UNIVERSITY OF CAPE COAST

# EFFECTS OF TWO-STEP FERMENTATION ON PROTEIN ENHANCEMENT AND NUTRITIONAL COMPOSITION OF RICE (*Oryza sativa* L.) AND WHEAT (*Triticum aestivum* L.) USING *Lactobacillus bulgaricus* AND *Saccharomyces cerevisiae*

BERNICE SARSAH

2023

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BY

BERNICE SARSAH

Thesis submitted to the Department of Molecular Biology and Biotechnology, School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Molecular Biology and Biotechnology

NOVEMBER 2023

# DECLARATION

## Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature: Date:……..

Name: Bernice Sarsah

## Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the University of Cape Coast.

Supervisor's Signature: Date:……..

Name: Dr. Levi Yafetto

## ABSTRACT

The effects of two-step fermentation on the protein content and nutritional composition of rice and wheat and their formulated blends (1:1, 1:3, and 3:1) have been studied *in vivo*. The rice and wheat substrate and their formulated blends were subjected to two treatments; (i) fermentation with mono-cultures of *S. cerevisiae* and *L. bulgaricus* and (ii) two-step fermentation for up to 10 days at 25 °C. After 10 days of fermentation, the protein content increased in all substrate. Fermentation with *S. cerevisiae* only and *L. bulgaricus* only increased the protein content varying from 22.56 % - 77.50 %, whereas, two-step fermentation increased the protein content varying from 43.48 % - 86.67 %. Therefore, two-step fermentation produced a synergistic effect in increasing the protein content in all substrate. The ash content increased in all substrates while carbohydrate content reduced. These findings show that employing a solid-state fermentation technique using *S. cerevisiae* and *L. bulgaricus* either as mono-culture or in two-step fermentation, could effectively enhance the protein contents and the nutritional composition of rice and wheat and their formulated blends for possible use as weaning and supplementary food for infants, adults and the aged. Results obtained in this study are being reported for the first time for rice and wheat and their amendments in a solid-state fermentation using *S. cerevisiae* and *L. bulgaricus* singly or in reverse order to improve nutritional status of grains and cereals in Ghana.

## ACKNOWLEDGEMENTS

My profound gratitude goes to my noble supervisor, Dr. Levi Yafetto, for his guidance, and words of encouragement throughout my studies. My sincere gratitude goes to Mr. Stephen Adu of the School of Agriculture, University of Cape Coast. A big thank you also goes to my family and Rev. Eddie Ebo Annan for their support and for making this new chapter of my life possible. Finally, thank you to all the laboratory technicians of the Department of Molecular Biology and Biotechnology.

## DEDICATION

To my parents, Mr. Jude Sarsah and Mrs. Cecilia Kowfie, and Rev. Eddie Ebo Annan.

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# CHAPTER ONE

# INTRODUCTION

## 1.0 Background to the Study

One of humankind's earliest traditional food processing method, fermentation, dates back to 10,000 BC (Wang et al., 2016b). Food is transformed and preserved throughout the fermentation process using common microorganisms, bacteria, yeasts, and fungi. The fermentation-based metabolic processes of these microorganisms contribute significantly to the addition of proteins, vital amino acids, fatty acids, and vitamins to the dietary substrate (Soni and Dey, 2014). These metabolic processes increase food digestibility, improve the sensory qualities and bioactive chemicals, and detoxify anti-nutritional components in food (Simango, 1997). Since ancient times, fermentation processes have been utilized by people to enhance flavor, preserve food, and get rid of toxins (Marco et al., 2017). Most foods, including cereals, legumes, vegetables, dairy, nuts, fruits, meat, seed, and fish are processed and preserved by fermentation. Presently, fermented food products are widely patronized by people due to their health benefits (Bell et al., 2017).

## 1.1 Introduction

Cereals are edible grains that belong to the grass family Gramineae and are a main source of nutrition in both developed and developing countries (McKevith, 2004). More than 73.5 % of the world's harvested cropland is made

up of cereals, and they contribute more than 60 % of the world's food (Sandhu et al., 2017). The key crops that provide about 90 % of the world's cereal grain production are wheat (28 %), corn (27 %), rice (25 %), and barley (10 %), whilst sorghum, oats, millet, and rye together only make up about 10 % of the total (Chavan and Kadam, 1989). More than 80 % of the cereal grains in the world are produced in Asia, America, Africa, and Europe (Chavan and Kadam, 1989). Asia produces a vast amount of rice, wheat, sorghum, and millet; America produces corn and sorghum; Africa produces sorghum, pearl millet, finger millet, teff, African rice, maize, and wheat; and Europe produces barley, oats, and rye (McKevith, 2004). However, the use of these food grains varies between developed and developing countries; in developed countries, over 70 % of total cereal production is utilized as animal feed, while in developing countries, human consumption accounts for 68 to 98 % of cereal production (Betschart, 1982).

Cereals and cereal products are widely cultivated and used in Africa and Asia, providing more food energy globally than any other food crop. The most common grains consumed worldwide include rice, wheat, oats, millet, sorghum, barley, etc. These foods provide the body with the energy and nutrients it needs for daily nutrition, growth, and maintenance. Cereals provide a variety of nutrients, including proteins, carbohydrates, dietary fiber, vitamins, and minerals (Gani et al., 2012). Cereals, which account for 50 % of the world's energy supply, contain approximately 75 % carbohydrates and 6-15 % proteins (Laskowski et al., 2019). Based on nutritional value and consumption level, researchers frequently examine the function of cereal and cereal products (Papanikolaou and Fulgoni,

2017). Cereal products such as bread, ready-to-eat cereals, flour, rolls, pastries, porridge, and biscuits are well-known sources of proteins, dietary fiber, lipids, vitamins, and minerals (Rubio et al., 2009; Yamada et al., 2014). Cereals also contain chemical substances known as phytochemicals, usually referred to as bioactive compounds. These compounds have antioxidant properties and are a good source of nutrients that promote human health. Phytochemicals are mostly present in the bran and germ of cereals and, as a result, a large quantity is found in whole grains than in refined grains where the bran and germ have been removed by milling (McIntosh, 2001). A few examples of phytochemicals are phenolic acid, carotenoids, phytosterols, ferulic acid, flavonoids, phytic acid, anthocyanins, proanthocyanidins, etc (McIntosh, 2001).

Furthermore, cereals contain a variety of anti-nutritional factors (ANFs) that prevent the absorption of minerals including iodine, iron, calcium, and zinc as well as other nutrients like proteins (Popova and Mihaylova, 2019). ANFs decrease the bioavailability, digestion, and utilization of nutrients and may have other adverse effects on the human body (Samtiya, Aluko, and Dhewa, 2020). Some common symptoms of a high level of ANFs in the body include nutrient deficiencies, nausea, headache, bloating, allergies, and rashes (Khokhar and Apenten, 2001). Some ANFs found in cereals are tannins, protease inhibitors, saponins, phytic acid, polyphenol, amylase inhibitors, trypsin, lectins, gossypol, goitrogens, etc. (Nadeem et al., 2010).

The nutritional value of cereals has been improved using a variety of techniques. These comprise genetic engineering, the fortification of amino acids,

mutational breeding, conventional plant breeding, nutrigenomics, the fortification of minerals, etc. (Jansen, 1974). Additionally, heating, sprouting, grinding, and fermentation are among the processing techniques used to improve the nutritional contents of cereals. In the Orient and Far East Asia, legumes are frequently processed using sprouting and fermenting techniques (Chavan and Kadam, 1989). In the case of cereals, sprouting and fermentation processes are uncommon (Tsafrakidou et al., 2020). Numerous studies have recently demonstrated that microbial activities of bacteria, yeasts, and other fungi during fermentation modify the bioactive chemicals, nutrient composition, and ANFs in the grain, thereby altering product properties such as digestibility, bioactivity, and bioavailability (Nambi et al., 2017). Additionally, fermentation modifies the product's flavor, aroma, texture, and organoleptic qualities. Cereals processed with solid-state fermentation (SSF) have a higher nutritional profile with improved organoleptic (sensory) properties (Verni et al., 2019).

SSF is a simple and low-cost technique that involves the introduction of microorganisms (bacteria, yeasts, and fungi) onto a solid matrix or substrate (cereals, legumes, tuber, agro-industrial wastes) in the absence or near absence of free-flowing aqueous phase (Rani et al., 2009). Microbial growth and metabolism in SSF are influenced by the substrate’s moisture content (Krishna, 2005). Additionally, the solid matrix gives the microorganism nutrient and acts as an anchor (Couto and Sanromán, 2006). SSF has made significant advancements in the field of research and has broadened the focus of numerous researchers studying the conversion of crops and leftover residues into value-added products.

