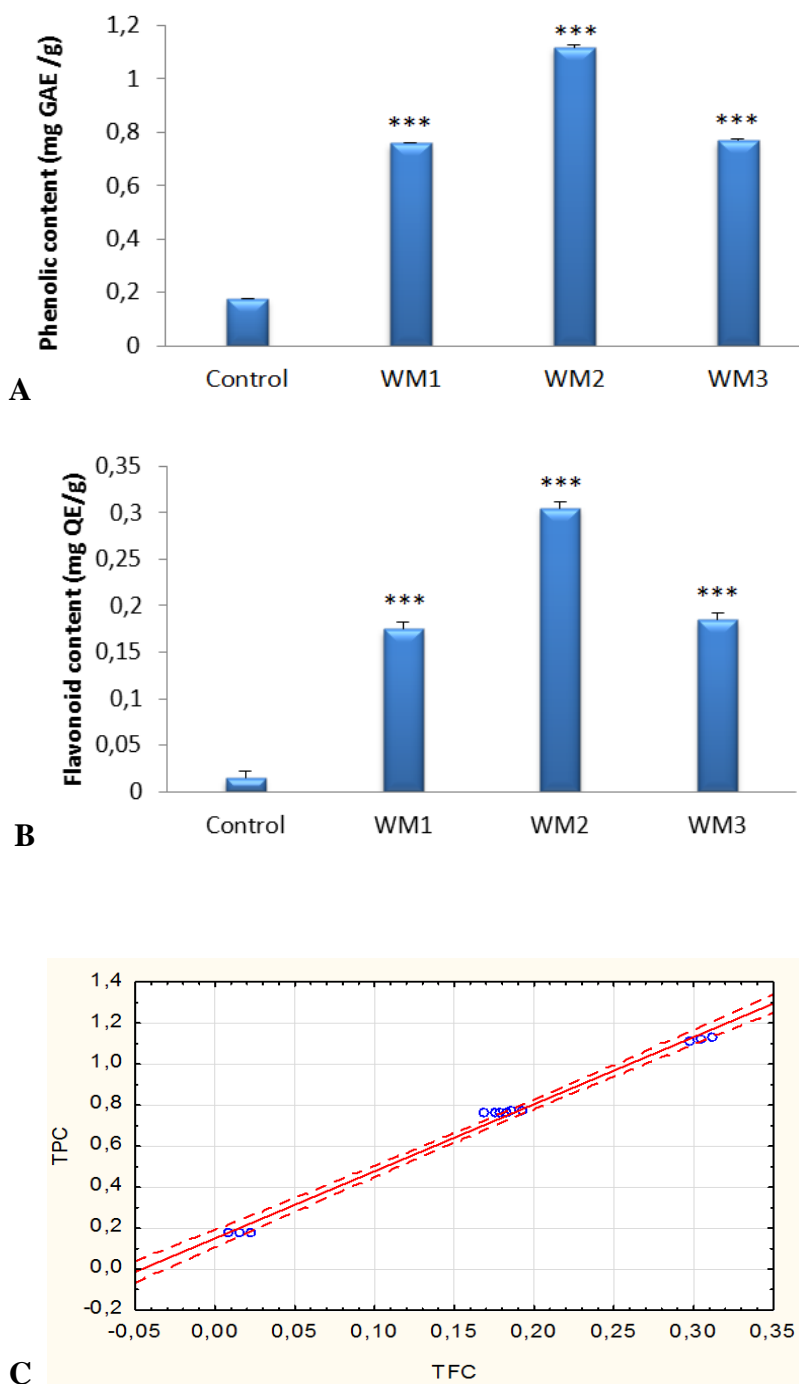
Fig. 18. Phenolic (A) and flavonoid (B) content in fermented and unfermented wheat samples. (C): correlation between TPC and TFC. WM1, WM2 and WM3: fermented wheat samples, TPC: total phenolic content, TFC: total flavonoid content, ANOVA test was used (n = 3), *** indicates significant difference at $P < 0.001$. Pearson's r correlation was assessed by IBM SPSS version 22. Correlation matrices were evaluated using STATISTICA 10.

According to **Blandino et *al*. (2003)**, natural fermentation involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may act simultaneously, while others act sequentially, with a changing dominant flora during fermentation. Spontaneous fermentation in the fermented wheat samples involves all of these microorganisms, which act to increase the level of the antioxidant compounds. Furthermore, it is reported that proteases, amylases and xylanases derived from fermenting microbes induce structural breakdown of the cell wall during fermentation of the grain, which leads to the synthesis of various bioactive compounds **(Wang et *al*., 2019; Adebo and Medina-Meza, 2020).**

From **Fig .18** (**A** and **B**), it is very clear that the increase in TPC was associated with the increase in TFC, which is confirmed by a positive correlation with a very high significance between the two parameters ($r = 0.996$ ** ≈ 1**, $p < 0.001$ ***) (**Fig. 18.C**).

II.2.3. Antioxidant activity of fermented wheat extracts

II.2.3.1. 2,2-diphenyl-1-picrylhydrazyl(DPPH) assay

Fig. 19 shows the DPPH antioxidant activity of traditionally fermented wheat extract samples (TFWE) with a very high significant difference ($p < 0.001$). It was found that the DPPH scavenging effect of TFWEs increased as their concentrations increase. Such a scavenging effect depends on the used concentrations.

The highest DPPH antioxidant capacity is attributed to the WM1 fermented wheat sample (21.82 ± 0.99 % and 28.64 ± 1.85 for the concentrations of 0.12 and 0.25 mg/ml respectively), followed by WM2 and WM3 (20.41 ± 0.01 %, 26.75 ± 1.98% and 15.49 ± 1.99 %, 23.94 ± 0.00 % respectively for the concentrations of 0.12 and 0.25 mg/ml), against only (3.5 ± 0.98 and 3.51 ± 0.99%) for the control sample.

The result of the DPPH scavenging activity assay indicates that fermented wheat was potently active and that the TFWEs contain compounds that are capable of donating hydrogen to a free radical in order to eliminate the odd electron, which is responsible for the radical's reactivity **(Aiyegoro and Okoh, 2010)**.

DPPH antioxidant activity is effective in traditionally fermented wheat. This can be explained by the enhancement of antioxidants like polyphenols and flavonoids through fermentation. Phenolic compounds are considered major antioxidants that contribute to the activity of wheat. These components, which contain hydroxyl groups connected to aromatic rings, can efficiently react

with free radicals **(Di Silvestro et *al*., 2017).**

According to **Zhang et *al*. (2012)**; DPPH antioxidant activity was more effective in wheat fermented using *Cordyceps militaris* than in unfermented wheat. Higher antioxidant activity was due to high total phenolic content **(Duan et *al*., 2020).** On the other hand, **Dey and Kuhad (2014)** reported that solid-state fermentation (SSF) at 30 °C for 72 h with four filamentous fungi generally recognized as safe (GRAS) (*Aspergillus oryzae* NCIM 1212, *Aspergillus awamori* MTCC No. 548, *Rhizopus oligosporus* NCIM 1215 and *Rhizopus oryzae* RCK2012) revealed a high ability for the enhancement of water-soluble total phenolic content (TPC) and antioxidant properties, including DPPH scavenging capacity. Such an increase is related to the release of more soluble bioactive compounds such as peptides and oligosaccharides.

In the same way, **Zhu et *al*. (2006)** and **Liu et *al*. (2017)** proved that fermentation of defatted wheat germ released peptides (180 - 1000 Da) which, after hydrolysis into low molecular weight peptides and free amino acids, showed relatively high activity against the DPPH radical.
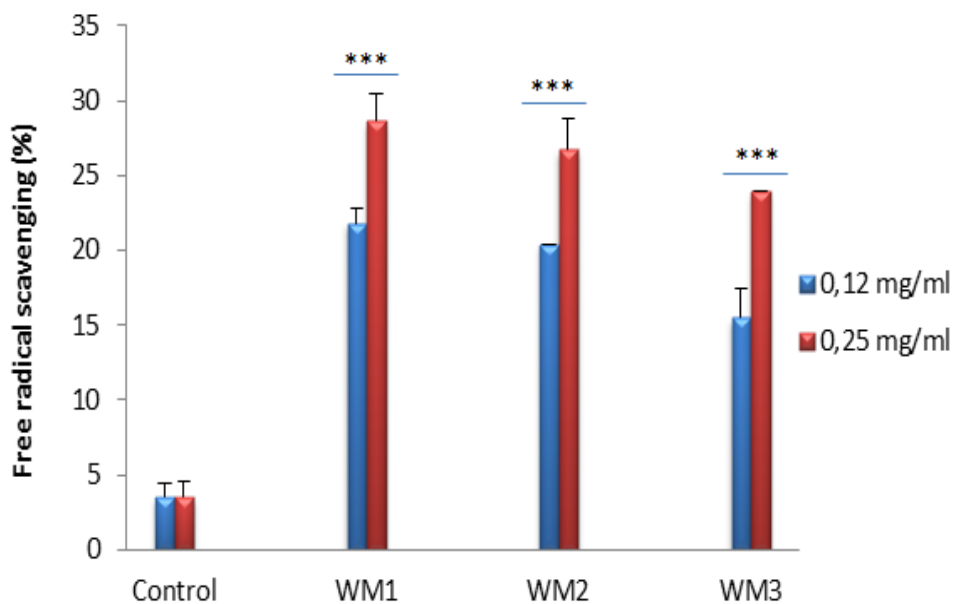
Fig. 19. DPPH scavenging activity of different concentrations of TFW and control extracts. WM1, WM2 and WM3: fermented wheat samples, ANOVA test was used (n = 3), *** indicates significant difference at $P < 0.001$

Dordevic et *al*. (2010) reported that fermentation in some cereals, including wheat, using *Lactobacillus rhamnosus* enhanced antioxidant activity and had a positive effect on DPPH inhibitory effect. However, the fermentation with *Saccharomyces cerevisiae* had no significant effect on DPPH inhibitory influence.

It has been demonstrated that fermentation has a positive influence on the TPC and the antioxidative activity of cereals, and that the degree of this influence depends on the microorganism species. In fact, an earlier study confirmed that LAB are involved in the spontaneous fermentation of durum wheat **(Gourchala et *al*., 2014).** All of these findings support the above results.

II.2.3.2. Ferric reducing antioxidant power (FRAP) assay

Fig. 20.A shows the reducing power potentials of the aqueous extract of TFW in comparison to the control at 700 nm ($p < 0.001$ ***).

It is clear that all sample extracts show reducing power potential, but WM2 proved to be more active (0.95 ± 0.07) followed by WM3 and WM1 (0.82 ± 0.007 and 0.7 ± 0.007 respectively). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The results show that there has been an increase in reducing power in the samples. The reducing capacity of fermented wheat is more significant than the reducing capacity of control (0.14 ± 0.02). This result indicates that fermented wheat is rich in antioxidant compounds that act as electron donors and can reduce the oxidized intermediates of lipid peroxidation processes **(Medhe et *al*., 2014)**. The same result was found by **Zhang et *al*. (2012)** in fermented wheat using *Cordyceps militaris*.

According to **Dey and Kuhad (2014)**, the increase in antioxidant properties like FRAP in tempe (fermented wheat product) is related to the composition of phenolic compounds, unidentified compounds, and other water-soluble bioactive compounds like small peptides and xylo-oligosaccharides produced during fermentation.

The capacity of the extracts to reduce Fe^{3+} into Fe^{2+} is due to their content of antioxidant molecules, such as phenolic compounds and flavonoids. The findings are proved by the positive correlation between these parameters. The results of the reducing power test correlate positively with polyphenolic contents (r = 0.972**, $p < 0.001$***) and with flavonoid contents (r = 0.958**, $p < 0.001$***) **(Fig. 20.B, C)**.
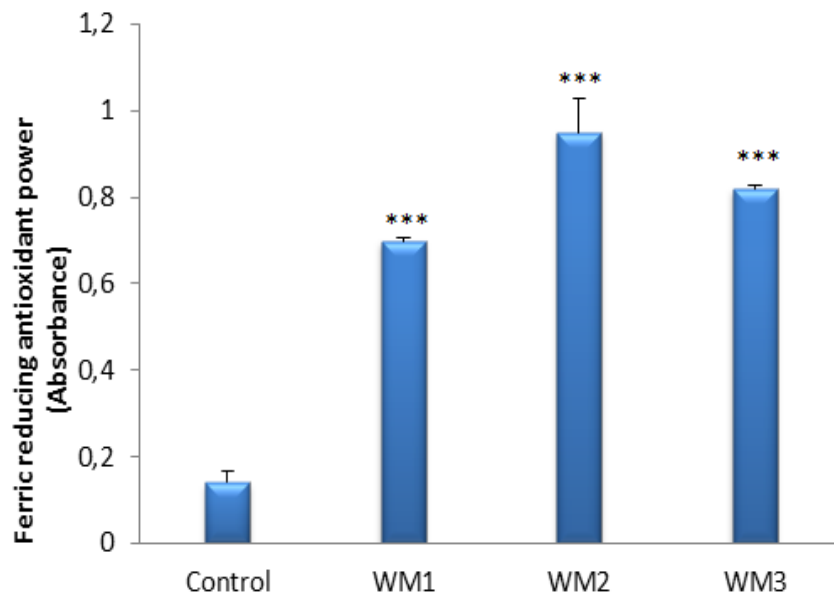
A
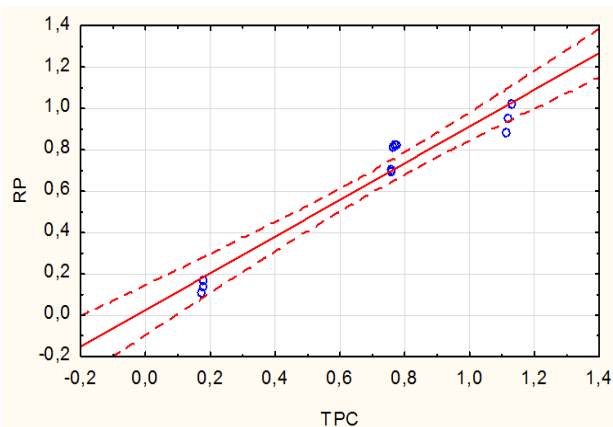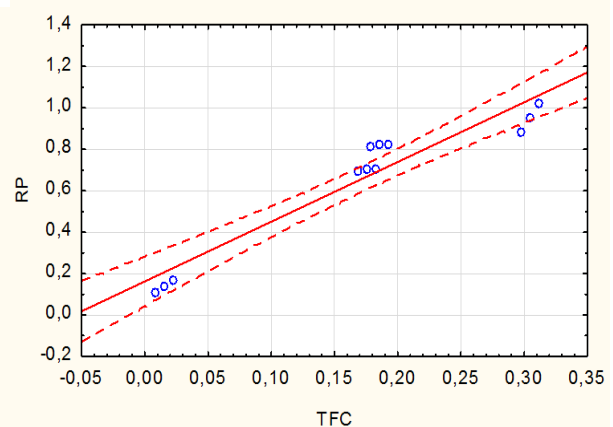
B   **C**

Fig. 20. Reducing power of TFW and control extracts (A), (B): correlation between FRAP and TPC, (C): correlation between FRAP and TFC. WM1, WM2, WM3: fermented wheat samples, RP: reducing power, TPC: total phenolic contents, TFC: total flavonoids content, ANOVA test was used (n = 3), *** indicates significant difference at $P < 0.001$, Pearson's r correlation was assessed by IBM SPSS statistics 22. Correlation matrices were evaluated using STATISTICA 10.

II.2.3.3. Hydrogen peroxide scavenging assay

Hydrogen peroxide is an important reactive oxygen species because it is able to penetrate

biological membranes and it may be toxic if converted to hydroxyl radicals in the cell (**Pagano et al., 2014; Zhao et al., 2016; Lv et al., 2018**).

The sample extracts show appreciable scavenging activity on hydrogen peroxide, compared to the control ($p = 0.003$ **). The highest value is obtained with sample WM3 (84.6 ± 7.00 %) followed by WM1 (81.90 ± 8.90 %) and WM2 (77.4 ± 0.00 %), while the control represents only the value 18.19 ± 0.00 % (**Fig. 21**).

According to our results, the ability of TFWEs to scavenge H_2O_2 may be attributed to their antioxidant compounds such as phenolics, which donate electrons to H_2O_2, thus reducing it to water (**Aiyegoro and Okoh, 2010**). In a study conducted by **Dey and Kuhad (2014)**, the increase in hydrogen peroxide scavenging activity in tempe (a fermented wheat product) was attributed to phenolic compounds, small peptides and xylo-oligosaccharides produced during fermentation.
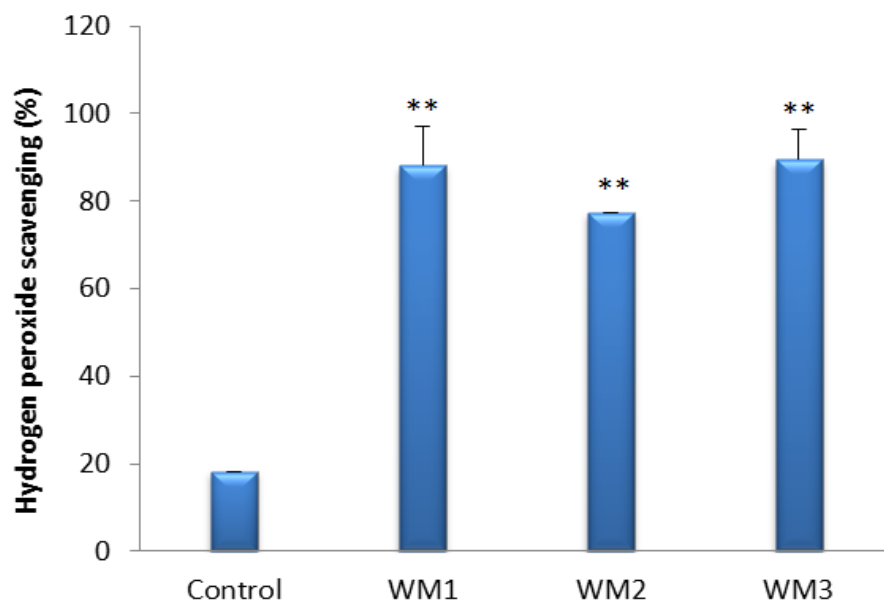
Fig. 21. Hydrogen peroxide scavenging activity of TFW and control extracts. WM1, WM2, WM3: fermented wheat samples, ANOVA test was used (n = 3), ** indicates significant difference at $P < 0.01$

II.2.3.4. Relationship between bioactive compounds and antioxidant activity of traditionally fermented wheat water extracts

The relationship between total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (DPPH capacity, reducing power (RP) and hydrogen peroxide scavenging capacity (HP)) was evaluated by the Pearson correlation **(Fig. 22, Table 9).** TPC is positively and significantly correlated with DPPH, RP and HP. Indeed, the highest correlation is observed between TPC and RP ($r = 0.97**$). On the other hand, we have also observed a positive significant correlation between TFC and DPPH, RP and HP. The strongest correlation was found between TFC and RP ($r = 0.96**$), while the weakest was found between TFC and HP ($r = 0.77**$).