SSF has aided in the production of beneficial products such as biofuels, chemicals, enzymes, single-cell proteins, food, pharmaceuticals, biofertilizers, and animal feeds that benefit both humans and livestock (Pandey, 2003). Furthermore, SSF has created new opportunities for bioprocessing including bioleaching, bio-beneficiation, and bioremediation, especially in the mining industry. There are numerous advantages to SSF over other fermentation processes such as submerged fermentation (SmF). Because of the low moisture content of the substrate in SSF, the rate of microbial growth is high, with low microbial contamination (Ghadi et al., 2011). Also, SSF processes and equipment involved are less complex and less expensive, product yield is high, it's eco-friendly and it delivers a high yield of enzymes, etc. (Nambi et al., 2017).

Fermented foods often contain a variety of microorganisms, some of which may be beneficial (such as those that preserve food) and others that may be detrimental (those that cause human diseases and food spoilage) to the human system (Doyle et al., 2013). Food-grade microorganisms (FGMs) are bacteria and yeasts that are utilized in industrial food fermentation processes to produce value-added products. FGMs are essential for boosting the nutraceutical content (food ingredient with therapeutic or health benefits) and nutrients of raw agricultural food through fermentation processes (Hugenholtz and Smid, 2002). These microorganisms are non-pathogenic and non-toxic, and have the status “Generally Recognized as Safe” (GRAS), and, therefore, are used to fortify foods (Lakshmi et al., 2019). Examples include *Saccharomyces cerevisiae* and Lactic acid bacteria (LAB).

LAB are industrially important microbes that are found in a wide range of industrial food fermentation processes around the world. They primarily belong to the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Hugenholtz and Smid, 2002). The most commonly and widely used LAB species is *Lactobacillus bulgaricus*. The most significant feature in the metabolism of *L. bulgaricus* is the efficient carbohydrate fermentation coupled with phosphorylation at the substrate level (Castellone et al., 2021). *L. bulgaricus* is responsible for the breaking down of carbohydrates into lactic acid as the product. *L. bulgaricus* is used to produce fermented foods with improved nutrients, desirable appearance, texture, flavor, and long shelf life (Hugenholtz and Smid, 2002). Some examples of commercially fermented foods obtained via the processes of *L. bulgaricus* fermentation include dairy products (yogurt, kefir, vili, sour cream, cheese, dried milk, cultured butter), meat products (sucuk, summer sausage, pepperoni, fuet, lup cheong, salchichon) (Keenan, 2016), vegetable products (pickles, olives, kimchi, sauerkraut) (Snyder et al., 2020), sea foods and bakery products (Castellone et al., 2021). Food preservation by *L. bulgaricus* involves the production of an antimicrobial compound, bacteriocin, which acts as a growth inhibitor to food spoilage bacteria (Patel and Shah, 2014). *L. bulgaricus* is a probiotic microorganism that provides numerous health benefits. Some health advantages of probiotic microorganisms include protection against intestinal pathogens (*Campylobacter*, *Shigella*, *Vibrio*, *Salmonella*, *Escherichia*), improving digestion, stimulating the gut immune

system, and optimizing intestinal peristalsis (Sanlier et al., 2017). *L. bulgaricus* is well known for its fermentation process in yogurt and fermented milk products. Due to the health benefits obtained from these fermented milk products, it has led to the utilization of *L. bulgaricus* to ferment other kinds of foods such as cereals, fruits, legumes, etc. (Doyle et al., 2013).

Baker's yeast, *Saccharomyces cerevisiae*, is a species of yeast in the Family Saccharomycetaceae. It is an important part of human civilization due to its widespread application in food and beverage fermentation, where it has high commercial relevance (Parapouli et al., 2020). An outstanding property of *S. cerevisiae* is its ability to convert sugars into carbon dioxide and alcohol using enzymes during fermentation (Walker and Stewart, 2016). Bread, cheese, coffee, chocolate, cider, beers, wines, spirits, and industrial alcohol are all products of yeast fermentation (Reis et al., 2013). Additionally, *S. cerevisiae* is sold commercially as a food product which serves as the primary source of yeast extract and nutritional yeast. It is well-known among vegans and vegetarians as an ingredient in cheese substitutes or as a general dietary supplement serving as a source of vitamins and minerals, particularly amino acids and vitamin B-complex. In the baking industry, *S. cerevisiae* serves as a leavening agent through the generation of carbon dioxide during fermentation. Also, *S. cerevisiae* plays a crucial role in the fermented beverages industry. Some examples of fermented beverages are beer, wine, whisky, rum, gin, brandies, vodka, tequila, cheese whey-derived beverages, kefir, soda, lemonades, etc. (Walker and Stewart, 2016).

Yeast fermentation also preserves and improves the organoleptic properties of the final product (Kelesidis and Pothoulakis, 2011).

In developing countries, the decline in quality and quantity of crops from harvesting to storage and consumption has been the main cause of loss in agricultural food crops. This has led to food insecurity and the rise in malnutrition among children, adults, and the aged in less developed countries such as Ghana (Batool et al., 2015). The modern biotechnological technique has been employed by researchers to decrease the loss of agricultural food crops, thereby increasing the availability of food and improving the nutritional quality (Verni et al., 2019). SSF of agricultural food crops has broadened the scope of many researchers. However, cereal grains such as rice and wheat have not been well explored in terms of their nutritional quality after it has gone through SSF processes (Kasote et al., 2021). The combinations of these rice and wheat fermented with *L. bulgaricus,* and *S. cerevisiae* are not well known in Ghana. This study, therefore, investigates the effects of two-step fermentation on protein enhancement and nutritional composition of rice and wheat using *L. bulgaricus* and *S. cerevisiae*.

## 1.2 Statement of the Problem

Poor diet affects human health and well-being, and food is an important aspect of human life. In developing countries, malnutrition and undernutrition are major health problems that contribute to infant and maternal mortality (Adepoju and Allen, 2019). Insufficient intake of nutritious food has a negative impact on the physical and intellectual development of infants and also lowers resistance to

diseases among infants, pregnant women, adolescents, and the aged (Batool et al., 2015). It is estimated that more than 460 million people in the world are severely malnourished (Chavan and Kadam, 1989). This is a result of inadequate feeding, consumption of foods with low energy and nutrient density, low bioavailability of nutrients, poor access to food, use of poor processing methods, and microbial contamination (Mugula and Lyimo, 2000). One of the major challenges facing humanity is the global eradication of malnutrition (Lidon, 2018); malnutrition causes over 60 % of deaths in developing nations (Ritche and Roser, 2017). There are widespread problems posed by malnutrition among infants and other vulnerable groups in developing countries of the world, especially protein-energy deficiency (PED) (Temba et al., 2016).

Whole grains (cereals that contain endosperm, germ, and bran) are rich in proteins, carbohydrates, vitamins, minerals, oils, proteins, and fat. When cereals are processed (refined grain), brans and germs are removed leaving the endosperm which is mostly rich in carbohydrates but low in vitamins, minerals, fats, oils, and protein (Gupta et al., 2017). Refined grains such as rice, wheat, millet, maize, etc. serve as the main food consumed daily in most developing countries and are low in protein, and therefore contribute to PED. This situation may be improved by the use of appropriate and sustainable interventions, which include the use of affordable, bio-enrichment processing techniques, such as fermentation, to improve and enhance the protein content of cereals. To this end, this study is driven by the biotechnological potential of using *S. cerevisiae* and *L. bulgaricus*, through two-step fermentation, to enhance the protein content and

nutritional composition of rice and wheat for possible use as food ration for infants and other vulnerable groups.

## 1.3 Objectives

### 1.3.1 Main objective

The overall objective of this study is to investigate the effects of two-step fermentation on protein enhancement and nutritional composition of rice and wheat using *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*.

### 1.3.1 Specific objectives

Specifically, the study aims to

1. Determine the nutritional composition of rice substrate before and after fermentation by (i) mono-cultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) a two-step fermentation process by the same isolates.
2. Determine the nutritional composition of wheat substrate before and after fermentation by (i) mono-cultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) a two-step fermentation process by the same isolates.
3. Determine the nutritional composition of formulated blends (1:1, 1:3, and 3:1) of rice and wheat substrates before and after fermentation by (i) mono-cultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) a two-step fermentation process by the same isolates.

### 1.4 Hypotheses

1. *S. cerevisiae* enhances protein content and nutritional composition in rice and wheat substrates and their formulated blends after fermentation.
2. *L. bulgaricus* enhances protein content and nutritional composition in rice and wheat substrates and their formulated blends after fermentation.
3. The use of *S. cerevisiae* as the first fermenter in a two-step fermentation with *L. bulgaricus* enhances the protein content and nutritional composition of rice and wheat substrates and their formulated blends.
4. The use of *L. bulgaricus* as the first fermenter in a two-step fermentation with *S. cerevisiae* enhances the protein content and nutritional composition of rice and wheat substrates and their formulated blends.

### 1.5 Significance of the Study

Cereals and cereal-based products are very popular and consumed by children and adults in developing countries. In Ghana, rice and wheat serve as daily sustenance and weaning food for infants, adults, and other vulnerable groups. However, the nutritional content of cereals consumed by infants and other vulnerable groups is usually low and has led to the rise in malnutrition in most developing countries such as Ghana. Utilizing fermentation biotechnology techniques will help improve the nutritional content of rice and wheat. This study, therefore, aims to improve the protein content and nutritional composition of rice and wheat with fermentation technique.

### 1.6 Organization of the Study

The work is divided into Five Chapters. The Introduction, the background of the study, the statement of the problem, the objectives, the hypotheses, and the significance of the study are covered in Chapter One. Chapter Two includes literature relevant to the study. These are research works on rice, wheat, fermentation processes (LAB and Yeast), the empirical review of related results, and conclusions that will help discuss and draw conclusions on the current study. Chapter Three deals with the research methods used. It includes sample collection and preparation, laboratory preparation of microbiological media, culturing of microorganisms, subculturing for new isolates, preparation of inoculum suspension, fermentation process, and proximate analysis. Chapter Four focuses on the results and interpretation of data from the research. Chapter Five outlines the findings, suggestions, and conclusions drawn on the key findings of the study.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

Rice, wheat, rye, oats, barley, maize, triticale, millet, and sorghum are grown in different countries around the globe. Wheat, maize, and rice are the world's most important crops, accounting for over 50 % of global cereal production (McKevith, 2004). Wheat and rice are cultivated predominantly in Africa and Asia, serving essentially as the primary food source for human consumption and nutrient intake (Kushi et al., 1999). Raw unprocessed rice and wheat are always richer in nutrients than processed rice because they have extra nutrients such as proteins, amino acids, fat, vitamins, and minerals. A low-protein diet is detrimental to human health, well-being, and development. Therefore, there is a need to apply a biotechnological approach using microorganisms (LAB and yeasts) under fermentation conditions to improve the nutritional content, sensory properties, and functional properties of rice and wheat.

## 2.2 Rice

Rice (*Oryza sativa* L.) is an edible starchy cereal grain produced by the grass plant in the family Poaceae. Rice is the most important and the third highest-produced food crop in the world after maize and wheat (Shiferaw et al., 2011). Almost 40 % of the world's population consumes rice as a staple food (Fukagawa and Ziska, 2019). Most of the people who depend on rice as their primary food live in less developed countries (Vijay, 2013). In terms of human

nutrition and caloric intake, rice provides more than a fifth of the calories consumed by humans worldwide (Smith, 1998).

### 2.2.1 History of Rice Cultivation

The origin of rice has long been a matter of debate. The plant is so old that the exact time and place of its first evolution may never be known (Sweeney and McCouch, 2007). What is certain, however, is that the domestication of rice represents one of the most important developments in history (Callaway, 2014). Rice has long fed more people than any other crop. The inhabitant of the Korat region of Thailand, specifically non-Nok Tha, discovered engraved grains and husks of the cultivated rice species *Oryza sativa* on a pottery shard (Hirst, 2020). Rice plant remains that date back to 10,000 BC have been found in the Spirit Cave on the border between Thailand and Myanmar (Vijay, 2013). Rice and agricultural tools that are at least 8,000 years old have also been found in China (Maclean et al., 2002). The originally grown rice species was *Oryza rufipogon*. The first domestication took place 9500-6000 years ago in the Yangtse River Basin and the rice species cultivated was *Oryza japonica* (Ma et al., 2018). In the Yangtse River Basin, China, the wet rice field started about 7000 years ago (Deng et al., 2015). Second and third domestication brought rice to Asia, India/Indonesia, and Africa, *Oryza sativa indica* about 4000 years ago, and *Oryza glaberima* about 3200 years ago (Linares, 2002).

Rice comes in various varieties and has a long history of supporting dense populations and civilizations throughout East, South, and Southeast Asia (Higham

and Lu, 1998). Rice fed more people for longer periods than any other crop (Fuller et al., 2016). Rice is documented in history books as a food source and also for traditional use. The cultivation of rice started in China and spread throughout Sri Lanka and India (Muthukumaram, 2022). It was then passed on to Greece and the Mediterranean spreading throughout Southern Europe and parts of North Africa. Rice was brought to North and South America from Europe and then to Brazil from Portugal (Faisca et al., 2021). This grain was brought to many parts of the world because of its versatility. It can grow in the desert conditions of Saudi Arabia and the wetland deltas of Southeast Asia (Talhelm and English, 2020). Therefore, rice, originating in Asian regions during ancient times, has spread its cultivation to encompass every continent worldwide, except Antarctica (Thomas, 1997).

In western Africa, rice is grown as the main staple crop by 10- 15 million people living in coastal areas stretching from Casamance in Senegal to Bandama River in Ivory Coast (Linares, 2002). In addition, rice is an important but not dominant crop in the drier savanna zones from the Senegal River to Lake Chad (Linares, 2002). Rice is now cultivated in Ghana (Upper East, North East, and Volta regions) and Nigeria. Rice is cultivated using these three methods; controlled flooding, upland, and valley-bottom rice (Rodenburg and Johnson, 2009). In the Volta region of Ghana, rice is cultivated in the mountainous areas between Lake Volta and the Togolese border using the upland method of rice cultivation. The rice field stretches between Ho and Nkwanta. Almost all traditional rice grown in the Volta region is the African rice *Oryza glaberrima*

(Adomako, 2018). Both controlled flooding and valley-bottom methods are used in cultivating rice in the Upper East and North East regions of Ghana. Vea, Nasia, Fumbisi, Tono, Yagaba and Nalerigu valleys are the large-scale commercial rice growing areas and these areas are mainly communities in the Upper East and North East regions of Ghana (Kyei and Matsui, 2021). *Oryza glaberrima* is also cultivated and produced in the coastal areas of Guinea Bissau, Guinea, Sierra Leone, Liberia, and the Casamance region in Southern Senegal, as well as from inland areas in Benin, Burkina Faso, Cameroon, Chad, Mali, Niger, Nigeria and Togo (Meyer et al., 2016).

### 2.2.2 Nutrition and Health Benefits of Rice

Rice is cooked by boiling or can be blended into flour. It is eaten alone and also with a variety of soups, side dishes, and main courses in Asian, Middle Eastern, and many other cuisines (Augustyn, 2019). Other products obtained from rice include breakfast cereals, noodles, and alcoholic beverages such as Japanese rice wine and beer. Rice grains and rice products are composed of high energy, calorie content, and high protein content with biological value essential for humans. In recent years, several varieties of rice and rice products have been used in different countries of the world including the USA, China, Indonesia, Japan, Sri Lanka, India, etc. (Verma and Shukla, 2014). Presently, scientists and researchers are introducing new technologies to increase the nutritional value of rice, which will benefit people (Verni et al., 2019). Some of these nutrient compositions in rice (white rice, brown rice, and parboiled rice) include protein, fat, crude fiber,

carbohydrates, ash, minerals, and vitamins (Table 2.1; Anjum et al., 2007). The nutrients contained in rice play an important role in the health and prevention of diseases such as cancer, Alzheimer's disease, hypertension, coronary heart disease, skincare, dysentery, etc. (Verma and Shukla, 2014).

**Table 2.1:** Nutrient composition of rice (mg per 100 g)

| Nutrient Composition | Rice Type | | |
|---|---|---|---|
| | White | Brown | Parboiled |
| Moisture | 12.0 | 12.0 | 10.0 |
| Ash | 0.5 | 1.2 | 0.7 |
| Crude Fibre | 0.3 | 0.9 | 0.2 |
| Fat | 0.4 | 1.9 | 0.3 |
| Calories | 363 | 360 | 369 |
| Carbohydrate | 80.1 | 76.5 | 81.1 |
| Protein | 6.7 | 7.5 | 7.4 |
| **Minerals** | | | |
| Calcium | 24.0 | 32.0 | 60.0 |
| Phosphorus | 94.0 | 221 | 200 |
| Iron | 0.8 | 1.6 | 2.9 |
| Sodium | 6.0 | - | - |
| Potassium | 5.0 | 9.0 | 9.0 |
| **Vitamins** | | | |
| Tocopherol | 0.13 | 29.0 | - |
| Thiamine | 0.07 | 0.34 | 0.44 |
| Niacin | 1.6 | 4.7 | 3.5 |
| Riboflavin | 0.03 | 0.05 | - |
| Folate | 0.02 | 0.05 | - |
| **Fat** | | | |
| Saturated | 0.85 | 0.74 | - |
| Monounsaturated | 0.91 | 0.66 | - |
| Polyunsaturated | 1.29 | 0.98 | - |

Source: (Verma and Shukla, 2014)

### 2.2.3 **Processing and Uses of Rice**

Rice in its unprocessed state (paddy) is encased in the husk or hull. Processes such as dehulling and milling most often remove both the husk and bran of the kernel; sometimes after removing the husk and bran a coating of glucose and talc is applied to give the kernel a polished finish (Ajala and Gana,

2015). Rice processing produces two types of rice; brown rice, and white rice. Brown rice has the husk removed leaving the bran and contains around 8 % protein and a small amount of fat. Brown rice is a good source of vitamins and minerals. White rice, on the other hand, is milled to remove the bran and is very low in nutrients. High consumption of white rice increases the risk of developing Beriberi, which is due to lack of thiamine and minerals (Oghbaei and Prakash, 2016). Another type of rice is parboiled white rice which is partially boiled before milling to preserve most of the nutrients. There is also enriched white rice fortified with iron and vitamin B.