According to **Kumar et *al*. (2014),** the high correlations confirm the role of phenolic compounds. The types and quantities of phenolic compounds might contribute to the varying antioxidant activity of the extracts. Furthermore, the highly significant correlations obtained in this study support the hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity of the examined wheat samples. Several publications were in line with the above findings (**Horvat et *al*., 2020; Kim and Lee, 2020; Dobrinas et *al*., 2021; Osman et *al*., 2021; Lyu et *al*., 2022).**

Many factors can affect the relationship between antioxidant activity and total phenolic content; in fact, total phenolic content does not include all antioxidants. Indeed, it must be taken into account the synergism between the antioxidants in the mixture that makes the antioxidant activity not only dependent on the concentration but also on the structure and the interaction between the antioxidants. This can explain why samples with similar concentration of total phenolics, vary in their antioxidant activities. Also, it is worth considering that the antioxidant activity of plant extracts may be related to the presence of some individual active phenolic compounds (**Piluzza and Bullitta, 2011).**
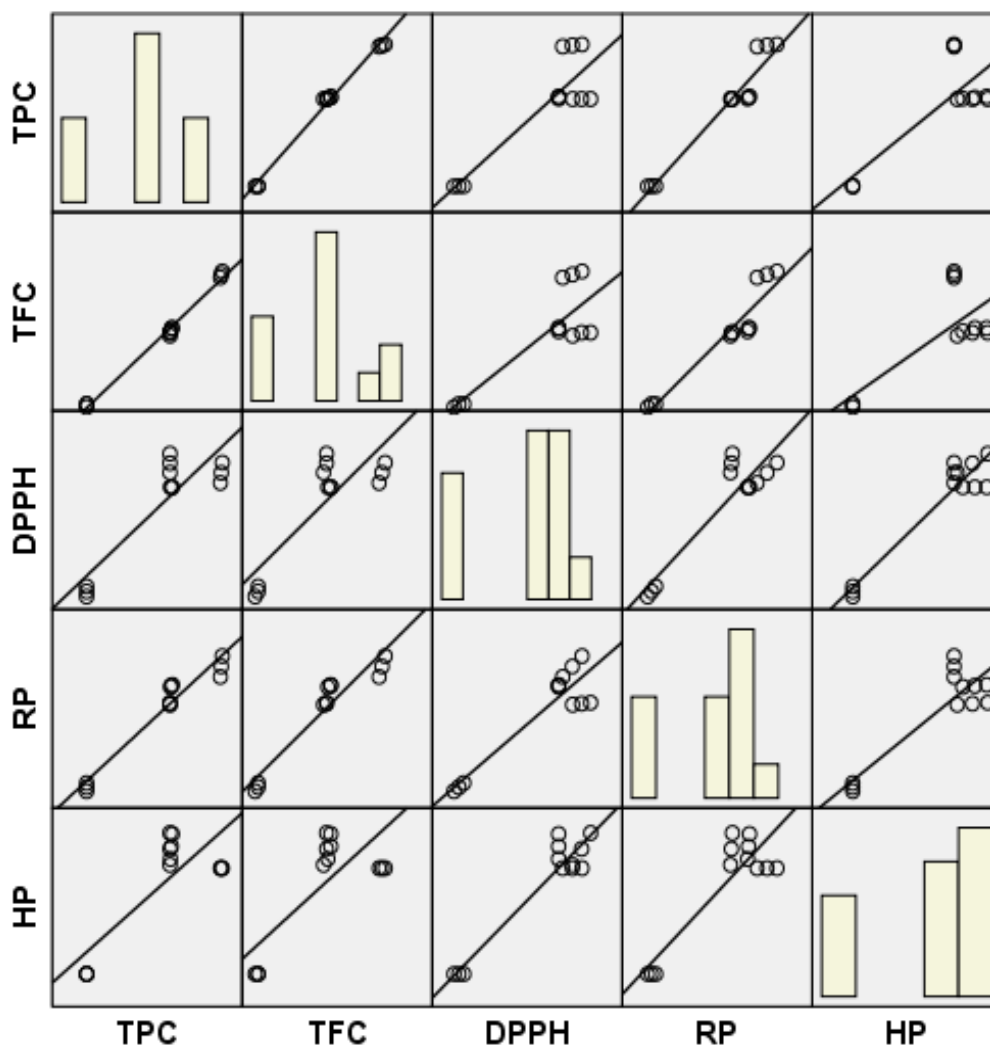
Fig. 22. Correlation matrices between TPC/TFC and antioxidant capacity of traditionally fermented wheat water extracts.TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, RP: ferric reducing power, HP: hydrogen peroxide scavenging capacity.

A possible relationship between DPPH, RP and HP was tested. All of the correlations were significant. The highest one was observed between DPPH and HP (r = 0.96**) followed by the correlation between DPPH and RP (r = 0.93**), RP and HP (r = 0.89**).

Table 9. Pearson's correlations between antioxidant activities measured using different assays and total phenolic/flavonoid contents

	TPC	TFC	DPPH	RP	HP
TPC	1,00				
TFC	,99**	1,00			
DPPH	,89**	,85**	1,00		
RP	,97**	,96**	,93**	1,00	
HP	,82**	,77**	,96**	,89**	1,00

TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, RP: ferric reducing power, HP: hydrogen peroxide scavenging capacity, ** indicates significant difference at $P < 0.01$

Article 3. Enhancement of antioxidant properties of *Triticum durum* obtained by traditional spontaneous fermentation in underground silos.

III. Antifungal activity of lactic acid bacteria isolated from traditionally fermented wheat

In the present part of the study, we present the ability of lactic acid bacteria (LAB) isolated from traditionally fermented wheat, to inhibit the growth of *Aspergillus niger* and *Aspergillus flavus*.

III.1. Materials and methods

III.1.1. Fungal strains

During our study, two reference fungal strains were mainly used. They were provided by Dr. Bourzama G. (University of Annaba, Algeria). These strains are *Aspergillus niger* ATCC 10577 and *Aspergilus flavus* ATCC 9643.

The *Aspergillus* strains used were grown on potato dextrose agar (PDA) and were kept at 4 °C in slant agar tubes. Before each use, they are subcultured on agar plates and incubated for 5-7 days at 25 °C.

III.1.2. Isolation of LAB strains

Eight (08) samples of traditionally fermented durum wheat were used for the isolation of LAB. To prepare the stock solution, 10 g of traditionally fermented wheat were crushed and then homogenized with 90 ml of physiological water. The solution was left in contact for 30 min, then decimal dilutions were prepared. 0.1 ml of dilution 10^{-3} was deposited on the surface of the Man, Rogosa and Sharpe (MRS) agar. After incubation at 37 °C for 2-3 days, 10 % of small shiny white colonies were transferred to MRS broth, and then they were subcultured several times for better purification. Isolated LAB strains were verified by Gram staining and catalase tests. All isolates were kept at - 20 °C in MRS broth with 30 % glycerol **(Nionelli, 2014)**.

III.1.2.1. Gram staining

This test was performed on young cultures less than 24 hours old. A smear of cells was made on a slide. Crystal violet and lugol solutions were respectively applied to the smear for 1 min, followed by a water wash. Ethanol 90 % was applied to the smear, which was then treated with fuchsine for 30 seconds. The smear was observed under a microscope (Olympus, Japan) under immersion at 100 X magnification. Isolates with purple staining are Gram-positive (+) while those with pink staining are Gram-negative (-) **(Ismail et *al*., 2018).**

III.1.2.2. Catalase test

A colony of a culture less than 24 hours old of each isolate was deposited via a loop on a slide; a drop of hydrogen peroxide 3 % was then added. The presence of catalase is indicated by the

appearance of effervescence. Isolates without catalase are unable to degrade hydrogen peroxide; they are catalase negative, characteristic of LAB (**Fevria and Hartanto, 2020**).

III.1.3. Screening for antifungal activity

III.1.3.1. Preparation of fungal spore solution

Aspergillus niger and *Aspergillus flavus* were used in this study for the assay of antifungal activity *in vitro*. The spore suspensions required to inoculate the media are freshly prepared. To prepare the spore solution, the fungi strains were grown on PDA medium at 25 °C for 5 days, the conidia were collected in physiologic water and the concentration of spores in the obtained stock solution was determined by counting at the microscope on the Malassez cell counting chamber, then adjusted to 10^6 spores / ml (**Kivanc et *al.*, 2014**).

Two different assays were employed: the overlay method and the dual-culture agar overlay method.

III.1.3.2. Qualitative test: Streak method

The antifungal activity of LAB was assessed using the overlay method described by **Magnusson et *al*. (2003)**.

Isolated LAB were inoculated in 2 cm lines on MRS agar plates and allowed to grow at 30 °C for two days. 10 ml of PDA agar (0.8 % w/w) containing 10^6 spores / ml of each strain of *A. niger* and *A. flavus* was then poured onto the agar plates and incubated at 25 °C for three days. The inhibition zone was measured around the bacterial streaks and was scored as follows: (-) no suppression of fungal growth; (+) no fungal growth on 0.1 % to 3 % of the plate area per bacterial streak; (++) no fungal growth on 3 % to 8 % of the plate area per bacterial streak; or (+++) no fungal growth on > 8 % of the plate area per bacterial streak. The experiment was carried out in triplicate.

III.1.3.3. Quantitative test

a. Co- cultrure on MRS agar: The method used is a variant of one of those described by **Florianowicz (2001).** 200 µl of young bacterial culture (12 to 16 h) were inoculated in depth of 15 ml of MRS agar. After the solidification of the culture medium, one sterile disk was deposited in the center of the plate and then saturated with 10 µl of sporal suspension (10^6 spores / ml). Cultures were incubated at 25 °C for two weeks. Linear growth was determined every two days, through measuring the diameter of the colonies in two perpendicular directions.

b. Determination of inhibitory activity of cell-free supernatants: The method described by **Cabo et *al.* (2000)** was applied. 100 ml of MRS broth were inoculated with 1 % (v/v) young bacterial culture (12 to 16 h) and incubated at 30 °C for 72 to 120 h. 20 ml of culture medium were removed and centrifuged at 10 000 g for 10 min then sterilized by filtration (0.22 μm Millipore). 2 ml of the supernatant was collected and mixed with 15 ml of PDA medium (concentration x 2). The medium obtained was homogenized and poured into a Petri dish. After solidification, 3 μl of spore suspension (10^6 spores / ml) were deposited on the surface of the medium and the whole was incubated at 25 °C for 5 days. The colony of the fungal strain was compared to that of the control, in which the supernatant was replaced with sterile distilled water. The experiment was carried out in triplicate. Antifungal activity was expressed in terms of colony growth inhibition as follows:

$$(13)\ldots\ldots\ldots\ldots\ldots\textbf{\textit{Inhibition (I) or antifungal activity (A.F.A)}}(\%) = \textbf{100}\,.\,(\textbf{1} - \frac{DE}{DT})$$

Where;

DE: the diameter of a fungal colony in the presence of LAB;

DT: the diameter of a fungal colony without LAB (as a control).

III.1.4. Influence of inhibitory metabolites on mycelium development

To determine the influence of the extracellular metabolites produced by the selected strains on radial growth of the mycelium, 100 ml of MRS broth medium were inoculated with young cultures (12 to 16 hours) at a rate of 1 % (v/v). After incubation at 30 °C for 120 hours, a 10 ml aliquot was centrifuged at 10,000 g for 10 min. 2 ml of supernatant sterilized on a Millipore membrane (0.22 μm) were added to 15 ml of PDA medium (concentration x 2) then poured onto the agar plates for solidification. The fungal strain was grown separately for 5 days on PDA medium at 25 °C. An implant of 6 mm in diameter was taken and deposited on the surface of the solidified culture medium. In the control, the supernatant was replaced with sterile distilled water. The cultures were then incubated at 25 °C for a week while being monitored. Anti-*Aspergillus* activity was assessed by measuring the diameter of the implant in two perpendicular directions **(Florianowicz, 2001; Sathe et *al.*, 2007).**

III.1.5. Molecular characterization of the most active strain

III.1.5.1. 16 S rRNA gene amplification

The extraction of bacterial genomic DNA was performed using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn Bhd, Selangor DE, Malaysia) according to the manufacturer's instructions. The extracted DNA was kept at 4 °C until it was needed for PCR.

The 16 S rRNA gene primer set (27F: 5' – AGA GTT TGA TCC TGG CTC AG – 3' and 1492R: 5' – CCG TCA ATT CCT TTG AGT TT-3') was used for PCR amplification (using the iCycler BIO-RAD USA) **(Edwards et *al*., 1989).**

III.1.5.2. Agarose gel electrophoresis

After the PCR reaction, the PCR product was separated on a 1.5 % agarose gel (Sigma-Aldrich, USA). One hundred base pair (100 bp) DNA ladder (Solis Biodyne, Estonia) was used as a DNA molecular weight marker. Electrophoresis was done at 80 V for 1 h 30 min, and the gel was viewed under UV light after staining with Midori Green Advance (Nippon Genetics, Japan) and inspected with a UV transilluminator.

The PCR products were electrophoresed and purified (Clean-Up kit,Vivantis) and sent to a sequencing agency (Apical scientific Sdn. Bhd.). The generated sequences were analyzed using BLASTn which is available on the NCBI website (*http://blast.ncbi.nlm.nih.gov*).

III.1.5.3. Sequencing of 16 S rRNA bacteria

The purified DNA was sequenced using Sanger Sequencing: ABI 3730 XL DNA Analyzer. Sequences with < 97 % similarity to hits from the GenBank database were of poor quality and were excluded from this study **(Welinder-Olsson et *al*., 2007).**

III.1.6. Partial characterization of antifungal metabolites of the most potent LAB strain

III.1.6.1. Temperature effect

Aliquots of 10 ml of sterilized lactic strain supernatant (0.22 m Millipore) (0.22 µm Millipore) were heated at 25 °C, 50 °C, 70 °C, 90 °C during 1 hour in water bath and at 120 °C during 20 min in autoclave. After this treatment, the aliquots were quickly cooled and the anti-*Aspergillus* activity was evaluated using the procedure described in **(III.1.3.3.b).**

III.1.6.2. pH effect

The sensibility of the antifungal supernatant of a selected LAB strain was evaluated by adjusting the pH of 10 ml aliquots of the supernatant to 3, 5, 7 and 8. The anti-*Aspergillus* activity was evaluated using the procedure described in **(III.1.3.3.b).**

III.1.6.3. Trypsin effect

Aliquots of 10 ml of selected LAB strain supernatant were adjusted to the optimum pH of the enzyme (pH 8). 3 ml of the supernatant were then treated with 3 mg of the enzyme and incubated at 37 °C for 1 hour. After the incubation, the sample was heated at 70 °C for 20 min, and then sterilized by filtration (0.22 μm Millipore). The sample pH was adjusted to the initial value (pH 4.3) and the anti-*Aspergillus* activity was evaluated using the procedure described in **(III.1.3.3.b).**

III.1.7. Statistical analysis

Data analysis was performed using SPSS software version 22.0 for Windows. The data obtained from the analysis is expressed as the mean ± standard deviation (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) at $p < 0.05$. Correlation analysis between some parameters was performed using Pearson correlation at $p < 0.05$. The correlation matrices were assessed by STATISTICA 10.

III.2. Results and discussion

III.2.1. Isolation of lactic acid bacteria

Thirty-two different colonies of lactic acid bacteria were isolated from traditionally fermented wheat. The characterization of these colonies **(Table 10)** led to the following result: all isolates are both Gram positive and catalase negative. According to microscopic observation, 15.6 % of them are identified as rods and 84.3 % as cocci.

III.2.2. Screening of *anti-Aspergillus* activity

The potential of lactic acid bacteria to inhibit the growth of *Aspergillus* strains has been defined as "anti-*Aspergillus*" activity.

Table 10. Characterization of isolated strains

Isolated strain	Catalase test	Gram test
LS001 to LS010	-	Cocci +
LS011	-	Rod +
LS012 to LS020	-	Cocci +
LS021	-	Rod +
LS022	-	Cocci +
LS023	-	Rod +
LS024 to LS029	-	Cocci +
LS030	-	Rod +
LS031	-	Cocci +
LS032	-	Rod +

III.2.2.1. Double layer method

The results of the overlay assay show different effects of LAB on *Aspergillus* strains growth **(Table 11).** The antifungal activity of the isolates ranges from weak to strong. There are also some negative results. The plates are examined for clear zones of inhibition around the bacterial streaks, and the area of the zones is scored **(Fig. 23).**

As shown, 75 % of LAB isolates (LS001-LS002, LS004-LS009, LS011, LS013-LS015, LS017-LS020, LS022-LS023, LS026-LS030 and LS032) present antifungal activity against *A. niger*. However zone inhibition diameter is variable according to the strain (+, ++ or +++). While 25 % of LAB isolates (LS003, LS010, LS012, LS016, LS021, LS024, LS025 and LS031) didn't present any antifungal activity. A recent work of **Le Lay et *al*. (2016)** screened the antifungal activities of 270 LAB strains and only a low proportion of the screened strains could suppress the growth of *A. niger*.

Table 11. Antifungal activity of isolated strains against *A. niger* and *A. flavus.*

LAB Strains	Anti *A. niger* activity	Anti *A. flavus* activity
LS001	++	++
LS002	++	++
LS003	-	++
LS004	+	+
LS005	+	-
LS006	++	++
LS007	+	++
LS008	+++	++
LS009	+	ND
LS010	-	ND
LS011	+++	+++
LS012	-	+++
LS013	++	++
LS014	+	++
LS015	+++	ND
LS016	-	-
LS017	+++	++
LS018	+	ND
LS019	+	ND
LS020	+	++
LS021	-	+++
LS022	+	-
LS023	++	+
LS024	-	++
LS025	-	ND
LS026	++	++
LS027	+	ND
LS028	++	+++
LS029	+	-
LS030	+++	+++
LS031	-	+
LS032	+++	+++

ND: not determined, (-) no suppression of fungal growth; (+) no fungal growth on 0.1 % to 3 % of the plate area per bacterial streak; (++) no fungal growth on 3 % to 8 % of the plate area per bacterial streak; (+++) inhibition zone > 8 % of the plate area per bacterial streak

About 84 percent of LAB isolates (LS001-LS004, LS006-LS008, LS011-LS014, LS017, LS020, LS021, LS023-LS024, LS026, LS028, LS030-LS032) exhibit antifungal activity against *A.*

flavus, with variable zone inhibition diameter (+, ++, +++) depending on the strain. While 16 % of LAB strains (LS005, LS016, LS022, LS029) didn't present any antifungal activity.

| A | B | C | D |
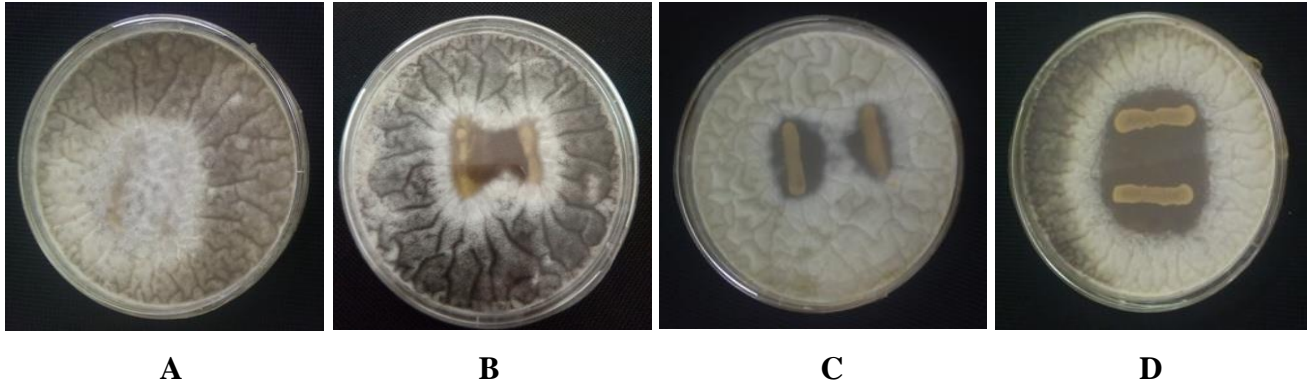
Fig. 23. Results of anti-*A. niger* activity of the strains: A (-): LS003, B (+): LS004, C (++): LS006, D (+++): LS011

According to the overlay assay results, we notice that *A. flavus* strain is more sensitive than *A. niger,* since 84 % of LAB isolates could inhibit its growth, compared to only 75 % in the case of *A. niger.*

LAB can produce many kinds of antifungal substances, which have been widely proven to have antifungal activity **(Zhao et *al.*, 2022).** Most importantly; inhibition differed with every fungal strain depending on the LAB used. According to the results, some LAB strains presented strong antifungal activity against the tested fungal species. Three of them are the most efficient, they are coded LS011, LS030 and LS032, which exerted an important antifungal activity on all mold strains that were examined **(Fig. 24).**

At the end of this test, these three strains were retained and their antifungal activity was then confirmed using a quantitative test.

III.2.2.2. Co-culture on MRS agar

The results of co-culture on MRS agar are presented in **Fig. 25** and **26**. These results indicate that there is a significant difference ($p < 0.001$) in fungal growth between the three LAB isolates, which explains the difference in their inhibitory efficacy.

| A | B | C |
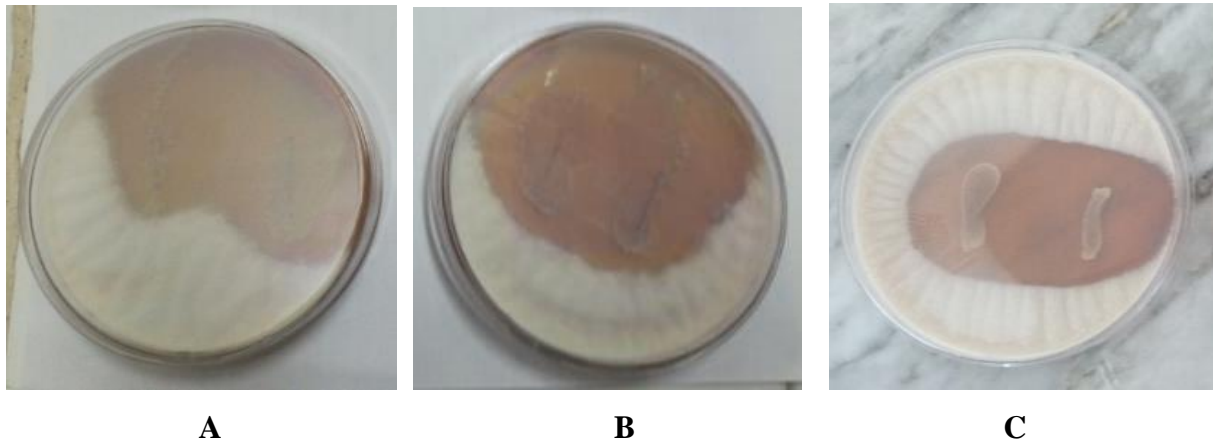
Fig. 24. Results of anti-*A. flavus* activity of the three selected LAB strains: A: LS011, B: LS030, C: LS032
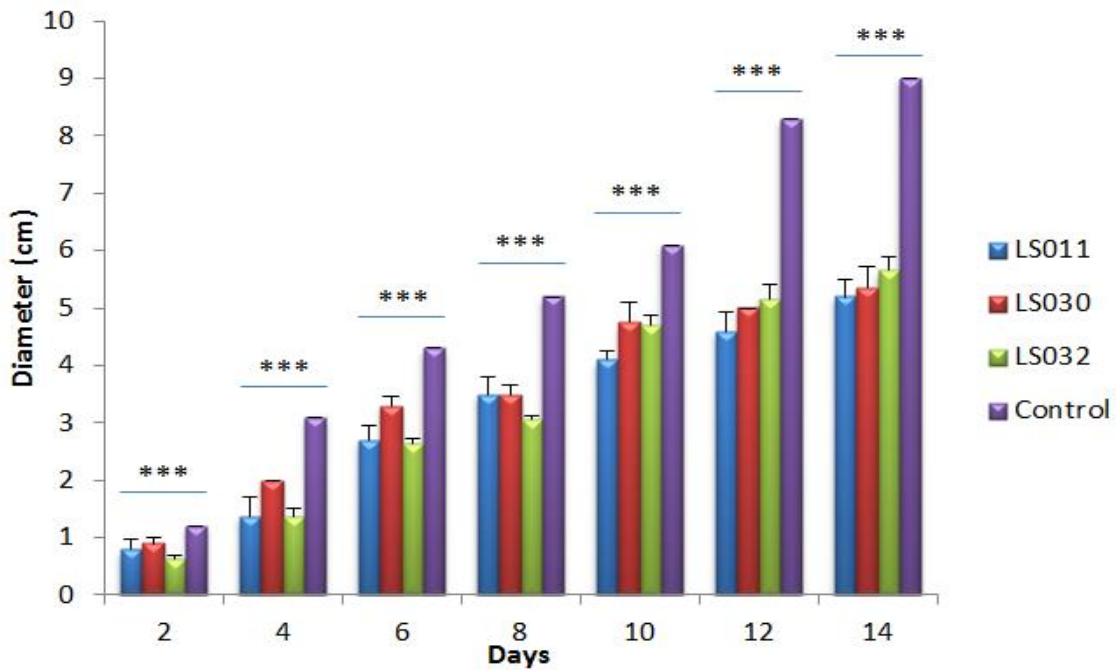
Fig. 25. Influence of the selected strains LS011, LS030 and LS032 on *A. niger* growth

According to **Fig. 25** and **26**, the three LAB strains reduce the growth of *A. niger* and *A. flavus* respectively in comparison with the control with a very high significance ($p < 0.001$). The MRS

control plate containing fungal spores without LAB showed increased growth after 48 h, and the plates were completely covered by the fungi on the fourteenth day.