Removal of the husks from paddy fields is mostly done manually with a pestle and mortar, by foot, or using hydropower. The residues left after milling, including the bran and broken rice, are used as feed for cattle. For industrial and food purposes, the bran is compressed through a mechanical screw press under mild heating and then filtered to produce refined functional oil (Müller et al., 2022). Milling broken rice is used to produce rice flour, whose filtrate, after being soaked in water, can be boiled to produce starch. The hulls are used for fuel, packing materials, industrial milling, the manufacture of fertilizers, and the manufacture of an industrial chemical called furfural. The straw is used as cattle feed, bedding for livestock, thatched roofs, mats, clothing, packaging material, and broom straw.

### 2.2.4 Production and Distribution of Rice

Rice is currently grown in over a hundred countries that produce more than 715 million tons of paddy rice annually (Muthayya et al., 2014). Approximately 90 % of the world's rice production comes from fifteen countries, and the combined contribution of China and India accounts for about 50 % of the total rice cultivation globally (Weisskopf et al., 2015). Together with Indonesia, Bangladesh, Vietnam, Myanmar, Thailand, the Philippines, Japan, Pakistan, Cambodia, the Republic of Korea, Nepal and Sri Lanka account for 90 % of the world's total rice production. Other major non-Asian rice-producing countries such as Brazil, the United States, Egypt, Madagascar, and Nigeria, account for 5 % of the rice produced globally. In Africa, rice is the fastest-growing staple food. Total cereal production in Africa has risen steadily from 9.3 % to 15.2 % from 1961 to 2007, respectively. Globally, rice production and yield levels over the years have increased significantly (Figures 2.1 and 2.2).

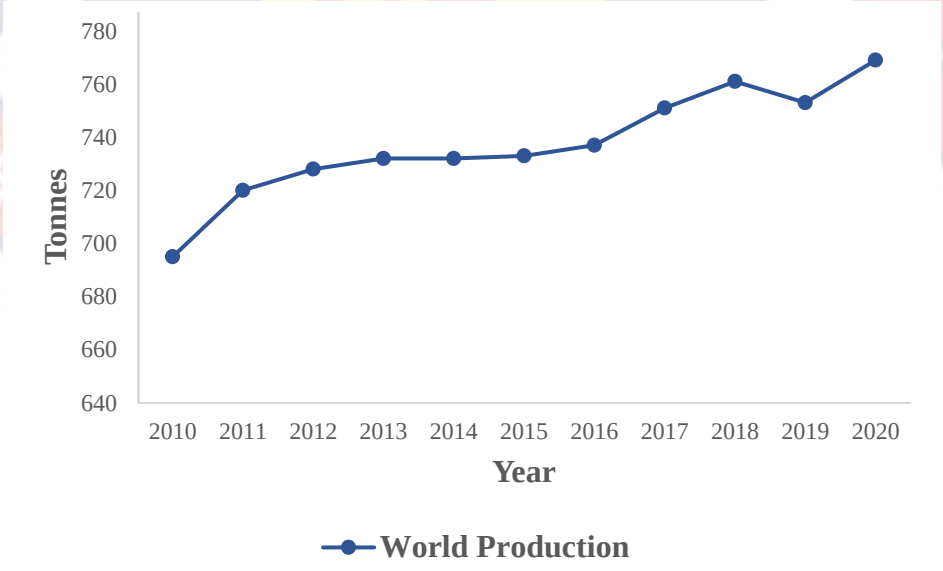

**Figure 2.1:** World production of rice from 2010-2020

Source: (FAOSTAT, 2022)

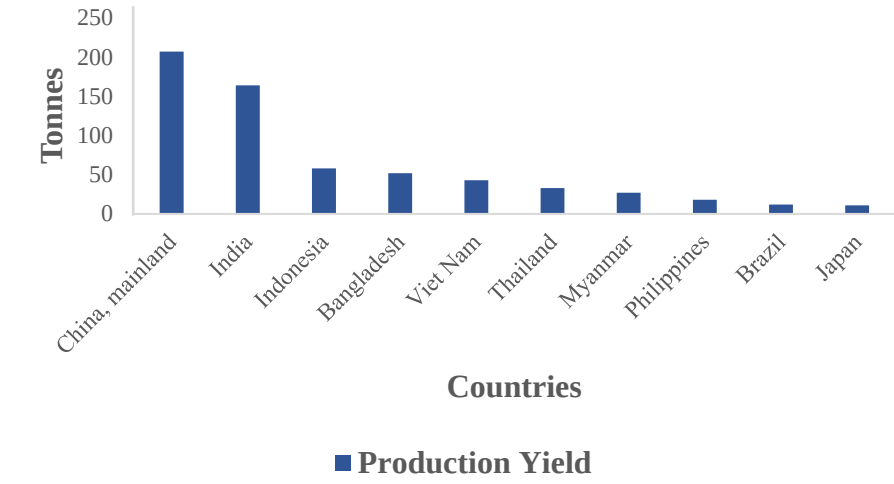


**Figure 2.2:** Top 10 producer countries of rice in the world from 2010-2020

Source: (FAOSTAT, 2022)

**2.3 Wheat**

Wheat is an important grain crop cultivated in many parts of the world including temperate countries, and it is used primarily as food and feed for livestock (Shewry, 2009). Wheat is the second highest-produced crop in the world followed by rice (Tadesse et al., 2015). It belongs to the genus *Triticum*, of which there are many species including *Triticum aestivum* subspecies *vulgare*, the commercially used ones, and *Triticum durum*, the durum wheat (McKevith, 2004).

### 2.3.1 History of Wheat Cultivation

Wheat was first cultivated around 10,000 years ago as part of the Neolithic Revolution also known as the Agricultural Revolution, which marked a transition from hunting and gathering food to settled agriculture (Shewry, 2009). The first wheat species cultivated were einkorn (diploid; genome AA) and emmer (tetraploid; genome AABB), whose genetic relationship indicates that they originated in the south-eastern part of Turkey (Dubcovsky and Dvorak, 2007). Wheat cultivation spread to the Middle East about 9000 years ago when hexaploidy bread wheat first appeared (Feldman, 2001).

### 2.3.2 Nutrition and Health Benefits of Wheat

Wheat is one of the oldest and most essential cereals grown in some parts of the world but can be found all over the world. There are numerous varieties of wheat, but one of the most important is common wheat, botanically known as *Triticum aestivum*. *T. aestivum* makes up most of the wheat flour commercially produced for making bread and is usually grown in arid climates. *T. aestivum* is usually known as the hard variety with a protein content of 11-15 % and also has a strong elastic protein (gluten) (de Sousa et al., 2021). Another wheat variety is *Triticum durum*, commonly known as low-gluten durum wheat. It is specially used to make couscous, burghul, macaroni, and spaghetti. Club wheat, botanically known as *Triticum compactum*, is a soft variety of wheat grown in a moist environment, with a protein content of 8 to 10 % and a weakly elastic protein (gluten). The soft wheat variety is mainly used for the production of household

flour, cakes, cookies, crackers, and pastries (Frisch and Severi, 1995). Other types of wheat are also essential in the food industry for the production of malt, gluten, alcohol, starch, dextrose, etc. The nutrient content of wheat grain varies slightly with climate and soil type. Wheat grain contains moisture, starch, fat, minerals, vitamins, proteins and crude fiber, phenolic compounds (Shewry and Hey, 2015). Table 2.2 shows the nutrient composition of wheat grains.

In infant and adult diets, wheat is widely recommended as a healthy food source that provides a range of nutrients and dietary fiber. Due to the high fiber content in wheat, it is recommended for infants and vulnerable groups to help achieve and maintain a healthy weight. Also, the soluble fiber content in wheat helps reduce the risk of coronary heart disease, lower cholesterol, increase digestion function and improve gut health or bowel function (Shewry and Hey, 2015). Wheat also serves as a natural and enriched nutritional supplement containing minerals, proteins, and bioactive chemicals which help reduce the risk of chronic diseases such as diabetes, hypertension, cardiovascular diseases, renal diseases, and nephrotic syndrome in infants (Shewry and Hey, 2015).

**Table 2.2:** Nutrient composition of wheat grain

| Nutrient Composition (g/ 100 g) | Wheat |
|---|---|
| Moisture | 13.0 |
| Energy | 1574 |
| Fat | 18.0 |
| Fibre | 14.0 |
| Carbohydrate | 82.0 |
| Protein | 14.5 |
| **Minerals (mg/ 100 g)** | |
| Calcium | 33.0 |
| Iron | 3.67 |
| Magnesium | 145 |
| Phosphorus | 331 |
| Sodium | 2.0 |
| Potassium | 417 |
| Zinc | 3.05 |
| Copper | 0.49 |
| Manganese | 4.59 |
| Selenium | 0.08 |
| **Vitamins (mg/ 100 g)** | |
| Vitamin C | 0.00 |
| Thiamine (B1) | 0.34 |
| Riboflavin (B2) | 0.14 |
| Niacin (B3) | 6.28 |
| Pantothenic (B5) | 1.09 |
| Vitamin B6 | 0.34 |
| Folate (B9) | 0.04 |
| Vitamin A | 10.0 |
| Vitamin E | 1.16 |
| Vitamin K | 0.00 |
| Betacarotene | 0.00 |
| **Fats (g/ 100 g)** | |
| Saturated fatty acid | 0.03 |
| Monounsaturated fatty acid | 0.23 |
| Polyunsaturated fatty acid | 0.72 |

Source: (McKevith, 2004)

### 2.3.3 Wheat Processing and its Use

Unprocessed wheat grains, the kernel, is encased in a hull. Soaking and milling processes are used in removing the hull. In the production of wheat flour, the kernel is first cleaned and then soaked to allow the breakdown. The kernel is then dried and pressed, after which it is milled to obtain fine flour. There are two varieties of flour; white flour and Graham flour. Graham flour is obtained from the milling of the whole kernel (bran, germ, and endosperm). Graham flour has a shorter shelf-life. When stored for a longer period, it produces an unpleasant odour and this is a result of the presence of germ oil. White flour, on the other hand, is obtained from the milling of only the endosperm and has an extended shelf life due to the lack of germ oil. The leftovers or residue (hull, bran, germ, and straw) from wheat after processing are used as animal feed, bedding for livestock, fuel, and for maintaining soil fertility and the organic content of soil (Saleem Khan and Mubeen, 2012).

### 2.3.4 Production and Distribution of Wheat

Wheat is cultivated as both a winter and summer crop in many countries around the globe (FAO, 2003). The major wheat-producing countries around the world include the US, China, and Russia. Wheat is also extensively cultivated in India, Pakistan, the European Union (EU), Canada, Argentina, and Australia (Figure 2.4; McKevith, 2004). Africa produces more than 25 million tons of wheat in a production area of 10 million hectares. The major wheat-producing countries in Africa, specifically Sub-Saharan Africa (SSA) include Ethiopia,

South Africa, Sudan, Kenya, Tanzania, Nigeria, Zimbabwe, and Zambia. SSA produced a total of 7.5 million tons on a total area of 2.9 million hectares accounting for 40 % of the wheat production in Africa (Tadesse et al., 2019). Ethiopia accounts for the largest production area (1.7 million hectares) followed by South Africa (0.5 million hectares). The major wheat type produced in SSA is the common wheat and it accounts for 95 % of the wheat production at the global level (Hailu et al., 1989). Wheat production and yield levels over the years have increased significantly (Figure 2.3).

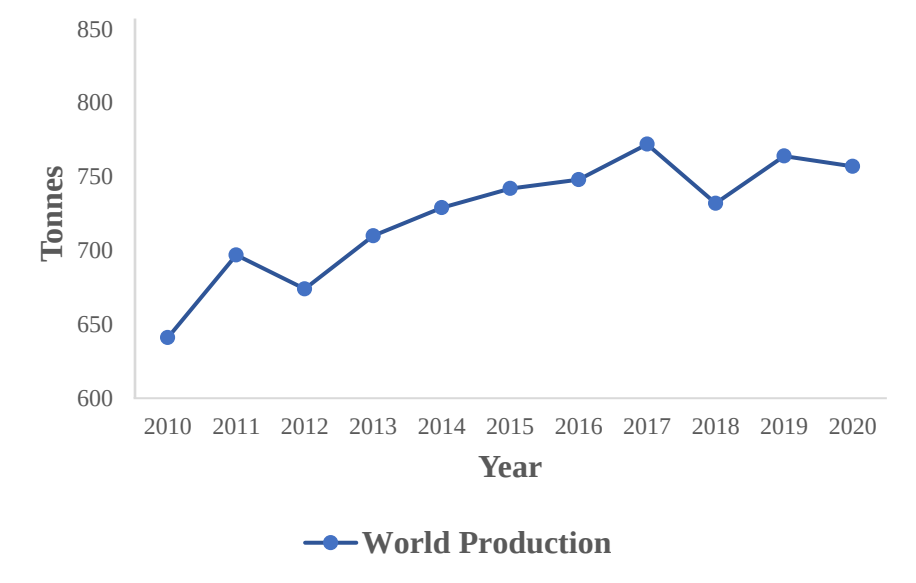


**Figure 2.3:** World production of rice from 2010-2020

Source: (FAOSTAT, 2022)

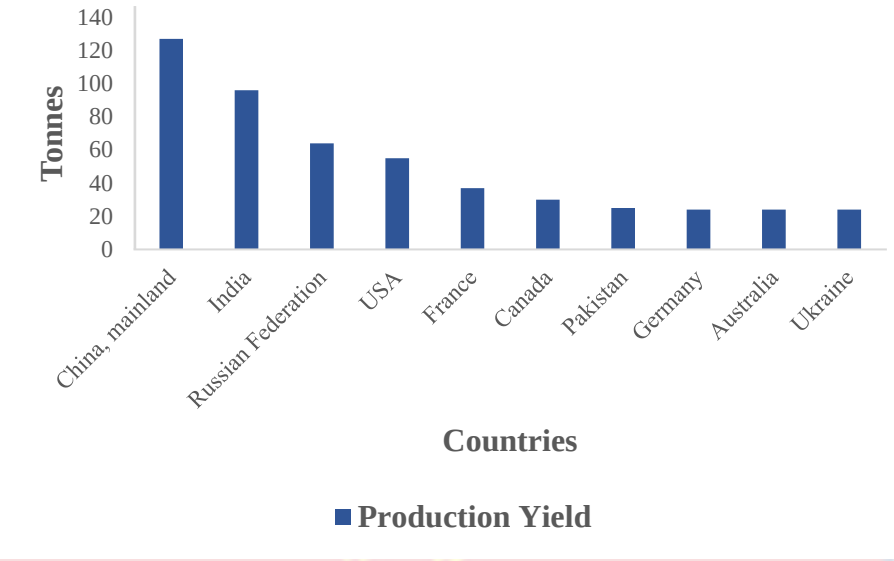


**Figure 2.4:** Top 10 producer countries of wheat in the world from 2010-2020

Source: (FAOSTAT, 2022)

## 2.4 Fermentation

Fermentation is an age-old food processing technique. Since the beginning of human civilization, there has been a close connection between humans, the fermentative activities of microorganisms, and the final value-added products. These fermentative activities by microorganisms have been utilized in the production of fermented foods and beverages, which are defined as those products that have been subjected to the action of microorganisms or enzymes to bring about desirable biochemical changes (Pswarayi and Ganzle, 2022). The earliest records of fermentation appear in the Fertile Crescent and date back to 10,000 before the common era (B.C.E.). However, in the mid-19th century, two events changed the way food fermentations were performed and the understanding of the process (Taveira et al., 2021). The Industrial Revolution initially led to the

concentration of large populations in cities (Doughty, 2013). As a result, food had to be mass-produced, requiring industrialization of the manufacturing process. Also, the advent of microbiology as a field of science in the 1850s provided the biological basis of fermentation, and the process was understood for the first time (Caplice and Fitzgerald, 1999). Since then, the technologies for the industrial production of fermented products for cereals, milk, meat, fruits, vegetables, and legumes have been widely developed and scientific work is actively carried out around the world (Blandino et al., 2003).