We notice that in the first eight days, the strain coded LS032 reduces the proliferation of *A. niger* more than the two other strains. But in the last six days, the fork turned to the LS011 strain. While for the inhibition of *A. flavus*, the strain LS011 is also the most effective, followed by LS030 and then LS032, and this is noticed throughout the experiment with very high significance.
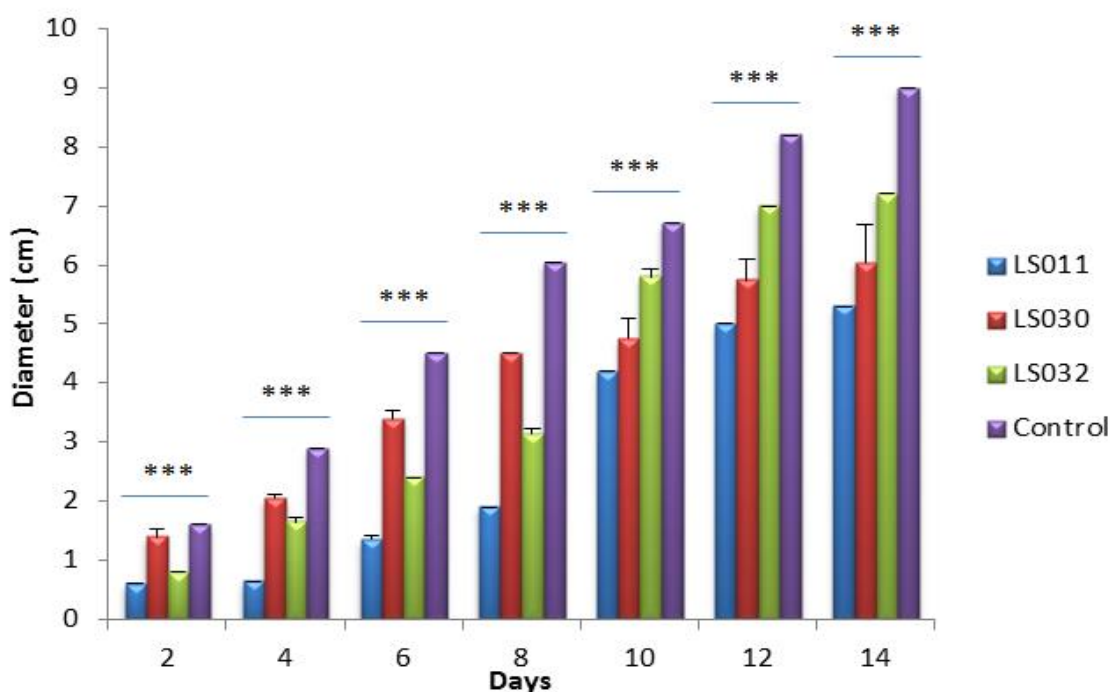
Fig. 26. Influence of the selected strains LS011, LS030 and LS032 on *A. flavus* growth

On the fourteenth day, the isolate LS011 induces an inhibition of about 40 % of the radial growth of *Aspergillus* strains. This inhibition is weak for the two other isolates. According to these results, and the results of the previous test, we confirmed that LS011 is the most effective and potent LAB strain because of its interesting antifungal activity.

III.2.2.3. Inhibitory activity of LAB cell-free supernatant

The results of the inhibitory activity of CFS of LAB isolates against fungal strains are presented in **Fig.27.** As shown in this figure, the CFS of the three LAB strains inhibits the growth of *A. niger* with a very high significance (*** $p < 0.001$), whereas the inhibition of *A. flavus* is very significant (** $p < 0.01$). The difference between the reduction of *A. niger* and *A. flavus* growth by LS011 CFS is not significant. However, LS030 and LS032 show remarkable significance (*** $p < 0.001$ and * $p < 0.05$, respectively).
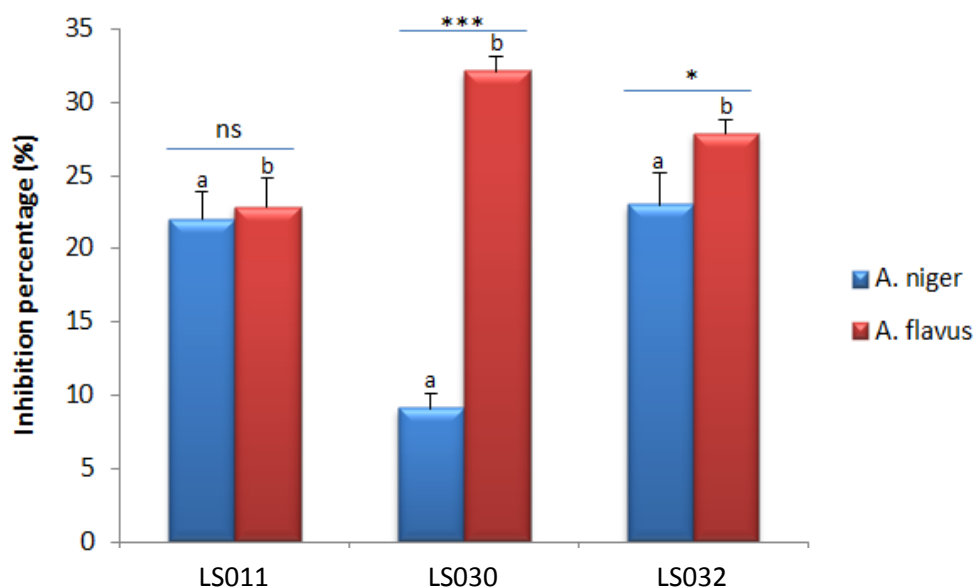
Fig. 27. Inhibitory activity of cell free supernatant of the selected LAB strains (LS011, LS030 and LS032). ** indicates significant difference at $P < 0.01$, * indicates significant difference at $P < 0.05$, ns: not significant, a: ***, b: **.

III.2.3. Influence of inhibitory metabolites on mycelium development

The results of the LAB inhibitory metabolites on mycelium development are presented in **Fig. 28**. According to this figure and in comparison with the control, we notice that LAB metabolites present inhibitory activity against the mycelium development of the fungal strains.

The highest inhibitory activity is attributed to LS011 with only a fungal growth estimated by an average of 1.8 ± 0.14 cm (*** $p < 0.001$) and 2.7 ± 0.42 cm ($p > 0.05$) for *A. niger* and *A. flavus,*

respectively. However, the growth of the two fungal strains in the presence of the two other LAB strains (LS030 and LS032) is higher with 2 ± 0.14 and 3 ± 0.14 cm for *A. niger* (*** $p < 0.001$) and 3.3 ± 0.00 and 3.05 ± 0.00 cm ($p > 0.05$) for *A. flavus* respectively. These results also indicate that the LAB strain coded LS011 is the most potent one.

A recent work screened the antifungal activity of 137 *Lactobacillus plantarum* isolates against six common spoilage indicator fungi, including *A. niger*. Among the *Lactobacillus plantarum* isolates, strain IMAU80174 was selected as the most effective based on the results of mycelium growth inhibition by its cell-free supernatant (**Li et *al*., 2020).**
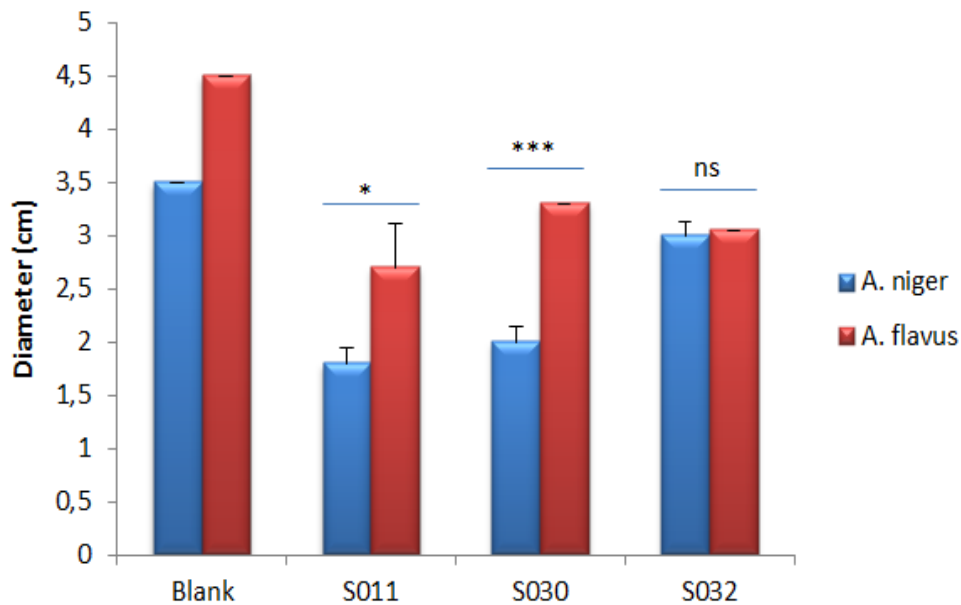
Fig. 28. Influence of inhibitory metabolites on mycelium development. *** indicates significant difference at $P < 0.001$, * indicates significant difference at $P < 0.05$, ns: not significant.

The antifungal properties of these LAB under study and their metabolites can make them a promising biological solution to reduce *A. niger* and *A. flavus* contamination and their possible secretion of mycotoxins in food and feed systems, especially for the LAB coded LS011. LAB have a long history of use in food and feed and are generally regarded as safe organisms, with reports that they effectively reduce mold growth (**Bourdichon et *al*., 2012)**. Significant progress

has been reported in the isolation and characterization of antimycotic compounds, which include various organic acids, cyclic dipeptides and fatty acids **(Crowley et al., 2013).** *Pediococcus pentosaceus* L006 was reported to inhibit the growth of *Fusarium verticillioides* **(Daliè et al., 2012). Kivanc et al. (2014)** reported that LAB isolated from Tarhana inhibited the growth of the mycelia and conidia germination of the fungi. Moreover, **Bulgasem et al. (2016)** demonstrated the reduction of *Candida* sp. growth by *Lactobacillus* sp. and *Pediococcus* sp. isolated from honey.

Also, in recent studies, **Nasrollahzadeh et al. (2020)** have shown the antifungal activity of some LAB isolated from masske, camel dough and local yogurt against *A. niger* and *A. flavus*. Both fungal strains were very sensitive to *L. plantarum* B38 and *L. rhamnosus*. **Zhao et al. (2022)** reported that *L. plantarum* presented antifungal activity against various fungal strains, including *A. niger* and *A. flavus*.

As shown in **Fig.29**, we note a non-significant correlation between the CFS activity of LS011 strain and the reduction of mycelium development of *A. niger* ($r = 0.444^{ns}$, $p = 0.231^{ns}$), whereas, a highly significant correlation is recorded between the CFS activity of LS011 strain and the reduction of mycelium development of *A. flavus* ($r = 0.894^{**}$, $p = 0.001^{**}$).

A   **B**
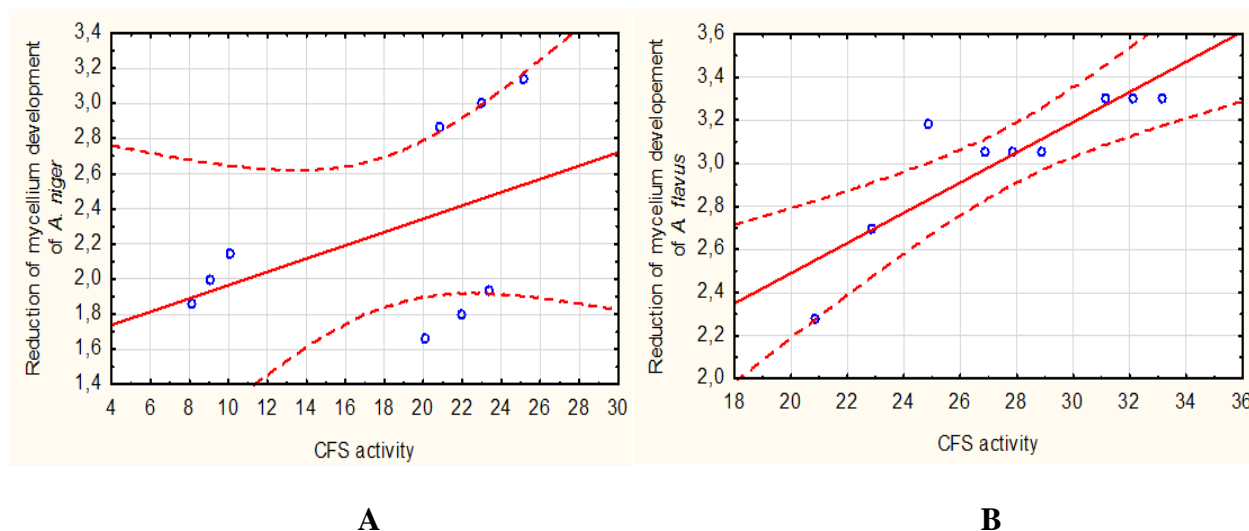
Fig. 29. Correlation between CFS activity of LS011 and mycelium development of *A. niger* (A) and *A. flavus* (B). Pearson's *r* correlation was assessed by IBM SPSS statistics 22. Correlation matrices were evaluated using STATISTICA 10.

III.2.4. Partial characterization of antifungal metabolites of *Lactiplantibacillus plantarum* **LS011**

The strain coded LS011 was identified by 16S rRNA sequencing as *Lactiplantibacillus plantarum.*

As indicated in **Fig.30.A**, CFS of the test culture, heated in a water bath or autoclaved at 120 °C for 20 min, loses partially the inhibitory activity against the indicator strain, suggesting that the antifungal activity of *L. plantarum* LS011 is heat sensitive.

A   B

C
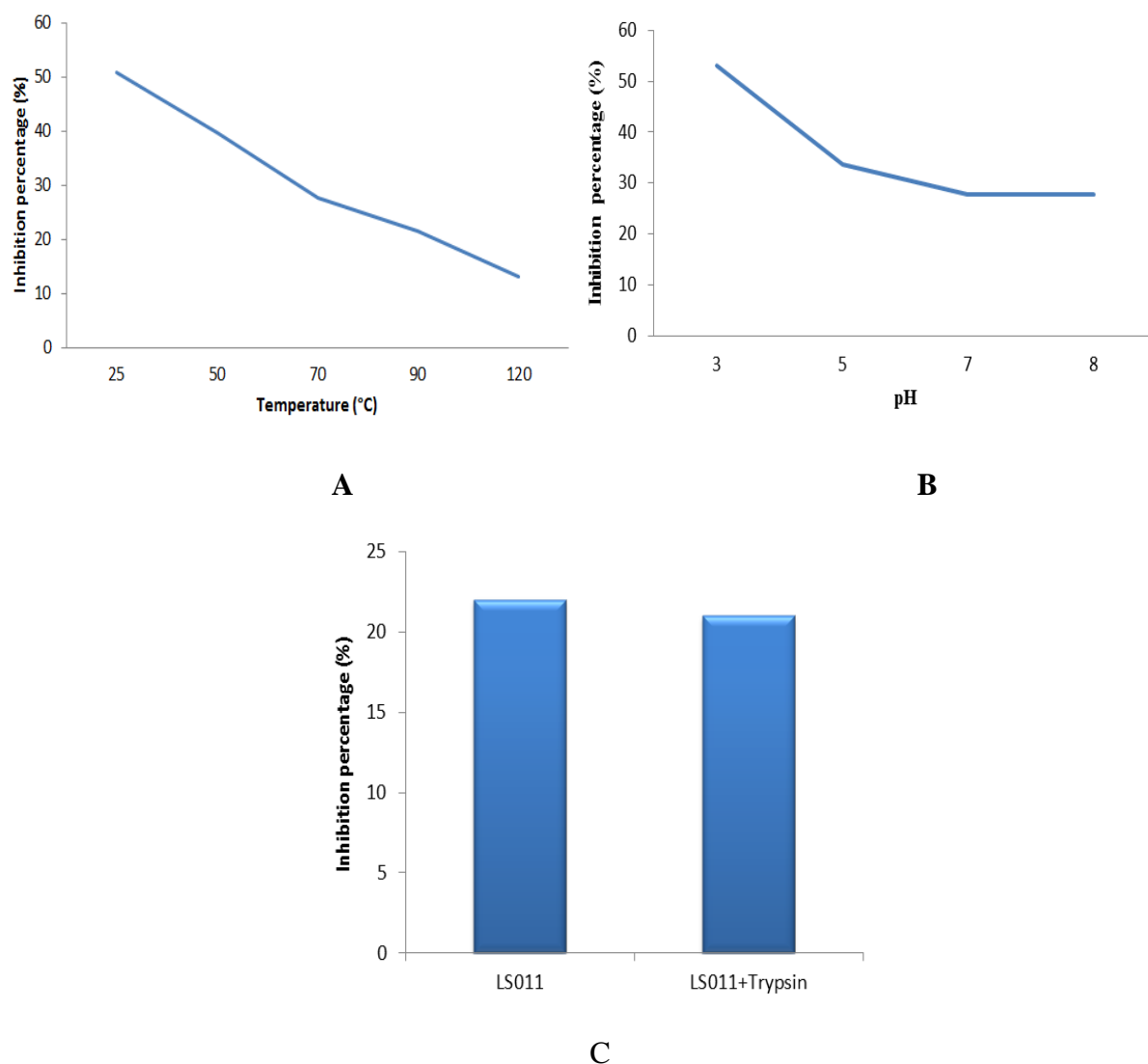
Fig. 30. Characterization of antifungal metabolites of *Lactiplantibacillus plantarum* LS011. A: effect of temperature, B: effect of pH, C: effect of trypsin.

The data in **Fig.30.B**, on the other hand, shows that the antifungal activity of the CFS rapidly decreases as the pH rises, indicating that the efficiency of the antifungal metabolite(s) is pH dependent. However, as it is shown in **Fig.30.C**, treatment of the CFS with the proteolytic enzyme (trypsin) does not affect the antifungal activity.

The proteolytic treatment did not affect the antifungal activity exhibited by *L. plantarum* LS011. However, this antifungal activity was not totally lost because an inhibition percentage of about 13 % was recorded at 120 °C. We suggest that the antifungal metabolites of LS011 are not protein in nature, but they may probally represent small peptides since their antifungal activity persists at 120 °C. On the other hand, the fact that the antifungal activity was reduced when the pH of the CFS was neutralized to pH 7 and pH 8 suggests that the antifungal metabolites may be organic acid compounds and leads us to assume that the organic acids in the CFS play a key role in inhibiting fungi. This result is consistent with the hypothesis that organic acids and low pH could be responsible for the reported inhibition **(Dalié et *al.*, 2010).**

Due to their lipophilic nature, organic acids readily diffuse across the fungal cell membrane and accumulate in the cytoplasm. Numerous studies have confirmed that organic acids are major metabolites of LAB that have a dramatic effect on fungi growth by inhibiting mycelial growth, and that the antifungal activity of LAB is generally associated with the production of organic acids **(Magnusson et *al*., 2003; Belguesmia et *al*., 2014; Mieszkin et *al*., 2017; Guimarães et *al*., 2018; Sadiq et *al*., 2019; Zhao et *al*., 2022).**

In a previous study, **Yoo et *al*. (2016)** demonstrated that the antifungal activity of CFS of LAB strains was caused by acidic compounds like phenyl lactic acid (PLA) or organic acids rather than proteins or peptide molecules. **Guimarães et *al*. (2018)** have reported that organic acids produced by *L .plantarum* UM55 and *L. buchneri* UTAD104 contribute to antifungal activity. The study showed that acetic acid, indole lactic acid (ILA) and phenyllactic acid (PLA) were the most effective in inhibiting *Penicillium nordicum* growth and ochratoxin A (OTA) production. According to **Matei et *al*. (2015)**, the mechanism of inhibitory action of *A. niger* and *A. flavus* by some LAB strains is most probably related to organic acid production (mainly lactic acid).