Long before the advent of refrigerators, fermentation was used as a means of preserving food and beverages. Locally available raw materials from plant sources such as wheat, rice, maize, millet, sorghum, etc., or animal sources such as fish are fermented either naturally or by adding a starter culture to produce fermented foods and beverages (Steinkraus, 1983). Today, fermented foods and beverages are greatly used and are part of the human diet. Fermented cereal foods and beverages with live and active microorganisms are much more common in Sub-Saharan Africa. The food products form a major component of staple foods, used as a part of weaning diets for infants and nutritional functional foods for children. Fermented cereal products commonly consumed across Africa include Abreh (Sudan), Akasa/Koko (Ghana), Asaana (Ghana), Brukutu (Benin, Ghana, Nigeria), Chikoki vana (Zimbabwe), Dalaki (Nigeria), Fura (Burkina Faso, Ghana, Nigeria), Kunnu (Nigeria), Maasa (Ghana), Mahewu (South Africa and Zimbabwe), Ogi (Nigeria), and Uji (Kenya), etc. Traditional fermented foods and beverages obtained from cereals are also common in Asia. In China, Japan, and

Southeast Asia, fermentation has been used to produce a wheat and soybean product called soy sauce and this dates back as far as 1000 years (Pandey, 1992). In traditional Japanese cuisine, koji rice, steamed rice inoculated with the koji mold, *Aspergillus oryzae* and fermented for just two days, is used to make a variety of products such as alcoholic beverages (brewed sake, amazake, distilled shōchū, and Okinawa-style awamori), spices for cooking (rice vinegar and mirin) and miso, shio koji, and shōyu. Miso has also been traditionally fermented and used as a condiment for cooking in China, Taiwan, the Philippines, and Indonesia (Pandey, 1992). Tables 2.3 and 2.4 show fermented cereal-based products in Africa and fermented cereal-based products in Asia, respectively.

Traditional fermented foods made from cereals are well-known in many parts of the world. Some are used as beverages, colorants, breakfast, and spices, while some of them are used as the main food in a diet. These fermented foods are produced worldwide using different manufacturing techniques, raw materials, and microorganisms. The most commonly used manufacturing technique is fermentation, and it comprises four main types (Soni and Sandhu, 1990). Alcohol fermentation is mainly carried out by yeasts which results in the production of ethanol. Some products obtained from alcohol fermentation include wines, beers, etc. Lactic acid fermentation is mainly carried out by lactic acid bacteria and is involved in the fermentation of milk and cereals. Acetic acid fermentation is mainly carried out by the acetic acid bacteria, *Acetobacter*. *Acetobacter* converts alcohol to acetic acid in the presence of excess oxygen. Some products obtained from acetic acid fermentation include vinegar. Alkaline fermentation often takes

place during the fermentation of fish and seeds, which are mainly used as condiments (McKay and Baldwin, 1990).

**Table 2.3:** Fermented cereal-based products in Africa

| Product | Substrate | Microorganism | Country | References |
|---|---|---|---|---|
| Banku | Maize, Cassava | Lactic Acid Bacteria, Molds | Ghana | (Blandino et al., 2003) |
| Ben-saalga | Millet | *Lactobacillus fermentum, Lactobacillus plantarum, Pediococcus pentosaceus* | Burkina Faso | (Wafula et al., 2022) |
| Bogobe | Sorghum | *Lactobacillus fermentum* | Botswana, South Africa | (Blandino et al., 2003) |
| Bouza | Maize, Millet, Rice, Wheat, | Lactic Acid Bacteria and Yeasts | Egypt | (Blandino et al., 2003) |
| Burukutu | Sorghum | Lactic Acid Bacteria, *Saccharomyces cerevisiae, Saccharomyces chavelier* | Benin, Ghana, Nigeria | (Iwuoha and Eke, 1996) |
| Busaa | Maize | Lactic Acid Bacteria, *Saccharomyces cerevisiae*, *Penicillum damnosus* | Nigeria, Ghana | (Blandino et al., 2003) |
| Dalaki | Millet | | Nigeria | (Onyango et al., 2004) |
| Doro | Finger Millet | Lactic Acid Bacteria and Yeasts | Zimbabwe | (Blandino et al., 2003) |
| Injera | Maize, Sorghum, Tef, Wheat | *Candida guilliermondii* | Ethiopia | (Wafula et al., 2022) |
| Iokubilisa | Maize | Lactic Acid Bacteria, Molds, Yeasts | Zimbabwe | (Blandino et al., 2003) |
| Koko | Maize, Millet | Lactic Acid Bacteria, Yeasts | Ghana | (Blandino et al., 2003) |
| Kunun-zaki | Maize, Millet | | Nigeria | (Iwuoha and Eke, 1996) |
| Mahewu | Maize | *Streptococcus lactis* | South Africa | (Wafula et al., 2022) |
| Mawe | Maize, Millet, Sorghum | Lactic Acid Bacteria and Yeasts | South Africa, Benin | (Blandino et al., 2003) |
| Merissa | Sorghum and Millet | Yeasts | Sudan | (Blandino et al., 2003) |
| Motoho | Sorghum | Lactic Acid Bacteria | South Africa | (Blandino et al., 2003) |
| Mutwiwa | Maize | Lactic Acid Bacteria, Molds | Zimbabwe | (Blandino et al., 2003) |
| Ogi | Maize, Millet, Sorghum | *Candida krusei, Candida tropicalis, Geotrichum candidum, Geotrichum fermentum, Lentilactobacillus buchneri, Lactobacillus cellobiosus, Lactobacillus fermentum, Lactobacillus plantarum, Pedicoccus acidilactici, Pedicoccus pentosaceus, Rhodotorula graminis, Saccharomyces cerevisiae* | Benin, Nigeria | (Iwuoha and Eke, 1996) |
| Pito | Maize, Millet, Sorghum | Lactic Acid Bacteria, Yeasts | Nigeria, Ghana | (Iwuoha and Eke, 1996) |
| Uji | Maize, Millet, Sorghum | *Candida krusei, Candida tropicalis, Geotrichum candidum, Geotrichum fermentum, Lentilactobacillus buchneri, Lactobacillus cellobiosus, Lactobacillus fermentum, Lactobacillus plantarum, Saccharomyces cerevisiae* | Benin, Kenya, Nigeria | (Onyango et al., 2004) |

**Table 2.4:** Fermented cereal-based products in Asia

| Product | Substrate | Microorganism | Country | References |
|---|---|---|---|---|
| Adai | Rice | Lactic Acid Bacteria | India | (Blandino et al., 2003) |
| Anarshe | Rice | Lactic Acid Bacteria | India | (Sathe and Mandal, 2016) |
| Ang-kak | Rice | *Monascus purpureus* | China, Syria, Southern Asia | (Chavan and Kadam, 1989) |
| Bangla maad | Rice | *Lactobacillus plantarum, Leuconostoc mesenteroides, Saccharomyces cerevisiae* | Bangladesh | (Chavan and Kadam, 1989) |
| Bibingka | Rice | | Philippines | (Reddy, 2019) |
| Brem | Rice | | Indonesia | (Reddy, 2019) |
| Dosa | Rice | *Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae* | India | (Reddy, 2019) |
| Idli | Rice | Lactic Acid Bacteria, Molds, and Yeasts | South India, Sri Lanka | (Reddy, 2019) |
| Jaand | Buckwheat, Millet, Rice | Aspergillus oryzae, Lactic Acid Bacteria, Yeasts | Nepal | (Chavan and Kadam, 1989) |
| Jaanr | Millet | *Hansenula anomala, Mucor rouxianus* | India | (Blandino et al., 2003) |
| Jalebies | Wheat | *Saccharomyces bayanus* | India, Nepal, Pakistan | (Reddy, 2019) |
| Kacep | Wheat, Soybean | *Aspergillus oryzae*, Lactic Acid Bcteria, and Yeasts | Indonesia | (Blandino et al., 2003) |
| Khanomjeen | Rice | Lactic Acid Bacteria | Thailand | (Blandino et al., 2003) |
| Khaomak | Rice | Molds | Thailand | (Blandino et al., 2003) |
| Kichudok | Rice, Takju | Yeasts | Korea | (Sathe and Mandal, 2016) |
| Kurdi | Wheat | | India | (Sathe and Mandal, 2016) |
| Mantou | Wheat | Yeasts | China | (Blandino et al., 2003) |
| Me | Rice | Lactic Acid Bacteria | Viet Nam | (Blandino et al., 2003) |
| Puto | Rice, Sugar | *Leuconostoc mesenteroides, Streptococcus faecalis,* Yeasts | Philippines | (Blandino et al., 2003) |
| Sake | Rice | *Aspergillus oryzae, Lactobacillus leichmanii, Lactobacillus plantarum Saccharomyces cerevisiae* | | (Blandino et al., 2003) |
| Selroti | Rice | *Debaryomyces hansenii, Escherichia faecium, Lactobacillus curvatus, Pichia burtonii, Pseudomonas pentosaceuts, Saccharomyces cerevisiae, Saccharomyces kluyveri, Zygosaccharomyces rouxii* | Nepal | (Chavan and Kadam, 1989) |
| Rakshi | Buckwheat, Millet, Rice | Lactic Acid Bacteria, Molds, and Yeasts | Nepal | (Chavan and Kadam, 1989) |

Lactic acid bacteria and yeast strains have been used successfully as starter cultures in several indigenous cereal-based fermented foods because of their desirable effects in such foods (Tables 2.3 and 2.4; Oyewole, 1990). These effects may include the ability to shorten fermentation times, minimize dry matter losses, avoid contamination by pathogenic and toxigenic bacteria and molds, and reduction in the risk of spoilage microorganisms causing off-flavors in foods (FAO, 1999). It has also been reported by several researchers that, the use of isolated strains of LAB and yeast during cereal dough fermentation minimizes dry matter losses, increases acid production or pH reduction, contributes to aroma and flavor formation, improves overall product acceptability, enhances product nutritional value, and producing preservative compounds or reducing mycotoxins (Agarry, Nkama, and Akoma, 2010; Annan et al., 2003; Halm et al., 1996). Common fermenting bacteria are the species *Bacillus, Lactobacillus, Leuconostoc, Micrococcus, Pediococcus,* and *Streptococcus*. The most common genera of fungi found in certain products include *Aspergillus, Cladosporium, Fusarium, Paecilomyces, Penicillium,* and *Trichothecium*. The common fermenting yeasts include *Candida tropicalis, Geotrichum fermentum, Rhodotorula graminis,* and *Saccharomyces cerevisiae*, (Steinkraus, 1998).

According to Adams et al. (2009), increasing the fermentation time in sorghum significantly reduced the number of harmful microorganisms *Escherichia coli, Staphylococcus aureus, Salmonella,* and coliform bacteria. Oyewole (1997) also stated that the fermentation process prevents food spoilage

and food-borne diseases in consumers living in a climate conducive to rapid food spoilage.

Fermented foods are of particular importance to ensuring adequate intake of protein and calories in the diet. Fermentation of corn, millet, and sorghum significantly improved protein and lysine levels (Hamad and Fields, 1979). In contrast, an examination of the nutritional value of sorghum kisra bread showed no increase in lysine content, although tyrosine and methionine levels increase (McKay and Baldwin, 1990). In the same line, it was reported that tryptophan levels increased during uji manufacture, while a significant decrease in lysine levels was measured (Blandino et al., 2003). It appears that the effect of fermentation on the nutritional value of foods varies, although the evidence for improvements is significant (Blandino et al., 2003). Yousif and El Tinay (2000) reported an increase in *in vitro* protein digestibility in maize after 16 hours of fermentation. Cuevas-Rodríguez et al. (2004) also reported an increase in protein content in maize flour from 9.1 to 13.4 g after fermentation. Likewise, Zaid and Taiwo (2008) reported an increase in crude protein (97 %) and a decrease in crude fiber (45 %) after the fermentation of rice husk.

The need for health-promoting foods and foods with improved organoleptic properties is growing worldwide due to increasing consumer awareness of the effect of food on health. Traditional fermented foods made from cereals are widespread in Asia and Africa. Fermenting these grains through traditional methods uses mixed cultures of various beneficial microorganisms known as probiotics (Achi and Asamudo, 2019). Probiotics are known to boost

the immune system, cure psoriasis, and chronic fatigue syndrome, and improve digestion (Zeng et al., 2021). Eating fermented foods can help replenish beneficial bacteria and even fight off pathogenic bacteria in the gut (Wang et al., 2016). Fermentation also results in an overall improvement in the shelf life, texture, taste, and aroma of the end products (Kohajdova and Karovicova, 2007). During the fermentation of grain, several volatile compounds are formed that contribute to a complex blend of flavors in the products (Chavan and Kadam, 1989). The presence of flavors representative of diacetyl acetic acid and butyric acid makes fermented cereal-based products more appealing to taste (Obafemi et al., 2022).

### 2.4.1 Lactic Acid Bacteria (LAB) Fermentation

*Lactobacillus* spp. are the most important bacteria in food production and belong to the group of lactic acid bacteria (LAB) (Aguirre and Collins, 1993). LAB are gram-positive, catalase-negative, cocci, and sporeless rods, normally non-motile microorganisms with a distinctive property such as the production of lactic acid. LAB used for food fermentations belongs to the genera *Lactobacillus, Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus,* and the newly recognized *Carnobacterium*.

According to Hutkins (2006), LAB are divided into three groups based on the utilization of sugars (hexoses), namely homofermentative, heterofermentative, and facultative heterofermentative. Homofermentative genera include *Lactococcus, Pediococcus, Streptococcus* and some *Lactobacillus*. These organisms metabolize hexoses by enzymes of the Embden-Mayerhoff glycolytic

pathway, resulting in more than 90 % of the substrate being converted to lactic acid as the major or only end product during anaerobic metabolism (Wang et al., 2021). Heterofermentative genera include *Leuconostoc* sp., *Weisella* sp., and some *Lactobacilli*. Microorganisms of this group metabolize hexoses via the pentose phosphate pathway (Warburg-Dickens), resulting in the conversion of only 50 % of the substrate to lactic acid, while the rest is metabolized to acetic acid, lactate, $CO_2$, formic acid, and ethanol (Castillo Martinez et al., 2013). Facultative heterofermentative *Lactobacilli* can metabolize hexose by both pathways, with the pentose-phosphate pathway predominating in the absence of fermentable sugars (Aguirre and Collins, 1993; Tamime and O'Connor, 1995).

LAB fermentation is the oldest and most popular means to improve the safety, nutritional value, shelf life, taste, appearance, aroma, and acceptability of a wide range of fermented foods (Halm et al., 1996). Table 2.5 summarizes the LAB species involved in different varieties of fermented products. Holzapfel (1997) described LAB as organisms that contribute to the improvement of nutrient levels, taste, aroma, texture, appearance, and shelf-life of fermented food products.

**Table 2.5:** LAB species involved in food fermentation

| Food product | LAB species | References |
|---|---|---|
| **Dairy** | | |
| Cheese | *Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus lactis, Lactococcus lactis, Lactococcus cremoris, Leuconostoc cremoris, Streptococcus thermophilus* | (Raj et al., 2022) |
| Yogurt | *Lactobacillus bulgaricus, Streptococcus thermophilus* | (Raj et al., 2022) |
| Fermented milk | *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus rhamnosus, Streptococcus thermophilus* | (Raj et al., 2022) |
| Yakult | *Lactobacillus casei* | (Raj et al., 2022) |
| Butter and buttermilk | *Lactobacillus brevis, Lactobacillus kefiranofacies, Lactobacillus kefir, Lactobacillus plantarum, Lactococcus lactis, Lactococcus cremoris, Lactococcus lactis, Leuconostoc cremoris* | (Raj et al., 2022) |
| Kefir | *Lactococcus lactis, Lactobacillus paracasei, Leuconostoc mesenteroides* | (Raj et al., 2022) |
| **Meat** | | |
| Dry sausages | *Lactobacillus casei, Lactobacillus curvatus, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus sakei, Pediococcus acidilactici, Pediococcus pentosaceous,* | (McKay and Baldwin, 1990) |
| Salami Milano | *Lactobacillus plantarum, Lactobacillus sakei* | (McKay and Baldwin, 1990) |
| Greek dry fermented sausages | *Lactobacillus curvatus, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus sakei, Lactococcus lactis, Weisella hellenica, Weisella minor, Weisella paramesenteroides, Weisella viridescens* | (McKay and Baldwin, 1990) |
| **Fish** | | |
| Thai fish | *Lactobacillus plantarum, Lactobacillus reuteri* | (McKay and Baldwin, 1990) |
| **Pickled fruits and vegetables** | | |
| Cabbage (Sauerkraut) | *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum, Leuconostoc mesenteroides* | (Castellone et al., 2021) |
| Cucumber | *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus pentosus, Lactobacillus plantarum, Leuconostoc mesenteroides* | (Castellone et al., 2021) |
| Olives | *Lactobacillus brevis, Lactobacillus pentosus, Lactobacillus plantarum* | (Castellone et al., 2021) |
| **Cereals** | | |
| Sourdough | *Lactobacillus alimentarius, Lactobacillus amylovorus, Lactobacillus brevis, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus hilgardii, Lactobacillus panis* | (McKay and Baldwin, 1990) |
| **Bakery** | *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus sanfrancisco* | (McKay and Baldwin, 1990) |
| **Alcoholic beverages** | *Lactobacillus bulgaricus, Leuconostoc oenos* | (McKay and Baldwin, 1990) |
| **Soy sauce** | *Lactobacillus bulgaricus, Pediococcus soyae* | (McKay and Baldwin, 1990) |