Apart from these findings, **Muhialdin et *al*. (2018)** reported that *Leuconostoc mesenteroides* DU15, *L. plantarum* TE10, *L. plantarum* IT10 and *L. plantarum* IS10 inhibited the growth of six

fungi commonly associated with bread spoilage, including *A. niger* and *A. flavus*. This antifungal activity was associated with the production of low molecular peptides. Also, **Roy et *al.* (1996)** have shown that the antifungal activity of the CFS of *Lc. lactis* CHD-28.3 against *A. flavus* disappeared after treatment with chymotrypsin, trypsin and pronase E. They concluded that the antifungal compounds are protein in nature.

From another point of view, it's not easy to study interactions between LAB and fungi, especially because the bacteria create fermentation end products that are either active individually or act synergistically with antifungal chemicals. The synergistic effect of the decrease in pH caused by the formation of organic acids and other antifungal metabolites of LAB results in a more effective antifungal action **(Cortés-Zavaleta et *al.*, 2014).** In previous studies, it was reported that there are synergetic effects between organic acids and other molecules all of them secreted by LAB. **Peyer et *al.*, (2016)** demonstrated that phenolic acids released by *L. plantarum* FST1.7 and *L. brevis* R2 act in synergy with organic acids at low pH against *Fusarium culmorum*. Synergistic effect between organic acids and antifungal peptides produced by LAB was also demonstrated by **Axel et *al.*, (2016).**

From the partial characterization of antifungal metabolites of *L. plantarum* LS011, a synergetic effect between organic acids and antifungal peptides at low pH is possible. Further study is therefore required to elucidate the real chemical nature of these antifungal metabolites.

Article 4. Article under revision

ANTIFUNGAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM TRADITIONALLY FERMENTED WHEAT

Nabila Benhamada[*1,2]**, Ouahiba Benhamada**[1,2]**, Ghania Bourzama**[3]**, Meryem Bouchekrit**[2]**,
Djahida Zerroug**[2]**, Farida Boulahia**[2]**, Sarra Er-rahmani**[4]**, Safae Er-raouan**[4]**, Saad Ibn
Souda Koraichi**[4,5]**, Tayeb Idoui**[1,2]

[1] Laboratory of Biotechnology, Environment and Health, Nature Sciences and Life Faculty, University of Mohammed Seddik Ben Yahia, Jijel 18000, Algeria.

[2] Applied Microbiology and Food Sciences, Nature Sciences and Life Faculty, University of Mohammed Seddik Ben Yahia, Jijel 18000, Algeria.

[3] Biochemistry Department, Faculty of Sciences, University of Badji-Mokhtar, Annaba, 23000, Algeria.

[4] Laboratory of Microbial Biotechnology and Bioactive Molecules, Sciences and technologies Faculty, University of Sidi Mohamed Ben Abdellah, Fès, Morocco.

[5] City of Innovation, University of Sidi Mohamed Ben Abdellah, Fès, Morocco.

[*] Author for correspondence. E-mail: n.benhamada@yahoo.fr

Abstract

The growth of spoilage fungi has been a global concern because of the economic loss and the health hazard of the mycotoxins produced by these fungi. Consumers are looking for effective and healthy ways to preserve food and maintain a good shelf life. The present study was conducted to evaluate the antifungal activity of 32 strains of lactic acid bacteria (LAB) isolated from traditionally fermented wheat against *Aspergillus niger* and *A. flavus*. Initially, a qualitative test was assayed and three potent LAB strains were selected and tested using a quantitative test. Afterwards, the most potent LAB strain was identified and a partial characterization of the cell-free supernatant metabolites was conducted. The results showed that the majority of LAB strains presented interesting antifungal activity and the most potent strain, identified as *Lactiplantibacillus plantarum* LS011, had the highest activity against the fungal species. Moreover, the partial characterization of the antifungal compounds suggests that they are organic acids. Thus, our results showed the possibility of using LAB isolates in biopreservation and the use of *L. plantarum* LS011 may be a good solution for food decontamination and spoilage reduction.

Key words: lactic acid bacteria; *L. plantarum*; biopreservation; *Aspergillus*; antifungal activity.

Conclusion and perspectives

Fermentation of wheat in underground silos gives the grains special organoleptic characteristics and allows the production of the Algerian traditional fermented product (Mzeyet or Elhammoum), which is much appreciated by Algerians. The results showed that samples of traditional fermented wheat represent a satisfactory quality from a biochemical and nutritional point of view. The use of the flour obtained after milling the samples as supplementation in bread technology represents a very interesting task and makes it possible to obtain breads of good quality and organoleptic acceptability through the use of a proportion of incorporation at a ratio of 90:10 (unfermented wheat : traditional fermented wheat).

The spontaneous fermentation of durum wheat can enhance the content of total polyphenols and flavonoids as well as the antioxidant activity of wheat. This was demonstrated by comparing the traditionally fermented wheat with the control (unfermented wheat). In this work, we have also reported a correlation between the total phenolic content, flavonoid content and reducing power. Therefore, spontaneous fermentation often improves the safety of foods by reducing pH and through detoxification, and can be applied as a way to transform wheat, in particular, and cereals in general, into healthy foods or ingredients in the food industry and then increase their antioxidant content.

According to our results, lactic acid bacteria have the ability to inhibit fungi, and this depends on strain's capability to produce antifungal compounds like organic acids, and those acids may differ from strain to strain. The results obtained in this study indicated that lactic acid bacteria isolated from traditionally fermented wheat produced bioactive compounds that can be used to inhibit growth of the pathogenic strains of *Aspergillus.* Therefore, these findings demonstrate the possibility of using *Lactiplantibacillus plantarum* LS011 and/or its antifungal metabolites as natural preservatives in food products to control the growth of spoilage fungi.

Finally, we have evaluated the quality of traditionally fermented wheat and determined the beneficial effect of spontaneous fermentation in improving its nutritional quality. Also, the isolation of lactic acid bacteria and the study of its antifungal activity were achieved. Whereas, new perspectives are emerging, such as:

- ✓ Applying the most relevant lactic strain in the food matrix (stored wheat in underground silos) and studying its antifungal activity;

- ✓ Conducting the antifungal study against other genera of fungi (such as: *Penicillium, Alternaria, Fusarium*);

- ✓ Using other proteolytic treatments of LS011 lactic strain cell free supernatant (such as: chymotrypsin, pepsin, proteinase K) to elucidate the real biochemical nature of the antifungal metabolite(s).

- ✓ Purifying and characterizing the LS011 lactic strain antifungal metabolite(s);

- ✓ Studying the capacity of this (these) antifungal metabolite(s) to biodegrade or sequester mycotoxins produced by *Aspergillus* strains;

- ✓ Monitoring the antifungal metabolite(s) impact on the expression of some genes involved in the mycotoxin biosynthesis pathway.

References

AACC. (1983). American Association of Cereals Chemists. Approved Methods of the AACC, American Association of Cereal Chemists, St. Paul, Mc.

AACC. (2000). American Association of Cereals Chemists. Approved Methods of the AACC. 10th Edition, American Association of Cereal Chemists, St. Paul. Mc.

Abdel-Azeem, A. M., Abdel-Azeem, M. A., Abdul-Hadi, S. Y., & Darwish, A. G. (2019). *Aspergillus*: Biodiversity, ecological significances, and industrial applications. In: A., Yadav, S., Mishra, S. Singh, & A., Gupta, (Eds.). *Recent Advancement in White Biotechnology Through Fungi. Fungal Biology*. DOI: https://doi.org/10.1007/978-3-030-10480-1-4.

Adams, M. R. (1990). Topical aspects of fermented foods. *Trends in Food Science and Technology*, 8, 140-144.

Adebayo, C. O., & Aderiye, B. I. (2010). Antifungal activity of bacteriocins of lactic acid bacteria from some Nigerian foods. *Research Journal of Microbiology*, 5(11), 1070-1082. DOI: https://doi.org/10.3923/jm.2010.1070.1082.

Adebiyi, J. A., Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2017). Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (Pennisetum glaucum) flour. *Food Chemistry*, 233, 210–217. DOI: https://doi.org/10.1016/j.foodchem.2017.04.020.

Adebo, J. A., Njobeh, P. B., Gbashi, S., Oyedeji, A. B., Ogundele, O. M., Oyeyinka, S. A., & Adebo, O. A. (2022). Fermentation of cereals and legumes: Impact on nutritional constituents and nutrient bioavailability. *Fermentation,* 8(2), 63. DOI: https://doi.org/10.3390/ fermentation8020063.

Adebo, O. A., & Medina-Meza, I. G. (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, 25(4), 927. DOI: https://doi.org/10.3390/molecules25040927.

Adebo, O. A., Njobeh, P. B., Adebiyi, J. A., & Kayitesi, E. (2018). Co-influence of fermentation time and temperature on physicochemical properties, bioactive components and microstructure of ting (a Southern African food) from whole grain sorghum. *Food Bioscience*, 25, 118–127. DOI: DOI: https://doi.org/10.1016/j.fbio.2018.08.007.

Adesuyi, S. A., Shejbal, J., Oyediran, J. O., Kuku, F. O., Sowunmi, O., Akinnusi, O., & Onayemi, O. (1980). Application of artificial control atmospheres to grain storage in the tropics: case study in Nigeria. *Developments in Agricultural Engineering*, 1, 259-279. DOI: https://doi.org/10.1016/B978-0-444-41939-2.50029-2.

AFNOR (Association Francaise de Normalisation). (1972). Recueil des normes françaises des produits dérivés des fruits et légumes. Jus de fruits (pp. 325). Paris.

AFNOR (Association Francaise de Normalisation). (1982). Recueil des normes françaises des produits dérivés des fruits et légumes. Jus de fruits (pp. 327), Paris.

Agriopoulou, S., Stamatelopoulou, E., & Varzakas, T. (2020). Advances in occurrence, importance, and mycotoxin control strategies: Prevention and detoxification in foods. *Foods*, 9(2), 137. DOI: https://doi.org/10.3390/foods9020137.

Ahangarkani, F., Rouhi, S., & Gholampour Azizi, I. (2014). A review on incidence and toxicity of fumonisins. *Toxin Reviews*, 33(3), 95-100. DOI: 10.3109/15569543.2013.871563.

Aiyegoro, O. A., & Okoh, A. I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine,* 10(21), 1-8. DOI: https://dx.doi.org/10.1186/1472-6882-10-21.

Alo, M. N., Eze, U. A., & Eda, N. E. (2012). Microbiological qualities of burukutu produced from a mixture of sorghum and millet. *American Journal of Food and Nutrition*, 2(4), 96–102. DOI: https://doi.org/10.5251/ajfn.2012.496.102.

Alshannaq, A., & Yu, J. H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environment Research. Public Health*, 14(6), 632. DOI: https://doi.org/10.3390/ijerph14060632.

Ameh, M. O., Gernah, D. I., & Igbadul, B. D. (2013). Physico-chemical and sensory evaluation of wheat bread supplemented with stabilized undefatted rice brean. *Food and Nutrition Sciences*, 4(43), 43-48.

Amir, R. M., Hussain, M. N, Ameer, K., Ahmed, A., Ahmad, A., Nadeem, M., Riaz, A., Chughtai, M. F. J., Khaliq, A., Ahsan, S., & Kausar, R. (2020). Comprehensive assessment and evaluation of selected wheat varieties for their relationship to chapatti quality attributes. *Food Science and Technology Campinas,* 40(2), 436-443. DOI: https://doi.org/10.1590/fst.31619.

Amjad, M., Safdar, M. N., Mumtaz, A. Naseem, K., Raza, S., & Khalil, S. (2010). Comparison of different wheat varieties grown in Punjab for leavened flat bread (Naan) production. *Pakistan Journal of Nutrition*, 9(12), 146-150. DOI: https://doi.org/10.3923/pjn.2010.146.150.

Andersson, A. A. M., Dimberg, L., Aman, P., & Langberg, R. (2014). Recent findings on certain bioactive components in whole grain wheat and rye. *Journal of Cereal Science*, 59(3), 294-311. DOI: https://doi.org/10.1016/j.jcs.2014.01.003.

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

AOAC (Association of Official Analytical Chemists). (1995). Official Methods of Analysis of the Association of Official Analystical Chemists (pp. 18-19). Horowitz W, Washington, DC: AOAC.

Apprich, S.,Tirpanalan, O., Hell, J., Reisinger, M., Böhmdorfer, S., Siebenhandl-Ehn, S., Novalin, S., & Kneifel, W. (2013). Wheat bran-based biorefinery 2: valorization of products. *LWT-Food Science and Technology*, 56(2), 222-231. DOI: https://doi.org/10.1016/j.lwt.2013.12.003.

Armanino, C., De Acutis, R., & Festa, M. R. (2002). Wheat lipids to discriminate species, varieties, geographical origins and crop years. *Analitica Chimica Acta,* 454(2), 315–326. DOI: https://doi.org/10.1016/S0003-2670(01)01548-3.

Arshad, M. S., Kwon, J., Muhammad Anjum, F., Sohaib, M., Saeed, F., Imran, M., Zaid Amjad, Z., Nadeem, M., & Hussain, S. (2017). Wheat antioxidants, their role in bakery industry, and health perspective. In: R., Wanyera, & J., Owuoche (Eds.), *Wheat improvement, management and utilization*. DOI: https://doi.org/10.5772/67276.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International*, 99(1), 58–71. DOI: https://doi.org/ /10.1016/j.foodres.2017.07.022.

Atanda, S. A, Pessu, P. O., Agoda, S., Isong, I. U., Adekalu, O. A., Echendu, M. A., & Falade, T. C. (2011). Fungi and mycotoxins in stored foods. *African Journal of Microbiology Research*, 5(25), 4373-4382. DOI: https://doi.org/10.5897/AJMR11.487.

Aunsbjerg, S. D., Honoré, A. H., Marcussen, J., Ebrahimi, P., Vogensen, F. K., Benfeldt, C., Skov, T., & Knøchel, S. (2014). Contribution of volatiles to the antifungal effect of *Lactobacillus paracasei* in defined medium and yogurt. *International Journal of Food and Microbiology*, 194, 46-53. DOI: https://doi.org/10.1016/j.ijfoodmicro.11.007.

Awulachew, M. T. (2020). Understanding basics of wheat grain and flour quality. *Journal of Health and Environmental Research*, 6(1), 10-26. DOI: http://doi.org/10.11648/j.jher.20200601.12.

Axel, C., Brosnan, B., Zannini, E., Furey, A., Coffey, A., & Arendt, E. K. (2016). Antifungal sourdough lactic acid bacteria as biopreservation tool in quinoa and rice bread. *International Journal of Food Microbiology*, 23, 86–94. DOI: https://doi.org/10.1016/j.ijfoodmicro.2016.05.006.

Ayivi, R. D., Gyawali, R., Krastanov, A., Aljaloud, S. O., Worku, M., Tahergorabi, R., Claro da Silva, R., & Ibrahim, S. A. (2020). Lactic acid bacteria: food safety and human health applications. *Dairy*, 1(3), 202–232; DOI: https://doi.org/10.3390/dairy1030015.

Bakker-Arkema, F. W., Maier, D. E., & Schisler, I. P. (2007). Drying rates and dryer capacities of different seed grains. *Drying Technology,* 5(4), 527-540. DOI: https://doi.org/10.1080/07373938708916561.

Bala, B. K. (2016). *Temperature and moisture changes during storage*. In: B. K., Bala, (Ed.). *Drying and storage of cereal grains* (2nd ed.). DOI: https://doi.org/10.1002/9781119124207.ch10.

Balla, A., Blecker, C., Oumarou, M., Paquot, M., & Deroanne, C. (1999). Mise au point de pains composites à base de mélanges de farines de sorgho-blé et analyse texturale. *Biotechnologie Agronomie Société et Environnement*, 3(2), 69-77.

Barac, A. M. (2019). Mycotoxins and human disease: A pratical approach. In: E., Presterl (Ed.). *Clinically relevant mycoses* (pp. 213-225). Amazon, France: Springer.

Bazukyan, I. Matevosyan, L. Toplaghaltsyan, A., & Trchounian1, A. (2018). Antifungal activity of lactobacilli isolated from Armenian dairy products: an efective strain and its probable nature. *AMB Express*, 8(1). DOI: https://doi.org/10.1186/s13568-018-0619-y.

Begum, A. N., Nicolle, C., Mila, I., Lapierre, C., Nagano, K., Fukushima, K., Heinonen, S. M., Adlercreutz, H., Remesy, C., & Scalbert, A. (2004). Dietary lignins are precursors of mammalian lignans in rats. *The Journal of Nutrition*, 134(1), 120-127. DOI: https://doi.org/10.1093/jn/134.1.120.

Bekhouche, F., Kermiche, M., & Merabti, R. (2014). Study of the microbial flora in fermented wheat and estimation of their hydrolytic activities. *Journal of Biotechnology and Biomater*, the 5th World Congress on Biotechnology, Valencia, Spain.

Belderok, B., Mesdag, H., & Donner. D. A. (2000). *Bread-making quality of wheat*. New York, Springer.

Beleggia, R., Platani, C., Nigro, F., De Vita, P., Cattivelli, L., & Papa, R. (2013). Effect of genotype, environment and genotype-by-environment interaction on metabolite profiling in durum wheat (*Triticum durum* Desf.) grain. *Journal of Cereal Science*, 57(2), 183–192. DOI: https://doi.org/10.1016/j.jcs.2012.09.004.

Belguesmia, Y., Rabesona, H., Mounier, J., Pawtowsky, A., Le Blay, G., Barbier, G., Haertlé, T., & Chobert, J. M. (2014). Characterization of antifungal organic acids produced by *Lactobacillus harbinensis* K.V9.3.1Np immobilized in gellan–xanthan beads during batch fermentation. *Food Control*, 36(1), 205–211. DOI: https://doi.org/10.1016/j.foodcont.2013.08.028.

Benayad, A., Taghouti, M., Benali, A., Aboussaleh, Y., & Benbrahim, N. (2021). Nutritional and technological assessment of durum wheat-faba bean enriched flours, and sensory quality of developed composite bread. *Saudi Journal of Biological Sciences*, 28(1), 635–642. DOI: https://doi.org/10.1016/j.sjbs.2020.10.053.

Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: mechanisms of action. *International Journal of Environmental Research and Public Health*, 17(2), 423. DOI: https://doi.org/10.3390/ijerph17020423.

Beristain-Bauza, S. C., Mani-López, E., Palou, E., & López-Malo, A. (2016). Antimicrobial activity and physical properties of protein films added with cell-free supernatant of *Lactobacillus rhamnosus*. *Food Control*, 62, 44-51. DOI: https://doi.org/10.1016/j.foodcont.2015.10.007.

Berthiller, F., Krska, R., Domig, K. J., Kneifel, W., Juge, N., Schuhmacher, R., & Adam, G. (2011). Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. *Toxicology letters*, 206(3), 264–267. DOI: https://doi.org/10.1016/j.toxlet.2011.08.006.

Bhardwaj, S. (2014). Grain storage structures for farmers. *Popular Kheti*, 2(2), 202-205. DOI: https://doi.org/10.13140/2.1.1972.0323.

Bin, X., & Ying, D. (2011). Present situation and trends of wheat germ industrialization developing. *Transactions of the Chinese Society of Agricultural Engineering,* 27(14), 341-345.

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiology*, 2018, 4(4), 665–684. DOI: https://dx.doi.org 10.3934/microbiol.2018.4.665.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal,* 5(1), 9-19. DOI: http://dx.doi.org/10.1097/WOX.0b013e3182439613.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International,* 36(6), 527-543. DOI: https://dx.doi.org/10.1016/S0963-9969(03)00009-7.

Bonestroo, M. H., Dewit, J. C., Kusters, B. J. M., & Rombout, F. M. (1993). Inhibition of the growth of yeasts in fermented salads. *International Journal of Food Microbiology*, 17(4), 311-320. DOI: http://dx.doi.org/10.1016/0168-1605(93)90201-Q.

Boo, Y. C. (2019). Can plant phenolic compounds protect the skin from airborne particulate matter? *Antioxidants,* 8(9), 379. DOI: http://dx.doi.org/10.3390/antiox8090379.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I. B., Prajapati, J. B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven,

V., Zgoda, A., Tuijtelaars, S., & Hansen, E. B. (2012). Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology.* 154(3), 87–97. DOI: https://doi.org/10.1016/j.ijfoodmicro.2011.12.030.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmitel–Wissenschauft und Technologie,* 28, 25-30.

Bruneton, J. (1993). *Pharmacognosie-phytochimie, plantes medicinales* (2nd ed.). Paris, France : Technique et Documentation Lavoisier.

Bulgasem, B. Y., Lani, M. N., Hassan, Z., Yusoff, W. M. W., & Fnaish, S. G. (2016). Antifungal activity of lactic acid bacteria strains isolated from natural honey against pathogenic *Candida* species. *Mycobiology*, 44(4), 302–309. DOI: https://doi.org/10.5941/MYCO.2016.44.4.302.

Bullerman, L. B., & Bianchini, A. (2009). Food safety issues and the microbiology of cereals and cereal products. In: N., Heredia, I., Wesley, & S., Garcia (Eds.), *Microbiologically safe foods* (pp. 315–335). New York, U.S.A.: John Wiley & Sons.

Buriro, M., Oad, F. C., Keerio, M. I., Gandahi, A. W., & Laghari, G. M. (2012). Impact of storage sources on physico-chemical properties of various wheat varieties. *Sarhad Journal of Agriculture,* 28(2), 185-190.

Bushuk, W., & Rasper, V. F. (2012). *Wheat: production, properties and quality*. Berlin, Germany: Springer Science and Business Media.

Cabo, M. L., Murado, M. A., Gonzalez, M. P., & Pastoriza, L. (2000). Dose-response relationships. A model for describing interactions, and its application to the combined effect of nisin and lactic acid on *Leuconostoc mesenteroides*. *Journal of Applied Microbiology*, 88(5), 756-763. DOI: https://doi.org/10.1046/j.1365-2672.2000.01010.x.

Cai, L., Macfadyen, S., Hua, B., Zhang, H., Xu, W., & Ren, Y. (2022). Identification of biomarker volatile organic compounds released by three stored-grain insect pests in wheat. *Molecules*, 27(6), 1963. DOI: https://doi.org/10.3390/molecules27061963.

Carocho, M., Rodrigues, P., Ciudad-Mulero, M., Fernández-Ruiz, V., Ferreira, E., Heleno, S., Rodrigues, P., Barros, L., & Ferreira, I. C. (2020). Comparison of different bread types: Chemical and physical parameters. *Food Chemistry*, 310, 125954. DOI: https://doi.org/ 10.1016/j.foodchem.2019.125954.

Cheli, F., Pinotti, L., Novacco, M., Tretola, M., & Dell'Orto, V. (2017). Mycotoxins in wheat and mitigation measures. In: R., Wanyera, & J., Owuoche, (Eds.). *Wheat improvement, management and utilization*. DOI: https://doi.org/10.5772/67240.

Cheli, F., Pinotti, L., Rossi, L., & Dell'Orto, V. (2013). Effect of milling procedures on mycotoxin distribution in wheat fractions: A review. *LWT - Food Science and Technology*, 54(2), 307–314. DOI: https://doi.org/10.1016/j.lwt.2013.05.040.

Chen, Z., Wassgren, C., & Ambrose, K. (2020). A review of grain kernel damage: Mechanisms, modeling, and testing procedures. *American Society of Agricultural and Biological Engineers*, 63(2), 455-475. DOI: http://doi.org/10.13031/trans.13643.

Cheng, Z., Su, L., Moore, J., Zhou, K., Luther, M., Yin, J., & Yu, L. 2006. Effects of postharvest treatment and heat stress on availability of wheat antioxidants. *Journal of Agricultural and Food Chemistry*, 54(15), 5623-5629. DOI: https://doi.org/10.1021/jf060719b.

Cheong, E. Y. L., Sandhu, A., Jayabalan, J., Kieu Le, T. T., Nhiep, N. T., My Ho, H.T., Zwielehner, J., Bansal, N., & Turner, M. S. (2014). Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mould *Penicillium commune* and their potential as biopreservatives in cheese. *Food Control*, 46, 91-97. DOI: https://doi.org/10.1016/j.foodcont.2014.05.011.

Ciudad-Mulero, M., Matallana-González, M. C., Callejo, M. J., Carrillo, J. M., Morales, P., & Fernández-Ruiz, V. (2021). Durum and bread wheat flours. Preliminary mineral characterization and its potential health claims. *Agronomy,* 11, 108. DOI: https://doi.org/10.3390/agronomy11010108.

Cornell, H. (2003). The chemistry and biochemistry of wheat. In: S. P., Cauvain, (Ed.). *Bread Making: Improving Quality* (2nd ed., pp. 31-70). Cambridge: Woodhead Publishing.

Cortés-Zavaleta, O., López-Malo, A., Hernández-Mendoza, A., & García, H. (2014). Antifungal activity of *lactobacilli* and its relationship with 3-phenyllactic acid production. *International Journal of Food Microbiology,* 173, 30–35. DOI: https://doi.org/10.1016/j.ijfoodmicro.2013.12.016.

Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Câmara, R. B. G., Nobre, L., Costa, M. S. S. P., Almeida-Lima, J., de Farias, E. H. C., & Rocha, H. A. O. (2010). Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomedicine and Pharmacotherapy,* 64(1), 21-28. DOI: http://dx.doi.org/10.1016/j.biopha.2009.03.005.

Crowley, S., Mahony, J., & van Sinderen, D. (2013). Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. *Trends in Food Science and Technology,* 33(2), 93–109. DOI: https://doi.org/10.1016/j.tifs.2013.07.004.

Cubadda, F., Aureli, F., Raggi, A., & Carcea, M. (2009). Effect of milling, pasta making and cooking on minerals in durum wheat. *Journal of Cereal Science*, 49, 92–97. DOI: https://doi.org/10.1016/j.jcs.2008.07.008.

Czaja, T., Sobota, A., & Szostak, R. (2020). Quantification of ash and moisture in wheat flour by Raman spectroscopy. *Foods*, 9, 280. DOI: https://doi.org/10.3390/foods9030280.

Dalié, D. K. D., Deschamps, A. M., & Richard-Forget, F. (2010). Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control*, 21(4), 370-380. DOI: https://dx.doi.org/10.1016/j.foodcont.2009.07.011.

Dalié, D., Gadais-Pinson, L., Atanasova-Penichon, V., Marchegay, G., Barreau, C., Deschamps, A., & Richard-Forget, F. (2012). Impact of *Pediococcus pentosaceus* strain L006 and its metabolites on fumonisin biosynthesis by *Fusarium verticillioides*. *Food Control*, 23(2), 405-411. DOI: https://doi.org/10.1016/j.foodvont.2011.08.008.

Dalinova, A. A., Salimova, D. R., & Berestetskiy, A. O. (2020). Fungi of the Genera *Alternaria* as producers of biological active compounds and mycoherbicides. *Applied Biochemistry and Microbiology*, 56(3), 256–272. DOI: https://doi.org/10.1134/S0003683820030023.

Daou, R., Joubrane, K., Maroun, R. G., Khabbaz, L. R., Ismail, A., & El Khoury, A. (2021). Mycotoxins: Factors influencing production and control strategies. *AIMS Agriculture and Food*, 6(1), 416–447. DOI: https://doi.org/10.3934/agrfood.2021025.

Day, C. N., & Morawicki, R. O. (2018). Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Hindawi Journal of Food Quality*, 1–8. DOI: https://doi.org/10.1155/2018/3964392.

De Brier, N., Gomand, S. V., Joye, I. J., Pareyt, B., Courtin, C. M., & Delcour, J. A. (2015). The impact of pearling as a treatment prior to wheat roller milling on the texture and structure of bran-rich breakfast flakes. *LWT-Food Science and Technology*, 62(1), 668-674. DOI: https://doi.org/10.1016/j.lwt.2014.08.015.

De Santis, M. A., Soccio, M., Laus, M. N., & Flagella, Z. (2021). Influence of drought and salt stress on durum wheat grain quality and composition: A review. *Plants*, 10, 2599. DOI: https://doi.org/ 10.3390/plants10122599.

De Simone, N., Capozzi, V., de Chiara, M. L. V., Amodio, M. L., Brahimi, S., Colelli, G., Drider, D., Spano, G., & Russo, P. (2021). Screening of lactic acid bacteria for the bio-control of *Botrytis cinerea* and the potential of *lactiplantibacillus plantarum* for eco-friendly preservation of fresh-cut kiwifruit. *Microorganisms*, 9(4), 773. DOI: https://doi.org/10.3390/microorganisms9040773.

Demeke, T., Perry, D. J., & Scowcroft, W. R. (2006) Adventitious presence of GMOs: scientific overview for Canadian grains. *Canadian Journal of Plant Science*, 86, 1–23. DOI: https://doi.org/10.4141/P05-114.

Deshpande, S. S. (2000). *Fermented grain legumes, seeds and nuts: a global perspective*. California, USA: Food and Agricultute Organization.

Dey, T. B., & Kuhad, R. C. (2014). Enhanced production and extraction of phenolic compounds from wheat by solid-state fermentation with *Rhizopus oryzae* RCK2012. *Biotechnology Reports*, 4, 120-127. DOI: https://doi.org/10.1016/j.btre.2014.09.006.

Dhillon, B., Kaur, K., Sodhi, N. S., & Garg, R. (2020). Physicochemical, antioxidant and microbial properties of whole wheat breads formulated with the incorporation of vegetable paste. *Journal of Food Measurement and Characterization*, 15(2), 1-7. DOI: https://doi.org/10.1007/s11694-020-00708-6.

Di Loreto, A., Bosi, S., Montero, L., Bregola, V., Marotti, I., Sferrazza, R. E., Dinelli, G., Herrero, M., & Cifuentes, A. (2018). Determination of phenolic compounds in ancient and modern durum wheat genotypes. *Electrophoresis*, 39(15), 2001–2010. DOI: https://doi.org/10.1002/elps.201700439.

Di Silvestro, R., Di Loreto, A., Bosi, S., Bregola, V., Marotti, I., Benedettelli, S., Segura-Carretero, A., & Dinelli, G. (2017). Environment and genotype effects on antioxidant properties of organically grown wheat varieties: A three year study. *Journal of the Science of Food and Agriculture,* 97(2), 641–649. DOI: https://doi.org/10.1002/jsfa.7782.

Divyashree, S., Anjali, P. G., Somashekaraiah, R., & Sreenivasa, M. Y. (2021). Probiotic properties of *Lactobacillus casei* - MYSRD 108 and *Lactobacillus plantarum*-MYSRD 71 with potential antimicrobial activity against *Salmonella paratyphi*. *Biotechnology reports (Amsterdam, Netherlands)*, *32*, e00672. DOI: https://doi.org/10.1016/j.btre.2021.e00672.

Djaaboub, S., Moussaoui, A., Meddah, B., Makhloufi, S., Gouri1, S., & El Khatib, R. (2018). Antifungal activity of some indigenous lactic acid bacteria isolated from soft wheat. *Journal of Pure and Applied Microbiology*, 12(1), 111-118. DOI: https://dx.doi.org/10.22207/JPAM.12.1.14.

Djadouni, F., & Kihal, M. (2012). Antimicrobial activity of lactic acid bacteria and the spectrum of their biopeptides against spoiling germs in foods. *Brazilian Archives of Biology and Technology,* 55(3), 435-443. DOI: https://doi.org/10.1590/S1516-89132012000300015.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(4), 654–660. DOI: https://doi.org/10.1016/j.foodchem.2005.04.028.

Dobrinas, S., Soceanu, A., Popescu, V., Popovici, L. C., & Jitariu, D. (2021). Relationship between total phenolic content, antioxidant capacity, Fe and Cu content from tea plant samples at different brewing times. *Processes*, 9(8), 1311. DOI: https://doi.org/10.3390/pr9081311.

Donelson, J. R., Gaines, C. S., Andrews, L. C., & Finney, P. F. (2002). Prediction of test weight from a small volume specific gravity measurement. *Cereal Chemistry*, 79(2), 227-229. DOI: https://doi.org/10.1094/CCHEM.2002.79.2.227.

Dopazo, V., Luz, C., Quiles, J. M., Calpe, J., Romano, R., Mañesa, J., & Meca, G. (2021). Potential application of lactic acid bacteria in the biopreservation of red grape from mycotoxigenic fungi. *Journal of the Science of Food and Agriculture*, 102(3), 898-907. DOI: http://dx.doi.org/10.1002/jsfa.11422.

Đorđević, T. M., Šiler-Marinković, S. S., & Dimitrijević-Branković, S. I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo-cereals. *Food Chemistry,* 119(3), 957-963. DOI: https://dx.doi.org/10.1016/j.foodchem.2009.07.049.

Doukani, K., Tabak, S., Gourchala, F., Mihoub, F. Ounes, M., & Benbaguar, M. (2013). Caractérisation physico-chimique du blé fermenté par stockage souterrain (Matmora). *Revue Ecologie-Environnement*, (9), 2013, 1-9.

Duan, L., Zhang, C., Zhao, Y., Chang, Y., & Guo, L. (2020). Comparison of bioactive phenolic compounds and antioxidant activities of different parts of *Taraxacum mongolicum*. *Molecules*, 25(14), 3260.

Early, R. (2009). Pathogen control in primary production: crop foods. In: C., De Blackburm, & P., McClure (Eds.). *Foodborne pathogens* (2nd ed.). DOI: https://doi.org/10.1533/9781845696337.1.205.

Ed Nignpense, B., Francis, N., Blanchard, C., & Santhakumar, A. B. (2021). Bioaccessibility and bioactivity of cereal polyphenols: A review. *Foods (Basel, Switzerland)*, 10(7), 1595. DOI: https://doi.org/10.3390/foods10071595.

Edwards, U., Rogall, T., Blocker, H., Emde, M., & Bottger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research*, 17(19), 7843–7853. DOI : https://doi.org/10.1093/nar/17.19.7843.

El Khoury, A., & Atoui, A. (2010). Ochratoxin A: general overview and actual molecular status. *Toxins*, 2(4), 461-493. DOI: https://doi.org/10.3390/toxins2040461.

El-Hag, M. E., El-Tinay, A. H., & Yousif, N. E. (2002). Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and in vitro protein digestibility of pearl millet. *Food Chemistry*, 77(2), 193–196. DOI: https://doi.org/10.1016/S0308-8146(01)00336-3.

Erdenetsogt, U., Gorgo, Y. P., & Skorochod, I. O. (2019). Detection of seedborne mycoflora in wheat. *International Journal of Innovative Science and Research Technology*, 4(10), 532-536.

Errampalli, D. (2014). *Penicillium expansum* (Blue Mold). In: S., Bautista-Baños (Ed.). *Postharvest decay: Control strategies* (pp. 189-231). Academic Press: London, UK. DOI: https://doi.org/10.1016/B978-0-12-411552-1.00006-5.

Evers, A. D., Blakeney, A. B., & O'Brien, L. (1999). Cereal structure and composition. *Australian Journal of Agricultural Research*, 50(5), 629–650.

Falguni, P., Shilpa, V., & Mann, B. (2010). Production of proteinaceous antifungal substances from *Lactobacillus brevis* NCDC. *International Journal of Dairy Technology*, 63(1), 70–76. DOI: https://doi.org/10.1111/j.1471-0307.2009.00553.x.

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: What is beyond fibre? *Nutrition Research Reviews*, 23(1), 65-134. DOI: 10.1017/S0954422410000041.

Farhat, W. Z. E., Khedr, R. A, & Shaaban, S. A. (2021). Response of some agronomic, physiological and anatomical characters for some bread wheat genotypes under water deficit in North Delta region. *Scientific Journal of Agricultural Sciences,* 3 (2), 145-160. DOI: https://doi.org/10.21608/sjas.2021.97378.1156.

Feillet, P. (2000). *Le grain de blé : composition et utilisation*. Amazon France: Editions Quae.

Fevria, R., & Hartanto, I. (2020). Isolation and characterization of lactic acid bacteria (*Lactobacillus sp.*) from Sauerkraut. *Advances in Biological Sciences Research,* 10, 74-77. DOI: https://doi.org/10.2991/absr.k.200807.018.

Florianowicz, T. (2001). Antifungal activity of some microorganisms against *Penicillium expansum. European Food Research and Technology*, 212(3), 282-286. DOI: https://doi.org/10.1007/s002170000261.

Frivasd, J. C., Smedsgaard, J., Larsen, T. O., & Samson, R. A. (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium. Studies in Mycology*, 49, 201-241.

Fuchida, S., Tajima, S., Nishimura, T., & Tokoro, C. (2022). Kinetic modeling and mechanisms of manganese removal from alkaline mine water using a pilot scale column reactor. *Minerals*, 12, 99. DOI: https://doi.org/10.3390/min12010099.

Gacem, M. A., Ould al-Hajj Khalil, A., & Gacemi, B. A. (2011). Etude de la qualite physico-chimique et mycologique du ble tendre local et importe stocke au niveau de l'office Algérien interprofessionnel des céréales (OAIC) de la localite de Saida (Algerie). *Algerian Journal of Arid Environment,* 1(2), 67-76.

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology*, 37, 2-10. DOI: https://dx.doi.org/10.1016/j.fm.2013.04.007.

García-Gómez, B., Fernández-Canto, N., Vázquez-Odériz, M. L., Quiroga-García, M., Muñoz-Ferreiro, N., & Romero-Rodríguez, M. A. (2022). Sensory descriptive analysis and hedonic consumer test for Galician type breads. *Food Control*, 134, 108765. DOI: https://doi.org/10.1016/j.foodcont.2021.108765.

Garcia-Oliveira, A. L., Chander, S., Ortiz, R., Menkir, A., & Gedil, M. (2018). Genetic basis and breeding perspectives of grain iron and zinc enrichment in cereals. *Frontiers in Plant Science,* 9, 937. DOI: https://doi.org/10.3389/fpls.2018.00937.

Gartaula, G., Dhital, S., Netzel, G., Flanagan, B. M., Yakubov, G. E., Beahan, C. T., Collins, H. M., Burton, R. A., Bacic, A., & Gidley, M. J. (2018). Quantitative structural organisation model for wheat endosperm cell walls: Cellulose as an important constituent. *Carbohydrate Polymers*, 196, 199–208. DOI: https://doi.org/10.1016/j.carbpol.2018.05.041.

Gil-Serna, J., Vázquez, C., González-Jaén, M. T., Patiño, B. (2014). Mycotoxins. Toxicology. In: C. A., Batt, & M. L. Tortorilo (Eds.). *Encyclopedia of food microbiology* (pp. 887-892). Academic Press: Cambridge, MA, USA. DOI: https://doi.org/10.1016/B978-0-12-384730-0.00234-2.

Glenn, A. (2007). Mycotoxigenic *Fusarium* species in animal feed. *Animal Feed Science and Technology*, 137(3-4), 213–240. DOI: https://doi.org/10.1016/j.anifeedsci.2007.06.003.

Godon, B., & Loisel, W. (1997). *Guide pratique d'analyses dans les industries des céréales* (2nd ed.). Paris : Technique et Documentation – Lavoisier.

Gomes, A. A. B., Ferreira, M. E., & Pimentel, T. C. (2016). Bread with flour obtained from green banana with its peel as partial substitute for wheat flour: physical, chemical and microbiological characteristics and acceptance. *International Food Research Journal*, 23(5), 2214-2222.

Gonzalez-Thuillier, I., Salt, L., Chope, G., Penson, S., Skeggs, P., Tosi, P., Powers, S. J., Ward, J. L., Wilde, P., Shewry, P. R., & Haslam, R. P. (2015). Distribution of lipids in the grain of wheat (cv. Hereward) determined by lipidomic analysis of milling and pearling fractions. *Journal of Agricultural and Food Chemistry,* 63, 10705−10716. DOI: https://doi.org/10.1021/acs.jafc.5b05289.

Gotti, R., Amadesi, E., Fiori, J., Bosi, S., Bregola, V., Marotti, I., & Dinelli, G. (2018). Differentiation of modern and ancient varieties of common wheat by quantitative capillary electrophoretic profile of phenolic acids. *Journal of Chromatography A,* 1532, 208–215. DOI: https://doi.org/10.1016/j.chroma.2017.11.058.

Gourchala, F., Hobamahoro, A. F., Mihoub, F., & Henchiri, C. (2014). Effect of natural fermentation on the nutritional quality of 'El hamoum' durum wheat (*Triticum durum*) fermented product of the Algerian country. *International Journal of Bio-Technology and Research, 4*(4), 9-18.

Guimarães, A., Venancio, A., & Abrunhosa, L. (2018). Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*, *Food Additives & Contaminants: Part A,* 35(9), 1803-1818. DOI: https://doi.org/10.1080/19440049.2018.1500718.

Güven, M., & Kara, H. H. (2016). Some chemical and physical properties, fatty acid composition and bioactive compounds of wheat germ oils extracted from different wheat cultivars. *The Journal of Agricultural Science,* 22(3), 433–443. DOI: https://doi.org/10.1501/Tarimbil_0000001401.

Harris, L. J., Shebuski, J. R., Danyluk, M. D., Palumbo, M. S., & Beuchat, L. R. (2013). Nuts, seeds, and cereals. In: M. P., Doyle, & R. L., Buchanan (Eds.), *Food microbiology* (4th ed., pp. 203–221). Washington, DC: ASM Press.

Hayma, J. (2003). *The storage of tropical agricultural products* (4th ed.). Wageningen, Netherlands: Agromisa Foundation. ISBN: 90-77073-60-4.

Heussner, A. H., & Bingle, L. E. H. (2015). Comparative ochratoxin toxicity: A review of the available data. *Toxins*, 7(10), 4253–4282. DOI: https://doi.org/10.3390/toxins7104253.

Hocking, A. D. (2003). Microbiological facts and fictions in grain storage. In: E. J., Wright, M.C., Webb, & E. Highley (Eds.) *Stored grain in Australia. Proceedings of the Australian Postharvest Technical Conference* (pp. 55-58), Canberra.

Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 75, 197-212.

Horvat, D., Šimi´c, G., Drezner, G., Lali´c, A., Ledenˇcan, T., Tucak, M., Plavši´c, H., Andri´c, L., & Zduni´c, Z. (2020). Phenolic acid profiles and antioxidant activity of major cereal crops. *Antioxidants*, 9(6), 527. DOI: https://doi.org/10.3390/antiox9060527.

Hossain, K., Ulven, C., Glover, K., Ghavami, F., Simsek, S., Alamri, M. S., Kumar, A., & Mergoum M. (2013). Interdependence of cultivar and environment on fibre composition in wheat bran. *Australian Journal of Crop Science*, 7(4), 525-531.

Hung, P. V. (2016). Phenolic compounds of cereals and their antioxidant capacity. *Critical Reviews in Food Science and Nutrition,* 56(1), 25-35. DOI: https://doi.org/10.1080/10408398.2012.708909.

Ibrahim, S. A., Gyawali, R., & Fidan, H. (2020). Self-Defense: A practical approach to combatting COVID-19. *Acta Scientific Nutritional Health*, 4(7), 33-37. DOI: https://doi.org/10.31080/ASNH.2020.04.0715.

Igbabul, B., Hiikyaa, O., & Amove, J. (2014). Effect of fermentation on the proximate composition and functional properties of mahogany bean (*Afzelia africana*) flour. *Current Research in Nutrition and Food Science Journal,* 2(1), 1–7. DOI: https://doi.org/10.12944/CRNFSJ.2.1.01.

Ijah, U. J. J., Auta, H. S., Aduloju, M. O., & Aransiola, S. A. (2014). Microbiological, nutritional, and sensory quality of bread produced from wheat and potato flour blends. *International Journal of Food Science*, ID 671701, 1-6.

Iqbal, M., Moakhar, N. P., Strenzke, K., Haile, T., Pozniak, C., Hucl, P., & Spaner, D. (2016). Genetic improvement in grain yield and other traits of wheat grown in western Canada. *Crop Science*, 56(2), 613-624. DOI: https://doi.org/10.2135/cropsci2015.06.0348.

Iqbal, Z., Pasha, I., Abrar, M., Masih, S., & Hanif, M. S. (2015). Physico-chemical, functional and rheological properties of wheat varieties. *Journal of Agricultural Research*, 53(2), 253-267.

Ismaiel, A., & Papenbrock, J. (2015). Mycotoxins: Producing fungi and mechanisms of phytotoxicity. *Agriculture*, 5(3), 492-537. DOI: https://doi.org/10.3390/agriculture5030492.

Ismail, Y., Yulvizar, C., & Mazhitov, B. (2018). Characterization of lactic acid bacteria from local cow´s milk kefir. *Earth and Environmental Science,* 130(1), 012019. DOI: https://doi.org/10.1088/1755-1315/130/1/012019.

Janik, E., Niemcewicz, M., Ceremuga, M., Stela, M., Saluk-Bijak, J., Siadkowski, A., & Bijak, M. (2020). Molecular aspects of mycotoxins-A serious problem for human health. *International journal of molecular sciences*, *21*(21), 8187. DOI: https://doi.org/10.3390/ijms21218187.

Jesperson, L. (2003). Occurrence and taxonomic characteristics of strains of predominant in African indigenous fermented foods and beverages. *FEMS Yeast Research*, 3, 191-200.

Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J., & Shi, J. (2019). Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: A Review. *Food Production, Processing and Nutrition,* 1(1), 1-14. DOI: https://doi.org/10.1186/s43014-019-0007-2.

Johansson, E., Svensson, G., & Tsegaye, S. (2000). Genotype and environment effects on bread-making quality old Swedish-grown wheat cultivars containing highmolecular-weight glutenin subunits 2 + 12 or 5 + 10. *Acta Agriculturae Scandinavica*, *Section B — Soil & Plant Science*, 49(4), 225-233. DOI: https://doi.org/10.1080/713782024.

Joshi, A., Kumar, A., Budhlakoti, V., Bhatt, N., & Prasad, B. (2018). Analysis of variance and estimation of genetic variability parameters for quality traits in bread wheat (*Triticum aestivum* L. em.Thell) genotypes. *International Journal of Chemical Studies,* 6(5), 369-372.

Just´e, A., Malfliet, S., Lenaerts, M., De Cooman, L., Aerts, G., Willems, K. A., & Lievens B. (2011). Microflora during malting of barley: Overview and impact on malt quality. *Brewing Science*, 64(3–4), 22–31.

Kaliyan, N., Morey, R. V., & Wilcke, W. F. (2005). Mathematical model for simulating headspace and grain temperatures in grain bins. *American Society of Agricultural Engineers*, 48(5), 1851–1863. DOI: https://doi.org/10.13031/2013.19983.

Kamal-Eldin, A. (2012). Fermented cereal and legume products. In: M. M., Bhavbhuti, A., Kamal-Eldin, & R. Z., Iwanski (Eds.). *Fermentation: effects on food properties* (pp. 209-229), United Kingdom: *CRC press*.

Kasote, D., Tiozon, R. N. Jr., Sartagoda, K. J. D., Itagi, H., Roy, P., Kohli, A., Regina, A., & Sreenivasulu, N. (2021). Food processing technologies to develop functional foods with enriched bioactive phenolic compounds in cereals. *Frontiers in Plant Science*, 12, 771276. DOI: https://doi.org/10.3389/fpls.2021.771276.

Kechkin, I. A., Ermolaev, V. A., Ivanov, M. V., Romanenko, A. I., & Gurkovskaya, E. A. (2020). Dependence of fat acidity value on wheat grain storage conditions. *BIO Web of Conferences,* 17, 00107. DOI: http://doi.org/10.1051/bioconf/20201700107.

Khalid, K. (2011). An overview of lactic acid bacteria. *International Journal of Biosciences*, 1(3), 1–13.

Khan, S., Memon, A. N., Deverajani, B. R., & Baloch, S. (2015). Physicochemical characteristics of wheat grain and their relation with proteins in different varieties cultivated in Sindh. *Sindh University Research Journal (Science Series)*, 47 (4), 839-842.

Kim, J. S., & Lee, J. H. 2020. Correlation between solid content and antioxidant activities in *Umbelliferae* aalad alants. *Preventive Nutrition and Food Science,* 25(1), 84-92. DOI: https://doi.org/10.3746/pnf.2020.25.1.84.

Kivanc, M., Kivanc, S. A., & Pektas S. (2014). Screening of lactic acid bacteria for antifungal activity against fungi. *Journal of Food Processing and Technology*, 5(3), 1000310. DOI: https://doi.org/10.4172/2157-7110.1000310.

Kohajdova, Z., & Karovicova, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food and Nutrition Research*, 2(46), 51-57.

Kosiak, B., Torp, M., Skjerve, E., & Andersen, B. (2004). *Alternaria* and *Fusarium* in Norwegian grains of reduced quality—A matched pair sample study. *International Journal of Food Microbiology*, 93(1), 51–62. DOI: https://doi.org/10.1016/j.ijfoodmicro.2003.10.006.

Kotta-Loizou, I. (2021). Diversity of mycoviruses in *Aspergilli*. In: Encyclopedia of Virology (4th ed., pp. 450-456). DOI: https://doi.org/10.1016/B978-0-12-809633-8.21321-6.

Kovač, M., Bulaić, M., Nevistić, A., Rot, T., Babić, J., Panjičko, M., Kovač, T., & Šarkanj, B. (2022). Regulated mycotoxin occurrence and co-occurrence in Croatian cereals. *Toxins*, 14, 112. DOI: https://doi.org/10.3390/ toxins14020112.

Kumar, S., Sinha, A., Kumar, R., Singh, V., Houda, K. S., & Nath, K. (2020). Storage fungi and mycotoxins. In: R., Kumar, & A., Gupta (Eds.). *Seed-Borne diseases of agricultural crops: detection, diagnosis and danagement* (pp. 821-861). DOI: https://doi.org/10.1007/978-981-32-9046-4_29.

Kumar, V. (2018). Aflatoxins: properties, toxicity and detoxification. *International Journal of Food Science and Nutrition*, 6(5), 555696. DOI: https://doi.org/10.19080/NFSIJ.2018.06.555696.

Lafiandra, D., Masci, S., Sissons, M., Dornez, E., Delcour, J. A., Courtin, C. M., & Caboni, M. F. (2012). Kernel components of technological value. In: M. J, Sissons, M., Carcea, B., Marchylo, & J., Abecassis. *Durum wheat chemistry and technology* (2nd ed., pp. 85-124). St. Paul, MN, USA: AACC International Inc.

Lavermicocca, P., Valerio, F., & Visconti, A. (2003). Antifungal activity of phenyllactic acid against molds isolated from bakery products. *Applied and Environmental Microbiology*, 69(1), 634–640. DOI: https://doi.org/10.1128/AEM.69.1.634-640.2003.

Lawrence, D. P., Rotondo, F., & Gannibal, P. B. (2016). Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. *Mycological Progress,* 15(1), 3. DOI: https://doi.org/10.1007/s11557-015-1144-x.

Le Lay, C., Mounier, J., Vasseur, V., Weill, A., Le Blay, G., Barbier, G., & Coton, E. (2016). *In vitro* and *In situ* screening of lactic acid bacteria and propionibacteria antifungal activities against bakery product spoilage molds. *Food Control*, 60, 247–255. DOI: https://doi.org/10.1016/j.foodcont.2015.07.034.

Lecoq, R. (1965). *Manuel d'analyses alimentaires et d'expertises usuelles*. Paris, France: Doin.

Leyva Salas, M., Mounier, J., Valence, F., Coton, M., Thierry, A., & Coton, E. (2017). Antifungal microbial agents for food biopreservation - a review. *Microorganisms,* 5(3), 37. DOI: https://doi.org/10.3390/microorganisms5030037.

Li, B., Zhao, L., Chen, H., Sun, D., Deng, B., Li, J., Liu, Y., & Wang, F. (2016). Inactivation of lipase and lipoxygenase of wheat germ with temperature-controlled short wave infra-red radiation and its effect on storage stability and quality of wheat germ oil. *PLoS One*, 11(12), e0167330. DOI: https://doi.org/10.1371/journal.pone.0167330.

Li, K., Zhang, W., Kwok, L. Y., & Menghe B. (2020). Screening of *Lactobacillus plantarum* with broad-spectrum antifungal activity and its application in preservation of golden-red apples. *Czech Journal of Food Sciences*, 38 (5), 315-322. DOI: https://doi.org/10.17221/175/2020-CJFS.

Li, Y., Ma, D., Sun, D., Wang, C., Zhang, J., Xie, Y., & Guo, T. (2015). Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored wheat grains by three milling methods. *The Crop Journal*, 3, 328-334. DOI: https://doi.org/10.1016/J.CJ.2015.04.004.

Lindblab, C., & Druben, L. (1980). *Small farm grain storage - Preparing grain for storage*. (Volume I). Peace Corps Program & Training Manual, USA.

Liu, B. H., Yu, F. Y., Wu, T. S., Li, S. Y., Su, M. C., Wang, M. C., & Shih, S. M. (2003). Evaluation of genotoxic risk and oxidative DNA damage in mammalian cells exposed to mycotoxins, patulin and citrinin. *Toxicology and Applied Pharmacology*, 191(3), 255-263. DOI: https://doi.org/10.1016/s0041-008x(03)00254-0.

Liu, F., Chen, Z., Shao, J., Wang, C., & Zhan, C. (2017). Effect of fermentation on the peptide content, phenolics and antioxidant activity of defatted wheat germ. *Food Bioscience*, 20, 141-148. DOI: https://doi.org/10.1016/j.fbio.2017.10.002.

Logrieco, A., Bottalico, A., Mule, G., Moretti, A., & Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. *European Journal of Plant Pathology*, 109(7), 645-667. DOI: https://doi.org/10.1023/A:1026033021542.

Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T., & Salovaara, H. (2004). Degradation of HMW glutenins during wheat sourdough fermentation. *Journal of Cereal Chemistry,* 81(1), 87-93. DOI: https://doi.org/10.1094/CCHEM.2004.81.1.87.

Los, A., Ziuzina, D., & Bourke, P. (2018). Current and future technologies for microbiological decontamination of cereal grains. *Journal of Food Sciences*, 83(6), 1484-1493. DOI: https://doi.org/10.1111/1750-3841.14181.

Lv, W., Booz, G. W., Fan, F., Wang, Y., & Roman, R. J. (2018). Oxidative stress and renal fibrosis: Recent insights for the development of novel therapeutic strategies. *Frontiers in Physiology*, 9, 105. DOI: http://dx.doi.org/10.3389/fphys.2018.00105.

Lyu, J. I., Ryu, J., Seo, K. S., Kang, K. Y., Park, S. H., Ha, T. H., Ahn, J. W., & Kang, S. Y. (2022). Comparative study on phenolic compounds and antioxidant activities of Hop (*Humulus lupulus* L.) strobile extracts. *Plants,* 11(1), 135. DOI: https://doi.org/ 10.3390/plants11010135.

MacDonald, S., Prickett, T. J., Wildey, K. B., & Chan, D. (2004). Survey of ochratoxin A and deoxynivalenol in stored grains from the 1999 harvest in the UK. *Food Additives Contaminants*, 21(2), 172-181. DOI: https://doi.org/ 10.1080/02652030310001648180.

Magan, N., & Aldred, D. (2007). Post-harvest control strategies: minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, 119(1-2), 131-139. DOI: https://doi.org/10.1016/j.ijfoodmicro.2007.07.034.

Magnusson, J., & Schnürer J. (2001). *Lactobacillus Coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and Environmental Microbiology*, 67 (1), 1-5. DOI: https://doi.org/10.1128/AEM.67.1.1-5.2001.

Magnusson, J., Stro¨m, K., Roos, S., Sjo¨gren, J., & Schnu¨rer, J. (2003). Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiology Letters,* 219(1), 129–135. DOI: https://doi.org10.1016/S0378-1097(02)01207-7.

Mahato, D. K., Devi, S., Pandhi, S., Sharma, B., Maurya, K. K., Mishra, S., Dhawan, K., Selvakumar, R., Kamle, M., Mishra, A., & Kumar, P. (2021). Occurrence, impact on agriculture, human health, and management strategies of zearalenone in food and feed: A Review. *Toxins,* 13(2), 92. DOI: https://doi.org/10.3390/toxins13020092.

Mahmood, S. U., Bashir, M. H., Abrar, M., Sabri, M. A., & Khan, M. A. (2013). Appraising the changes in the nutritional value of stored wheat, *Triticum aestivum* L. infested with acarid mite, *Rhizoglyphus tritici* (Acari: Acaridae). *Pakistan Journal of Zoology*, 45(5), 1257-1261.

Mahmood, S. U., Bashir, M. H., Afzal, M., & Ashfaq, M. (2012). Evaluation of germination losses caused by mites in seeds of maize and mung from farmer's holdings in Tehsil Toba Tek Singh. *Pakistan Journal of Zoology*, 44(1), 117-121.

Mallick, S. A., Azaz, K., Gupta, M., Sharma, V., & Sinha, B. K. (2013). Characterization of grain nutritional quality in wheat. *Indian Journal of Plant Physiology*. 18(2), 183-186. DOI: https://doi.org/10.1007/s40502-013-0025-z.

Mansouri, A., Embarek, G., Kokkalou, E., & Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry,* 89(3), 411-420. DOI: https://dx.doi.org/10.1016/j.foodchem.2004.02.051.

Manthey, F. A., Wolf-Hall, C. E., Yalla, S., Viajayakumar, C., & Carlson, D. (2004). Microbial loads, mycotoxins, and quality of durum wheat from the 2001 harvest of the North Plains region of the United States. *Journal of Food Protection*, 67(4), 772-780. DOI: https://doi.org/10.4315/0362-028x-67.4.772.

Martin, T. N., Filho, A. C., Deak, E. A., Cechin, J., Burg, G. M., & Grün, E. (2022). Variation in the hectolitre weight of wheat grain for equipment and sample size. *Ciência Rural*, 52(6), 1–8.

Matei, A., Cornea, C. P., & Matei, G. M. (2015). Antifungal activity of lactic acid bacteria strains against *Aspergillus niger* and *Aspergillus flavus* isolated from brined vegetables. *Journal of Biotechnology,* 208, S79. DOI: https://doi.org/10.1016/j.jbiotec.2015.06.243.

Matevosyan, L., Bazukyan, I., & Trchounian, A. (2019). Antifungal and antibacterial effects of newly created lactic acid bacteria associations depending on cultivation media and duration of cultivation. *BMC Microbiology*. 19, 102. DOI: https://doi.org/10.1186/s12866-019-1475-x.

Matz, A. (1991). *Chemistry and technology of cereals as food and feed* (2nd ed.). USA: Springer Science and Business media.

Mauzé, C., Richard, M., & Scotti, G. (1972). *Guide pratique de contrôle de la qualité des blés*. Paris : Institut Technique des Céréales et de Fourrage (ITCF).

Mazur, W., & Adlercreutz, H. (1998). Naturally occurring oestrogens in food. *Pure and Applied Chemistry*, 70(9), 1759-1776. DOI: https://doi.org/10.1351/pac199870091759.

McIntyre, C. L., Seung, D., Casu, R. E., Rebetzke, G. J., Shorter, R., & Xue, G. P. (2012). Genotypic variation in the accumulation of water soluble carbohydrates in wheat. *Functional Plant Biology,* 39(7), 560–568. DOI: https://doi.org/10.1071/FP12077.

Medhe, S., Bansal, P., & Srivastava, M. M. (2014). Enhanced antioxidant activity of gold nanoparticle embedded 3, 6- dihydroxyflavone: A combinational study. *Applied Nanoscience*, 4, 153-161. DOI: http://dx.doi.org/10.1007/s13204-012-0182-9.

Mhiko, T. A. (2012). Determination of the causes and the effects of storage conditions on the quality of silo stored wheat (*Triticum aestivum*) in Zimbabwe. *Natural Products and Bioprospecting*, 2(1), 21-28. DOI: https://doi.org/10.1007/s13659-012-0004-5.

Mieszkin, S., Hymery, N., Debaets, S., Coton, E., Le Blay, G., Valence, F., & Mounier, J. (2017). Action mechanisms involved in the bioprotective effect of *Lactobacillus harbinensis* K.V9.3.1.Np against *Yarrowia lipolytica* in fermented milk. *International Journal of Food Microbiology,* 2(248), 47-55. DOI: https://doi.org/10.1016/j.ijfoodmicro.2017.02.013.

Miller, H. E., Rigelhof, F., Marquart, L., Prakash, A., & Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition*, 19(3), 312S-319S. DOI: https://doi.org/10.1080/07315724.2000.10718966.

Mokhtari, S., Taghouti, M., Saidi, D., & Kheroua, O. (2020). Traditional Algerian fermented food: First data on nutritional characteristics of wheat (*Triticum durum*) fermented in underground silos matmor (Mascara, Algeria) compared to unfermented wheat. *Advances in Biology and Earth Sciences*, 5(3), 176-192.

Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. Journal of Agricultural and Food chemistry, 54, 1265-1270. DOI: https://doi.org/10.1021/jf052683d.

Muhialdin, B. J., Hassan, Z., & Saari, N. (2018). *In vitro* antifungal activity of lactic acid bacteria low molecular peptides against spoilage fungi of bakery products. *Annals of Microbiology,* 68(9), 557–567. DOI: https://doi.org/10.1007/s13213-018-1363-x.

Munkvold, G. P. (2017). *Fusarium* species and their associated mycotoxins. In: A., Moretti, & A., Susca (Eds.). *Mycotoxigenic Fungi: Methods and Protocols, Methods in Molecular Biology* (1st ed., pp. 51-106). New York, NY: Humana Press: Springer. DOI: https://doi.org/ 10.1007/978-1-4939-6707-0_4.

Narducci, V., Finotti, E., Galli, V., & Carcea, M. (2019). Lipids and fatty acids in Italian durum wheat (*Triticum durum* Desf.) cultivars. *Foods (Basel, Switzerland)*, 8(6), 223. DOI: https://doi.org/10.3390/foods8060223.

Nasiroleslami, E., Mozafari, H., Sadeghi-Shoae, M., Habibi, D., & Sani, B. (2021). Changes in yield, protein, minerals, and fatty acid profile of wheat (*Triticum aestivum* L.) under fertilizer management involving application of nitrogen, humic acid, and seaweed extract. *Journal of Soil Science and Plant Nutrition*, 21(3), 2642-2651. DOI: https://doi.org/10.1007/s42729-021-00552-7.

Nasrollahzadeh, A., Khomeiri, M., Sadeghi, A., Mahmoudi, M., & Ebrahimi, M. (2020). Antifungal activity of lactic acid bacteria isolated from Masske, camel dough, and local yogurt against *Aspergillus flavus* and *Aspergillus niger*. *Journal of Food Hygiene*, 9 (4), 1-11. DOI: https://doi.org/10.30495/JFH.2020.670898.

Navarro, S., & Navarro, H. (2016). Emerging global technological challenges in the reduction of post-harvest grain losses. In: S., Navarro, D. S., Jayas, & K., Alagusundaram (Eds.) *Proceedings of the 10th international conference*

on controlled atmosphere and fumigation in stored products (CAF2016) (pp. 259-265). Winnipeg, Canada: CAF Permanent Committee Secretariat.

Navarro, S., & Noyes, R. T. (2001). *The mechanics and physics of modern grain aeration management* (1st ed.). Boca Raton: CRC Press. DOI: https://doi.org/10.1201/9781420040333.

Nierop, S.V. (2006). The impact of microorganisms on barley and malt quality - A review. *Journal of the American Society of Brewing Chemists,* 64(2), 69–78. DOI: https://doi.org/10.1094/ asbcj-64-0069.

Nionelli, L., Curri, N., Curiel, J. A., Di Cagno, R., Pontonio, E., Cavoski, I., Gobbetti, M., & Rizzello, C. G. (2014). Exploitation of Albanian wheat cultivars: characterization of the flours and lactic acid bacteria microbiota, and selection of starters for sourdough fermentation. *Food Microbiology*, 44, 96-107. DOI: https://doi.org/10.1016/j.fm.2014.05.011.

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition*, 6(8), 2449-2458. DOI: https:// doi.org/10.1002/fsn3.846.

Noots, I., Delcour, J. A., & Michiels, C. W. (1999). From field barley to malt: Detection and specification of microbial activity for quality aspects. *Critical Reviews in Microbiology*, 25(2), 121–153. DOI: https://doi.org/10.1080/10408419991299257.

Nuraida, L. A. (2015). Review: health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Science and Human Wellness,* 4(2), 47–55. DOI: https://doi.org/10.1016/j.fshw.2015.06.001.

Nwaigwe, K. N. (2019). An overview of cereal grain storage techniques and prospects in Africa. *International Journal of Bioengineering & Biotechnology*, 4(2), 19-25.

Odey, G. N., & Lee, W. (2019). Evaluation of the quality characteristics of flour and pasta from fermented cassava roots. *International Journal Food Science and Technology*, 55(2), 1–10. DOI: https://doi.org/10.1111/ijfs.14364.

Ogodo, A. C., Ugbogu, O. C., Onyeagba, R. A., & Okereke, H. C. (2017). Effect of lactic acid bacteria consortium fermentation on the proximate composition and in-vitro starch/protein digestibility of maize (*Zea mays*) flour. *American Journal of Microbiology and Biotechnology*, 4(4), 35–43.

Ojo, M., Ariahu, C., & Chinma, E. (2017). Proximate, functional and pasting properties of cassava starch and mushroom (*Pleurotus Pulmonarius*) flour blends. *American Journal of Food Science and Technology*, 5(1), 11–8. DOI: https://doi.org/10.12691/ajfst-5-1-3.

Okafor, J. N. C., Okafor, G. I., Ozumba, A. U., & Elemo, G. N. (2012). Quality characteristics of bread made from wheat and Nigerian oyster mushroom (*Pleurotus plumonarius*) powder. *Pakistan Journal of Nutrition*, 11, 5-10.

Okuyama, L. A., Caramori, P. H., & Kohli, M. M. (2020). New microchondrometer to measure hectoliter weight in small samples of wheat. *African Journal of Agricultural Research,* 15(4), 524-530. DOI: http://doi.org/10.5897/AJAR2019.14538.

Olaoye, O. A., Onilude, A., & Idowu, O. A. (2006). Quality characteristica of bread produced from composite flours of wheat, plantain and soy beans. *African Journal of Biotechnology*, 5(11), 1102-1106.

Ollivier, D., Artaud, J., Pinatel, C., & Guérère, M. (2006). Differentiation of french virgin oil RDOCS by sensory characteristic fatty acid and triacylglycerol composition and chemotrics. *Food Chemistry*, 97(3), 382-93.

Oloyede, O. O., Ocheme, O. B., & Nurudeen, L. M. (2013). Physical, sensory and microbiological properties of wheat fermented unripe plantain flour. *Nigerian Food Journal*, 31(2), 123-129.

Onoja, U. S., & Obizoba, I. C. (2009). Nutrient composition and organoleptic attributes of gruel based on fermented cereal, legume, tuber and root flour. *Journal of Tropical Agriculture, Food, Environment and Extension*, 8(3), 162–168. DOI: https://doi.org/10.4314/as.v8i3.51735.

Osborne, T. B. (1924). The vegetable proteins. *Nature,* 114, 822. DOI: https://doi.org/10.1038/114822c0.

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences*, 10(1), 1–6. DOI: https://doi.org/10.1016/j.jssas.2010.06.001.

Osman, M. A., Mahmoud, G. I., & Shoman, S. S. (2021). Correlation between total phenols content, antioxidant power and cytotoxicity. *Biointerface Research in Applied Chemistry*. 11(3), 10640 – 10653. DOI: https://doi.org/10.33263/BRIAC113.1064010653.

Osuna, M. B., Judis, M. A., Romero, A. M., Avallone, C. M., & Bertola, N. C. (2014). Improvement of fatty acid profile and studio of rheological and technological characteristics in breads supplemented with flaxseed, soybean, and wheat bran flours. *Scientific World Journal*, 2014, 1-10. DOI: https://dx.doi.org/10.1155/2014/401981.

Othman, A., Ismail, A., Ghani, N. A., & Adenan, I. (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry,* 100(4), 1523-1530. DOI: https://dx.doi.org/10.1016/j.foodchem.2005.12.021.

Padayachee, A., Day, L., Howell, K., & Gidley, M. J. (2017). Complexity and health functionality of plant cell wall fibers from fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 57(1), 59–81. DOI: https://doi.org/10.1080/10408398.2013.850652.

Pagano, G., Talamanca, A. A., Castello, G., Cordero, M. D., Dischia, M., & Gadaleta, M. N. (2014). Oxidative stress and mitochondrial dysfunction across broad-ranging pathologies: Toward mitochondria-targeted clinical strategies. *Oxidative Medicine and Cellular Longevity,* 54, 12-30. DOI: https://dx.doi.org/10.1155/2014/541230.

Palumbo, R., Crisci, A., Venâncio, A., Abrahantes, J. C., Dorne, J. L., Battilani, P., & Toscano, P. (2020). Occurrence and co-occurrence of mycotoxins in cereal-based feed and food. *Microorganisms*, 8, 74. DOI: https://doi.org/10.3390/microorganisms8010074.

Parada, J. L., Caron, C. R., Medeiros, A. B. P., & Soccol, C. R. (2007). Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. *Brazilian Archives of Biology and Technology*. 50, 521–542. DOI: https://doi.org/10.1590/S1516-89132007000300018.

Patriarca, A., & Pinto, V. F. (2018). *Alternaria*. Reference module. *Food Science,* 1-8. DOI: https://doi.org/10.1016/B978-0-08-100596-5.22572-9.

Pawlowska, E., Szczepanska, J., Koskela, A., Kaarniranta, K., & Blasiak, J. (2019). Dietary polyphenols in age-related macular degeneration: Protection against oxidative stress and beyond. *Oxidative Medicine and Cellular Longevity*, ID 9682318. DOI: https://doi.org/10.1155/2019/9682318.

Pekmez, H. (2016). Cereal storage techniques: A review. *Journal of Agricultural Science and Technology*, B 6, 67-71. DOI: https://doi.org/10.17265/2161-6264/2016.02.001.

Peña-Chocarro, L., Jordà, G. P., Mateos, J. M., & Zapata, L. (2015). Storage in traditional farming communities of the western Mediterranean: Ethnographic, historical and archaeological data. *Environmental Archaeology*, 20(4), 379-389. DOI: https://doi.org/10.1179/1749631415Y.0000000004.

Peng, Y., Chang, X., & Lang, M. (2021). Iron homeostasis disorder and lzheimer's disease. *International Journal of Molecular Sciences*, 22, 12442. DOI: https://doi.org/ 10.3390/ijms222212442.

Perez, R. H., Zendo, T., & Sonomoto, K. (2014). Novel bacteriocins from lactic acid bacteria (LAB), various structures and applications. *Microbial Cell Factories*, 13 (Suppl. 1), S3. DOI: https://doi.org/10.1186/1475-2859-13-S1-S3.

Perrone, G., Ferrara, M., Medina, A., Pascale, M., & Magan, N. (2020). Toxigenic fungi and mycotoxins in a climate change scenario: ecology, genomics, distribution, prediction and prevention of the risk. *Microorganisms*, 8(10), 1496. DOI: https://doi.org/10.3390/microorganisms8101496.

Petrie, J. R., Guzik, T. J., & Touyz, R. M. (2018). Diabetes, Hypertension, and cardiovascular disease: Clinical insights and vascular mechanisms. *Canadian Journal of Cardiology,* 34, 575-584. DOI: http://dx.doi.org/10.1016/j.cjca.2017.12.005.

Peyer, L. C., Axel, C., Lynch, K. M., Zannini, E., Jacob, F., & Arendt, E. K. (2016). Inhibition of *Fusarium culmorum* by carboxylic acids released from lactic acid bacteria in a barley malt substrate. *Food Control,* 69, 227–236. DOI: https://doi.org/10.1016/j.foodcont.2016.05.010.

Piluzza, G., & Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharmaceutical Biology,* 49(3), 240–247. DOI: 10.3109/13880209.2010.501083.

Pitt, J. I. (2000). Toxigenic fungi and mycotoxins. *British Medical Bulletin*, 56(1), 184 – 192.

Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage (3rd Ed.) N.Y., U.S.A.: Springer Science+Business Media. DOI: https://doi.org/10.1007/978-0-387-92207-2.

Pohl, F., & Lin, P. K. T. (2018). The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases : *In vitro*, *In vivo* and clinical trials. *Molecules,* 23(12), 3283. DOI: https://dx.doi.org/10.3390/molecules23123283.

Poole, N., Donovan, J., & Erenstein, O. (2020). Agri-nutrition research: Revisiting the contribution of maize and wheat to human nutrition and health. *Food Policy*, 100(3), 101976. DOI: https://doi.org/10.1016/j.foodpol.2020.101976.

Pranoto, Y., Anggrahini, S., & Efendi, Z. (2013). Effect of natural and *Lactobacillus plantarum* fermentation on *In vitro* protein and starch digestibilities of sorghum flours. *Food Bioscience*, 2, 46–52. DOI: https://doi.org/10.1016/j.fbio.2013.04.001.

Prasadi, N. P. V., & Joye, I. J. (2020). Dietary fibre from whole grains and their benefits on metabolic health. *Nutrients*, *12(10)*, 3045. DOI: https://doi.org/10.3390/nu12103045.

Prema, P., Smila, D., Palavesam, A., & Immanuel, G. (2010). Production and characterization of an antifungal compound (3-Phenyllactic Acid) produced by *Lactobacillus plantarum* strain. *Food and Bioprocess Technology, 3(3)*, 379–386. DOI: https://doi.org/10.1007/s11947-008-0127-1.

Quinto, E.J., Jiménez, P., Caro, I., Tejero, J., Javier Mateo, J., & Girbés, T. (2014). Probiotic lactic acid bacteria: A review. *Food and Nutrition Sciences*, 5(18), 1765-1775. DOI: https://dx.doi.org/10.4236/fns.2014.518190.

Rajendran, S. (2003). Grain storage: Perspectives and problems. In: A., Chakraverty, A. S., Majumdar, G. S. V., Raghavan, & H. S., Ramaswamy (Eds.). *Handbook of post-harvest technology* (pp. 183-214). USA: Marcel and Dekker Inc.

Raza, S., Khalil, S., Naseem, K., Gilani, M.A., Amjad, M., Masud, T., & Naqv, S. M. S. (2010). Effect of house hold storage receptacles on physico-chemical characteristics of wheat. *Sarhad Journal of Agriculture,* 26 (2), 275-287.

Rebolleda, S., González-San José, M. L., Sanz, M. T., Beltrán, S., & Solaesa, A. G. (2020). Bioactive compounds of a wheat bran oily extract obtained with supercritical carbon dioxide. *Foods*, 9(5), 625. DOI: https://doi.org/10.3390/foods9050625.

Rehman, Z. U., & Shah, W. H. (1999). Biochemical changes in wheat during storage at three temperatures. *Plant Foods for Human Nutrition*, 54 (2), 109-117. DOI: https://doi.org/10.1023/A:1008178101991.

Relina, L. I., Suprun, O. H., Boguslavsky, R. L., Didenko, S. Y., Vecherska, L. A., & Golik, O. V. (2020). Fatty acid composition of oil from grain of some tetraploid wheat species. *Biotechnologia Acta,* 13(2), 56-64. DOI: https://doi.org/10.15407/biotech13.02.056.

Righetti, L., Bhandari, D. R., Rolli, E., Tortorella, S., Bruni, R., Dall'Asta, C., & Spengler, B. (2021). Mycotoxin uptake in wheat-eavesdropping *Fusarium* presence for priming plant defenses or a trojan horse to weaken them? *Frontiers in Plant Science*, 12, 711389. DOI: https://doi.org/10.3389/fpls.2021.711389.

Rodriguez, J., Bartosik, R., Cardoso, L., & Crocce, D. (2008). Factors affecting carbon dioxide concentration in interstitial air of wheat stored in hermetic plastic bags (Silo-bag). *8th International Conference on Controlled Atmosphere and Fumigation in Stored Products* -Chengdu, China.

Rouse, S., Harnett, D., Vaughan, A., & Van Sinderen, D. (2008). Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology*, 104(3), 915-923. DOI: https://doi.org/10.1111/j.1365-2672.2007.03619.x.

Roy, U., Batish, V. K., Grover, S., & Neelakantan, S. (1996). Production of antifungal substance by *Lactococcus lactis* subsp. *lactis* CHD-28.3. *International Journal of Food Microbiology*, 32(1-2), 27-34. DOI: https://doi.org/10.1016/0168-1605 (96)01101-4.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis,* 10(6), 1003-1008. DOI:

https://dx.doi.org/10.1093/carcin/10.6.1003.

Ruibal-Mendieta, N. L., Rozenberg, R., Delacroix, D. L., Petitjean, G., Dekeyser, A., Baccelli, C., Marques, C., Delzenne, N. M., Meurens, M., & Habib-Jiwan, J. L. (2004). Spelt (*Triticum spelta* L.) and winter wheat (*Triticum aestivum* L.) whole meals have similar sterol profiles, as determined by quantitative liquid chromatography and mass spectrometry analysis. *Journal of Agricultural and Food Chemistry*, 52(15), 4802-4807. DOI: https://doi.org/10.1021/jf049772v.

Ruiz, L. M., Libedinsky, A., & Elorza, A. A. (2021). Role of copper on mitochondrial function and metabolism. *Frontiers in Molecular Biosciences*, 8, 711227. DOI: https://doi.org/10.3389/fmolb.2021.711227.

Sadiq, F. A., Yan, B. W., Tian, F. W., Zhao, J. X., Zhang, H., & Chen, W. (2019). Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), 1403–1436. DOI: https://doi.org/10.1111/1541-4337.12481.

Said, P. P., & Pradhan, R. C. (2014). Grain storage practices: A review. *Journal of Grain Processing and Storage*, 1(1), 1-5.

Salar, R. K., Certik, M., & Brezova, V. (2012). Modulation of phenolic content and antioxidant activity of maize by solid state fermentation with Thamnidium elegans CCF 1456. *Biotechnology and Bioprocess Engineering,* 17, 109–116. DOI: https://doi.org/10.1007/s12257-011-0455-2.

Salas, M. L., Thierry, A., Lemaitre, M., Garric, G., Harel-Oger, M., Chatel, M., Le S., Mounier, J., Valence, F., & Coton, E. (2018). Antifungal activity of lactic acid bacteria combinations in dairy mimicking models and their potentials as bioprotective cultures in pilot scale applications. *Frontiers in Microbiology*, 9, 1-18. DOI: https://doi.org/10.3389/fmicb.2018.01787.

Sanchez-Bragado, R., Molero, G., Reynolds, M. P., & Araus, J. L. (2014). Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ δ13C. *Journal of Experimental Botany*, 65(18), 5401–5413. DOI: https://doi.org/10.1093/jxb/eru298.

Sandhu, K. S., Punia, S., & Kaur, M. (2016). Effect of duration of solid-state fermentation by *Aspergillus awamorinakazawa* on antioxidant properties of wheat cultivars. *LWT-Food Science and Technology*, 71, 323-328. DOI: https://dx.doi.org/10.1016/j.lwt.2016.04.008.

Şanlier, N., Gökcen, B. B., & Sezgin, A. C. (2019). Health benefits of fermented foods. *Critical Reviews in Food Science and Nutrition,* 59(3), 506-527. DOI: https://dx.doi.org/10.1080/10408398.2017.1383355.

Sathe, S. J., Nawani, N. N., Dhakephalkar, P. K., & Kapadnis, B. P. (2007). Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. *Journal of Applied Microbiology*, 103, 2622-2628.

Sawant, A., Patil, S., Kalse, S., & Thakor, N. (2012). Effect of temperature, relative humidity and moisture content on germination percentage of wheat stored in different storage structures. *Agricultural Engineering International: CIGR Journal*, 14(2), 110-118.

Schillinger, U., Holzapfel, W. H., & Björkroth, K. J. (2006). Lactic acid bacteria. In: C. W., Blackburn (Ed.). *Food spoilage microorganisms* (1st ed., pp. 541-578). Cambridge, United Kingdom: Woodhead Publishing Limited.

Semple, R. L. (1992). Post-harvest technology: storage development and application in developing Asian countries. In: R. L., Semple, P.A., Hicks, J. V., Lozare, & A., Castermans (Eds.). *Grain Storage Systems in Selected Asian Countries* (pp. 9-59). REAPASIA.

Serna-Saldivar, S. O. (2012). *Cereal Grains: Laboratory reference and procedures manuel*. Food preservation technology. CRC Press.

Sevgi, E., & Tsveteslava, I. (2015). Antifungal activity of lactic acid bacteria, isolated from Bulgarian wheat and rye flour. *Journal of Life Sciences,* 9, 1-6. DOI: https://doi.org/10.17265/1934-7391/2015.01.001.

Shehata, M. G., Badr, A. N., El Sohaimy, S. A., Asker, D., & Awad, T. S. (2019). Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. *Annals of Agricultural Sciences*, 64(1), 71-78. DOI: https://doi.org/10.1016/j.aoas.2019.05.002.

Shekib, L. A. (1994). Nutritional improvement of lentils, chick pea, rice and wheat by natural fermentation. *Plant Foods for Human Nutrition*, 46, 201-205.

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: an elastic protein from wheat grain. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 357(1418), 133–142. DOI: https://doi.org/10.1098/rstb.2001.1024.

Shibanuma, K., Takeda, Y., Hizukuri, S., & Shibata, S. (1994). Molecular-structures of some wheat starches. *Carbohydrate Polymers*, 25(2), 111-116. DOI: https://doi.org/10.1016/0144-8617(94)90146-5.

Shongwe, S. G., Kidane, S. W., Shelembe, J. S., & Nkambule, T. P. (2022). Dough rheology and physicochemical and sensory properties of wheat–peanut composite flour bread. *Legume Science*, e138. DOI: https://doi.org/10.1002/leg3.138.

Siddiquee, S. (2018). Recent advancements on the role of biologically active secondary metabolites from *Aspergillus.* In: V. K., Gupta, & S., Rodriguez-Couto (Eds.). *New and future developments in microbial biotechnology and bioengineering* (pp. 69-94). Elsevier. DOI: https://doi.org/10.1016/B978-0-444-63501-3.00004-1.

Sieuwerts, S. (2016). Microbial interactions in the yoghurt consortium: Current status and product implications. *SOJ Microbiology & Infectious Diseases*, 4(2), 1–5. DOI: http://dx.doi.org/10.15226/sojmid/4/2/00150.

Singh, B., Sharma, H. K., & Sarkar, B. C. (2012). Optimization of extraction of antioxidants from wheat bran (*Triticum* spp.) using response surface methodology. *Journal of Food Science and Technology*, 49(3), 294–308. DOI: https://doi.org/10.1007/s13197-011-0276-5.

Sj̈ovall, O., Virtalaine, T., Lapvetel̈ainen, A., & Kallio, H. (2000). Development of rancidity in wheat germ analyzed by head space gas chromatography and sensory analysis. *Journal of Agricultural and Food Chemistry*, 48(8), 3522-3527. DOI: https://doi.org/10.1021/jf981309t.

Southgate, D. A. T. (1991). *Determination of Food Carbohydrates*. London: Elsevier Applied Science.

Spaggiari, M., Ricci, A., Calani, L., Bresciani, L., Neviani, E., Dall'Asta, C., Lazzi, C., & Galaverna, G. (2020). Solid state lactic acid fermentation: A strategy to improve wheat bran functionality. *Lebensmittel-Wissenschaft+Technologie,* 118, 108668. DOI: https://doi.org/10.1016/j.lwt.2019.108668.

Šramkováa, Z., Gregováb, E., & Šturdíka, E. (2009). Chemical composition and nutritional quality of wheat grain. *Acta Chimica Slovaca*, 2(1), 115-138.

Srivastava, A., Akoh, C. C., Yi, W., Fischer, J., & Krewer, G. (2007). Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. *Journal of Agricultural and Food Chemistry*, 55(7), 2705–2713. DOI: https://doi.org/10.1021/jf062914w.

Srivastava, R. P., Sanjeev, K., & Kumar, S. (2002). *Fruit and vegetable preservation principles and practices* (3rd ed.). International Book Distribution Co.

Stanton, C., Sanders, D., Krämer, U., & Podar, D. (2022). Zinc in plants: Integrating homeostasis and biofortification. *Molecular Plant*, 15(1), 65-85. DOI: https://doi.org/10.1016/j.molp.2021.12.008.

Stathers, T. E., Arnold, S. E. J., Rumney, C. J., & Hopson, C. (2020). Measuring the nutritional cost of insect infestation of stored maize and cowpea. *Food Security*, 12, 285–308. DOI:https://doi.org/10.1007/s12571-019-00997-w.

Stepien, A., Wojtkowiak, K., Pietrzak-Fiecko, R., Zalewska, M., & Grzywinska-Rapca, M. (2019). Effect of manganese and nitrogen fertilization on the content of some essential micronutrients and composition of fatty acids in winter wheat grain. *Chilean Journal of Agricultural Research,* 79(4), 616-627. DOI: https://dx.doi.org/10.4067/S0718-58392019000400616.

Stepien, L. (2020). *Fusarium*: mycotoxins, taxonomy, pathogenicity. *Microorganisms*, 8(9), 1404. DOI: https/doi.org/10.3390/microorganisms8091404.

Stojiljkovi´c, D., Arsi´c, I., & Tadi´c, V. (2016). Extracts of wild apple fruit (*Malus sylvestris* (L.) Mill., *Rosaceae*) as a source of antioxidant substances for use in production of nutraceuticals and cosmeceuticals. *Industrial Crops and Products,* 80, 165-176. DOI: http://dx.doi.org/10.1016/j.indcrop.2015.11.023.

Stone, B., & Morell, M. K. (2009). Carbohydrates. In: K., Khan, & P. R., Shewry (Eds.). *Wheat chemistry and technology* (4th ed., pp. 299-362). American Association of Cereal Chemists, St Paul, MN. DOI: https://doi.org/10.1094/9781891127557.fm.

Ström, K., Sjögren, J., Broberg, A., & Schnürer, J. (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo (L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. *Applied and Environmental Microbiology*, 68(9), 4322-4327. DOI: https://doi.org/10.1128/AEM.68.9.4322-4327.2002.

Studer, J. M., Schweer, W., Gabler, N., & Ross, J. W. (2022). Functions of manganese in reproduction. *Animal Reproduction Science*, 238, 106924. DOI: https://doi.org/ 10.1016/j.anireprosci.2022.106924.

Talbi, H., Boumaza, A., El-mostafa, K., Talbi, J., & Hilali, A. (2015). Evaluation of antioxidant activity and physico-chemical composition of methanolic and aqueous extracts of *Nigella sativa L. Materials and Environmental Science,* 6(4), 1111-1117.

Tamang, J. P. (2014). Biochemical and modern identification techniques. Microfloras of fermented foods. In: C. A., Batt, & M. L., Tortorello (Eds.). *Encyclopedia of Food Microbiology,* (2nd ed., pp, 250-258). Academic Press.

Tangyu, M., Muller, J., Bolten, C. J., & Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied Microbiology and Biotechnology,* 103, 9263-9275. DOI: https://dx.doi.org/10.1007/s00253-019-10175-9.

Thiele, C., Grassi, S., & Ganzle, M. (2004). Gluten hydrolysis and depolymerization during sourdough fermentation. *Journal of Agriculture and Food chemistry*, 52, 1307-1314.

Thrane, U. (2014). *Fusarium*. In: C., Batt, & P., Patel (Eds.). *Encyclopedia of food microbiology* (2nd ed., pp. 76-81). Elsevier. DOI: https://doi.org/10.1016/B978-0-12-384730-0.00141-5.

Todorov, S. D. (2009). Bacteriocins from *Lactobacillus plantarum* – Production, genetic organization and mode of action. *Brazilian Journal of Microbiology*, 40(2), 209-221. DOI: https://doi.org/ 10.1590/S1517-83822009000200001.

Topcu, A., Bulat, T., Wishah, R., & BoyacI, I. H. (2010). Detoxification of aflatoxin B1 and patulin by *Enterococcus faecium* strains. *International journal of food microbiology,* 139(3), 102-205. DOI: https://doi.org/10.1016/j.ijfoodmicro.2010.03.006.

Tutelyan, V. A. (2004). Deoxynivalenol in cereals in Russia. *Toxicology Letters*, 153(1), 173-9. DOI: https://doi.org/ doi: 10.1016/j.toxlet.2004.04.042.

Udofia, P. G., Udoudo, P. J., & Eyen, N. O. (2013). Sensory evaluation of wheat-cassava-soybean composite flour (WCS) bread by the mixture experiment design. *African Journal of Food Science*, 7(10), 368-374. DOI: https://doi.org/10.5897/AJFS09.108.

Udoh, J. M., Cardwell, K. F., & Ikotun, T. (2000). Storage structure and aflatoxin content in five agroecological zones of Nigeria. *Journal of Stored Products Protection*, 36(2), 187-201. DOI: https://doi.org/10.1016/S0022-474X(99)00042-9.

Varga, J., Baranyi, N., Chandrasekaran, M., Vágvölgyi, C., & Kocsubé, S. (2015). Mycotoxin producers in the *Aspergillus* genus: an update. *Acta Biologica Szegediensis*, 59(2), 151-167.

Vida Plavec, T., & Berlec, A. (2020). Safety aspects of genetically modified lactic acid bacteria. *Microorganisms,* 8(2), 297. DOI: http://dx.doi.org/10.3390/microorganisms8020297.

Viéra-Dalodé, G., Jespersen, L., Hounhouigan, J., Moller, P. L., Nago, C. M., & Jakobsen, M. (2007). Lactic acid bacteria and yeasts associated with gowé production from sorghum in Bénin. *Journal of Applied Microbiology,* 103(2), 342-349.

Vignola, M. B., Moiraghi, M., Salvucci, E., Baroni, M., & Perez, G. T. (2016). Whole meal and white flour from Argentine wheat genotypes: Mineral and arabinoxylan differences. *Journal of Cereal Science*, 71, 217–223. DOI: https://doi.org/10.1016/j.jcs.2016.09.002.

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Varga, J., Yaguchi, T., & Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium. Studies in Mycology*, 78, 343–371. DOI: https://doi.org/10.1016/j.simyco.2014.09.001.

Visconti, A., Pascale, M. (2010). An overview on *Fusarium* mycotoxins in the durum wheat pasta production chain. *Cereal Chemistry*, 87(1), 21–27. DOI: https://doi.org/10.1094/CCHEM-87-1-0021.

Wang, P., Yuan, Y., Xu, K., Zhong, H., Yang, Y., Jin, S., Yang, K., & Qi, X. (2021). Biological applications of copper-containing materials. *Bioactive Materials*, 6(4), 916-927. DOI: https://doi.org/10.1016/j.bioactmat.2020.09.017.

Wang, Y., Compaoré-Sérémé, D., Sawadogo-Lingani, H., Coda, R., Katina, K., & Maina, N. J. (2019). Influence of dextran synthesized in situ on the rheological, technological and nutritional properties of whole grain pearl millet bread. *Food Chemistry*, 285, 221–230. DOI: https://doi.org/10.1016/j.foodchem.2019.01.126.

Weinberg, Z., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G., & Navarro, S. (2008). The effect of moisture level on high-moisture maize (*Zea mays* L.) under hermetic storage conditions - *In vitro* studies. *Journal of Stored Products Research,* 44(2), 136-144. DOI: https://doi.org/10.1016/j.jspr.2007.08.006.

Welinder-Olsson, C., Dotevall, L., Hogevik, H., Jungnelius, R., Trollfors, B., Wahl, M., & Larsson, P. (2007). Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis. *Clinical Microbiology and Infection*, 13(9), 879-86. DOI: https://doi.org/10.1111/j.1469-0691.2007.01756.x.

Wicklow, D. T. (1995). The mycology of stored grain: an ecological perspective. In: D. S., Jayas, N. D. G., White, & W.E., Muir (Eds.). *Stored grain ecosystems*. New York, Marcel Dekker, pp., 197-249.

Wong, M. M. H., Chan, K. Y., & Lo, K. (2022). Manganese exposure and metabolic syndrome: A systematic review and meta-analysis. *Nutrients*, 14, 825. DOI: https://doi.org/10.3390/nu14040825.

Xiang, J., Apea-bah, F. B., Ndolo, V. U., Katundu, M. C., & Beta, T. (2019). Profile of phenolic compounds and antioxidant activity of finger millet varieties. *Food Chemistry,* 275, 361-368. DOI: https://dx.doi.org/10.1016/j.foodchem.2018.09.120.

Xu, R., Sa, R., Jia, J., Li, L., Wang, X., & Liu, G. (2021). Screening of antifungal lactic acid bacteria as bioprotective cultures in yogurts and whey beverage. *Journal of Food Protection*, 84, 953-61. DOI: https://doi.org/10.4315/JFP-20-441.

Yin, G., Zhang, Y., Pennerman, K. K., Wu, G., Hua, S. S. T., Yu, J., Jurick, W. M., Guo, A., & Bennett, J. W. (2017). Characterization of blue mold *Penicillium* species isolated from stored fruits using multiple highly conserved loci. *Journal of Fungi (Basel)*, 3(1), 12. DOI: https://doi.org/10.3390/jof3010012.

Yin, Z., Wu, W., Sun, C., Lei, Z., Chen, H., Liu, H., Chen, W., Ma, J., Min, T., Zhang, M., & Wu, H. (2018). Comparison of releasing bound phenolic acids from wheat bran by fermentation of three *Aspergillus* species. *International Journal of Food Science and Technology,* 53(5), 1120–1130. DOI: https://doi.org/10.1111/ijfs.13675.

Yoo, J. A., Lim, Y. M., & Yoon, M. A. (2016). Production and antifungal effect of 3-phenyllactic acid (PLA) by lactic acid bacteria. *Journal of Applied Biological Chemistry*, 59(3), 173−178. DOI: https://doi.org/10.3839/jabc.2016.032.

Yu, L. L. (2007). *Wheat antioxidants*. Amazon, France: John Wiley & Sons.

Yu, L., Perret, J., Harris, M., Wilson, J., & Haley, S. (2003). Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *Journal of Agricultural and Food Chemistry*, 51(6), 1566–1570. DOI: https://doi.org/10.1021/jf020950z.

Zacharof, M. P., & Lovitt, R. W. (2012). Bacteriocins produced by lactic acid bacteria: A review article. *APCBEE Procedia*, 2, 50–56. DOI: https://doi.org/10.1016/j.apcbee.2012.06.010.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), 129–144. DOI: https://doi.org/10.1016/j.jscs.2010.06.006.

Zarroug, Y., Mejri, J., Dhawefi, N., Ali, S. B. S., El Felah, M., & Hassouna, M. (2015). Comparison of chemical composition of two durum wheat (*Triticum durum* L.) and bread wheat (*Triticum aestivum* L.) germ oils. *Ekin Journal of Crop Breeding and Genetics*, 1(1), 69–76.

Zavala-López, M., & García-Lara, S. (2017). An improved microscale method for extraction of phenolic acids from maize. *Plant Methods*, 13, 81. DOI: https://doi.org/10.1186/s13007-017-0235-x.

Zebboudj, N., Yezli, W., Hamini-Kadar, N., & Kihal, M. (2020). Antifungal activity of lactic acid bacteria against *Fusarium* species responsible for tomato crown and root rots. *Environmental and Experimental Biology*, 18, 7-13. DOI: http://doi.org/10.22364/eeb.18.02.

Zhang, C., Zheng, B., & He, Y. (2022). Improving grain yield via promotion of kernel weight in high yielding winter wheat genotypes. *Biology,* 11(1), 42. DOI: https://doi.org/10.3390/biology11010042.

Zhang, Y., Li, C., & Ma, X. (2014). Experiment and numerical simulation of layer resistance parameters in dryer. *Chinese Society of Agricultural Machinery*, 45(7), 216–221. DOI: 10.6041/j.issn.1000-1298.2014.07.034.

Zhang, Y., Truzzi, F., D'Amen, E., & Dinelli, G. (2021). Effect of storage conditions and time on the polyphenol content of wheat flours. *Processes,* 9(2), 248. DOI: https://doi.org/10.3390/pr9020248.

Zhang, Z., Lv, G., Pan, H., Fan, L., Soccol, C. R., & Pandey, A. (2012). Production of powerful antioxidant supplements via solid-state fermentation of wheat (*Triticum aestivum* Linn) by *Cordyceps militaris*. *Food Technology Biotechnology,* 50(1), 32-93.

Zhao, J., Cheng, T. Xu, W., Han, X., Zhang, J., Zhang, H., Wang, C., Fanning, S., & Li, F. (2021). Natural co-occurrence of multi-mycotoxins in unprocessed wheat grains from China. *Food Control*, 130, 108321. DOI: https://doi.org/10.1016/j.foodcont.2021.108321.

Zhao, J., Wang, S., Zhong, W., Yang, B., Sun, L., & Zheng, Y. (2016). Oxidative stress in the trabecular meshwork (Review). *International Journal of Molecular Medicine,* 38, 995-1002. DOI: http://dx.doi.org/10.3892/ijmm.2016.2714.

Zhao, S., Hao, X., Yang, F., Wang, Y., Fan, X., & Wang, Y. (2022). Antifungal activity of *Lactobacillus plantarum* ZZUA493 and its application to extend the shelf life of Chinese steamed buns. *Foods*, 11(2), 195. DOI: https://doi.org/10.3390/ foods11020195.

Zhao, Y., Zhang, J., Wei, Y., Ali, L., Ying, D., & Xiao, X. (2020). Improvement of bread quality by adding wheat germ fermented with *Lactobacillus plantarum* dy-1. *Journal of Food Quality*, 2020(14), 1-8. DOI: https://doi.org/10.1155/2020/9348951.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B, Mattarelli, P., Toole, P. W. O., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70(4), 2782-2858. DOI: http://dx.doi.org/10.1099/ijsem.0.004107.

Zhou, Y., Jiang, Z., Lu, H., Xu, Z., Tong, R., Shi, J., & Jia, G. (2019). Recent advances of natural polyphenols activators for Keap1-Nrf2 signaling pathway. *Chemistry and Biodiversity,* 16(11), e1900400. DOI: https://dx.doi.org/10.1002/cbdv.201900400.

Zhu, K. X., Zhou, H. M., & Qian, H.F. (2006). Proteins extracted from defatted wheat germ: Nutritional and structural properties. *Cereal Chemistry*, 83(1), 69–75. DOI: https://doi.org/10.1094/CC-83-0069.

Žilić, S., Šukalović, V., Dodig, D., Maksimović, V., Maksimović, M., & Basić, Z. (2011). Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, 54(3), 417-424. DOI: https://doi.org/10.1016/j.jcs.2011.08.006.

Appendixes

1. PCR conditions

PCR reaction mixture contained 25 μl of master mix (1.25 U Taq DNA Polymerase (Solis Biodyne, Estonia), 3 μl of DNA template, 5 μl of each primer and made up to 50 μl reaction volume with distilled H_2O. The PCR runs were as follows: Initial denaturation at 94°C (2minutes), denaturation at 94°C (1 minute), annealing at 55°C (1 minute), and extension at 72°C (1 minute). The amplification was repeated in 30 cycles followed by a final extension at 72°C (7 minutes). PCR were carried out using a thermocycler (icycler Bio-Rad, USA).

The DNA concentrations were checked using Nanodrop Spectrophotometer (NanoDropTM 2000, USA).

2. *Lactiplantibacillus plantarum* **LS011 sequence**

>S11_27f_1   (26 .. 1214 = 1189 bp) *Lactiplantibacillus plantarum* strain JCM 1149 16S ribosomal RNA, partial sequence

AGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGT
GGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTG
GAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGAT
GGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAA
CGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGG
GACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAAT
GGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAA
AACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTAT
TTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGT
GCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAA
GAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAG
TATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGC
TAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGC
CTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGAC
ATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT

The traditional fermented wheat: quality and selection of lactic acid bacteria strains with antifungal activity

By: Nabila BENHAMADA

Abstract

The aim of this study can be summarized in three points: Firstly, the nutritional quality of traditionally fermented wheat (locally named: Mzeyet) was determined, followed by an assessment of the organoleptic acceptance of the bread produced from composite traditionally fermented wheat flour. Secondly, the impact of natural fermentation on its antioxidant properties was also conducted. Finally, 32 lactic acid bacteria strains were isolated and assessed for their antifungal activity against *Aspergillus niger* and *Aspergillus flavus*.

The results showed that samples of traditionally fermented wheat are more or less rich in proteins (4.59%), crude fibers (1%), fat (11.29%), polyphenols (10.48 mg AGE/g) and flavonoids (7.99 mg QE/g). They also show interesting antioxidant activity. Furthermore, the bread made with a mixture of 10% traditional fermented wheat flour was highly appreciated and had the best sensory qualities. The total phenolic and total flavonoid contents were significantly increased in fermented wheat. Moreover, the antioxidant activity was more effective in fermented wheat. Also, the results showed that the majority of LAB strains presented interesting antifungal activity and the most potent strain, identified as *Lactiplantibacillus plantarum* LS011, had the highest activity against the fungal species. Moreover, the partial characterization of the antifungal compounds suggests that they are organic acids.

Key words: Traditionally fermented wheat, quality, bread, antioxidant activity, lactic acid bacteria, *Lactiplantibacillus plantarum*, *Aspergillus*, antifungal activity, biopreservation.

Résumé

L'objectif de cette étude peut être résumé en trois points : Dans un premier temps, la qualité nutritionnelle du blé fermenté traditionnellement (nom local : Mzeyet) a été déterminée, suivie de l'évaluation de l'acceptabilité organoleptique du pain produit à partir de farine composite de blé fermenté traditionnellement. Dans un second temps, l'impact de la fermentation naturelle sur ses propriétés antioxydantes a été mené. Enfin, 32 souches de bactéries lactiques ont été isolées et évaluées pour leur activité antifongique contre *Aspergillus niger* et *Aspergillus flavus*.

Les résultats ont montré que les échantillons de blé traditionnellement fermenté sont plus ou moins riches en protéines (4,59 %), fibres brutes (1 %), lipides (11,29 %), polyphénols (10,48 mg AGE/g) et flavonoïdes (7,99 mg QE/g). Ils montrent également une activité antioxydante intéressante. De plus, le pain fait avec un mélange de 10 % de farine de blé fermenté traditionnel était très apprécié et présentait les meilleures qualités sensorielles. Les teneurs en phénols totaux et en flavonoïdes totaux étaient significativement augmentées dans le blé fermenté. De plus, l'activité antioxydante était plus efficace. Les résultats ont aussi montré que la majorité des souches de bactéries lactiques présentaient une activité antifongique intéressante et que la souche la plus puissante identifiée comme *Lactiplantibacillus plantarum* LS011 avait l'activité la plus élevée contre les espèces fongiques. En fin, la caractérisation partielle des composés antifongiques suggère qu'il s'agit d'acides organiques.

Mots clés : Blé traditionnellement fermenté, qualité, pain, activité antioxydante, bactéries lactiques, *Lactiplantibacillus plantarum, Aspergillus*, activité antifongique, bioconservation.

ملخص

يمكن تلخيص الهدف من هذه الدراسة في ثلاث نقاط: أولاً، تم تحديد الجودة الغذائية للقمح المخمر تقليديًا (المسمى محليًا: المزيت)، متبوعًا بتقييم القبول الحسي للخبز المنتج من دقيق القمح المركب المخمر تقليديًا. ثانياً، تم إجراء تأثير التخمر الطبيعي على خصائصه المضادة للأكسدة. أخيرًا ، تم عزل 32 سلالة من البكتيريا اللبنية وتقييم نشاطها المضاد للفطريات ضد *Aspergillus niger* و *Aspergillus flavus*.

أظهرت النتائج أن عينات القمح المخمر تقليديًا غنية بالبروتينات (4.59٪) والألياف الخام (1٪) والدهون (11.29٪) والبوليفينول (10.48 مجم AGE / جم) والفلافونويد (7.99 مجم QE / جم). كما أنها أظهرت نشاطًا مثيرًا للاهتمام كمضاد للأكسدة. علاوة على ذلك، فإن الخبز المصنوع من خليط 10٪ من دقيق القمح المخمر التقليدي كان محل تقدير كبير ولديه أفضل الصفات الحسية ، كما زاد محتوى الفينول الكلي والفلافونويد الكلي في القمح المخمر بشكل ملحوظ. علاوة على ذلك ، كان النشاط المضاد للأكسدة أكثر فعالية في القمح المخمر. أظهرت النتائج أيضًا أن غالبية سلالات البكتيريا اللبنية أظهرت نشاطًا مضادًا للفطريات مثيرًا للاهتمام وأن السلالة الأكثر فاعلية التي تم تحديدها على أنها LS011 *Lactiplantibacillus plantarum* كان لها أعلى نشاط ضد الأنواع الفطرية. و في الأخير، يوحي الوصف الجزئي للمركبات المضادة للفطريات بأنها أحماض عضوية.

الكلمات المفتاحية: قمح مخمر تقليديًا ، جودة ، خبز ، نشاط مضاد للأكسدة ، بكتيريا لبنية ، *Lactiplantibacillus plantarum* ، *Aspergillus* ، نشاط مضاد للفطريات ، حفظ حيوي.