The preservative and safety functions of LAB fermentation have been investigated by researchers in some fermented cereal products (Ziarno and Cichońska, 2021). It is known that LAB has an enormous function in bio-preservation as they are safe to eat and naturally dominate the food microflora during storage (Olukoya et al., 1994). The term bio-preservation refers to extended shelf-life and increased food safety using natural microflora and/or their antibacterial products such as organic acids and bacteriocins (Stiles, 1996). The well-known property of LAB related to preservative property is their ability to produce organic acid and bacteriocins, which in turn exhibit antimicrobial activity (Widyastuti et al., 2014). Breidt and Fleming (1997) also demonstrated the ability of *Lactobacillus* species to produce some metabolites such as lactic and acetic acids, which lower pH and inhibit competing bacteria including psychrotropic (15-20 °C) pathogens. The inhibitory properties of fermented foods are often considered for their ability to reduce diarrhoea and/or improve microbial quality and antimicrobial activity *in vitro*. The potential of fermented cereal porridge to reduce the incidence of diarrhoea in young children was demonstrated in Tanzania (Lorri and Svanberg, 1994).

The increase in demand and consumption of LAB-fermented foods due to their nutritional benefits has led to the introduction of lactic acid fermentation in other foods such as cereals, vegetables, and fruits (Doyle et al., 2013). The genus *Lactobacillus* with over a hundred species such as *L. acidophilus, L. bulgaricus*, *L. casei*, *L. helveticus*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, etc. are considered probiotics (Shah, 2007). LAB are very sensitive to many environmental stresses

such as temperature, acidity, and oxygen. LAB can be attach to the intestinal epithelium and colonize the lumen of the intestinal tract, hence, stabilizing the intestinal microbiota, counteract the effects of harmful microorganisms, producing antimicrobial agents, and stimulating the host's immune response (Heller, 2001; Parvez et al., 2006; Soccol et al., 2010). Various health benefits of LAB include protecting against gut pathogens (*Campylobacter*, *Shigella*, *Vibrio*, *Salmonella*, *Escherichia*), improving digestion, stimulating the gut immune system, and optimizing gut peristalsis (Sanlier et al., 2017).

Cereal-based products made from rice, wheat, millet, maize, and sorghum dough contribute to the protein needs of West Africans and are particularly important as weaning foods for children and as staple foods for adults (FAO, 1999). The above-mentioned cereals contain a significant amount of phytate. The phytate affects the bioavailability of vitamins and minerals, resulting in low absorption and utilization of minerals and vitamins, giving rise to nutrient deficiency in children in West African countries such as Burkina Faso, Mali, Niger, Nigeria, and Sierra Leone (Camara and Amaro, 2003). Other anti-nutrients, tannins, and α-galacto-oligosaccharides (α-GOS) such as stachyose and raffinose are important in cereal grains. These anti-nutrients are known to have anticancer, antiviral, anti-inflammatory, and antioxidant properties (Soro-Yao et al., 2014). A phytase, α-galactosidase or tannase-producing lactic acid bacterium is therefore useful during cereal dough fermentation to help reduce the amount of phytate or tannins and metabolize stachyose or raffinose, which have a greater influence on the nutritional quality of cereal grains. LAB fermentation also

provides optimum pH conditions for the enzymatic breakdown of phytate, which is present in cereals in the form of complexes with polyvalent cations such as calcium, proteins, iron, magnesium, and zinc (Coulibaly et al., 2011). Many studies have shown that LAB fermentation improves the nutritional value and sensory properties of fermented food products. Olukoya et al. (1994) demonstrated the potential of "*Dogik*", an improved "*Ogi*" prepared from starter culture strains of *Lactobacilli* isolated from local fermented foods, with strong antibacterial activity to control diarrhoea. Banigo and Muller (1972) reported the ability of a combined inoculum of *Lactobacillus plantarum*, *Lactococcus lactis*, and *Saccharomyces rouxii* to increase the rate of dough fermentation in *Ogi* (a fermented cereal gruel processed from maize) production. A mixed culture of *Lactobacillus* and *Acetobacter* improved the nutritional quality of maize by increasing the concentration of riboflavin and niacin (Akinrele, 1970). Likewise, Teniola and Odunfa (2001) observed a high increase in lysine and methionine levels in cereal porridge inoculated with mixed starter cultures of *S. cerevisiae* and *L. brevis*. A starter culture consisting of LAB (*L. fermentum*, *L. brevis*, and *L. amylovorus*) in combination with *S. cerevisiae* was able to reduce the fermentation time from 19 hours to 14 hours and the pH to 3.47 in traditional fermentation of sorghum flour (Asmahan and Muna, 2009). In a study by Chis et al. (2020) rice flour fermented with *L. spicheri* DSM 15429 increased the amino acid content, mineral, lactic acid, and total phenol and antioxidant activity by 70.75 % and 73.70 %, respectively.

### 2.4.2 Yeast Fermentation

Yeast fermentation is an important part of human civilization due to its widespread application in food and beverage industries, where it has high commercial relevance (Parapouli et al., 2020). An outstanding property of yeasts is their ability to convert sugars into carbon dioxide and alcohol using enzymes in the fermentation process (Walker and Stewart, 2016). The yeasts used to ferment sugar in the manufacture of bread, beers, wines, spirits, and industrial alcohol are all strains of a species, *Saccharomyces cerevisiae* (Reis et al., 2013). Aidoo et al. (2006) reported that a variety of yeasts are involved in traditional fermented foods and play a vital role in the production of these traditional fermented foods (bread, sourdough, kefir, cassava dough, etc.) and beverages (wine, beer, apple cider, etc.) worldwide. Owusu-Kwarteng et al. (2010) also reported that during the fermentation of *Fura* (fermented millet product in Ghana), different types of yeast species were isolated, *Candida tropicalis*, *Galactomyces geotricum*, *Issatchenkia orientalis*, *Pichia anomala*, *S. cerevisiae*, *S. pastorianus*, and *Yarrowia lipolytica.* Likewise, Omemu et al. (2007) reported the yeast species isolated from *Ogi* to include *Geotrichum fermentans*, *Geotrichum candidum*, *Rhodotorula graminis*, *S. cerevisiae*, *Candida krusei*, and *Candida tropicalis*. Tables 2.6 and 2.7 summarize the various yeast species involved in fermented foods and the various yeast species associated with the production of alcoholic beverages, respectively. Ojokoh et al. (2019) also reported that after 72 hours of fermentation of rice-mucuna beans flour, the predominant mold isolated were *Geotrichum candida*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Rhizopus stolonifer*.

Besides LAB, *S. cerevisiae* is a predominant species of yeast involved in food fermentation in Africa, Asia, and other parts of the world (Shetty et al., 2007). The functions of yeasts in fermented cereal foods and beverages have been described by several authors (Lara-Hidalgo et al., 2017). Some of the functions include the production of aromatic compounds through the conversion of carbohydrates into alcohols, esters, organic acids, and carbonyl compounds, inhibition of mycotoxin-producing molds, degradation of mycotoxins, production of tissue-degrading enzymes such as cellulases and pectinases (Jespersen, 2003). *S. cerevisiae* is also well-known for its probiotic properties such as protecting the normal microbiota of the gut and inhibiting the growth of the pathogen in the gut (Osorio-Cadavid., 2008). Other health benefits of *S. cerevisiae* include anti-inflammatory, antioxidant, antidiabetic, immune booster, and anticancer properties (Kohajdova and Karovicova, 2007).

Fermentation by *S. cerevisiae* alters the nutritional level, bioactive substances, and ANFs of fermented products obtained from cereals and other food materials such as nuts, seeds, fruits, vegetables, etc. Ojokoh et al. (2019) reported a significant reduction in tannin, phytate, and oxalate after 72 hours of fermentation of rice and mucuna beans formulated blend. Nicolau et al. (2011) reported that protein content doubled in fermented rice due to the growth of yeast on the surface of the rice substrate. Ojokoh et al. (2019) reported an increase in protein and ash content while carbohydrate, lipid, and fiber were reduced after 72 hours of fermentation of rice-mucuna beans flour. A similar observation was reported by Wang and Fields (1978) where fermentation of corn meal with *S.*

*cerevisiae* and *Candida tropicalis* increased the protein content from 7.6 to 16.4 % and 14.5 %, respectively. Likewise, Chavan et al. (1988) reported a protein increase in sorghum and that of sorghum and green gram formulated blend from 14.9 to 17.0 % within the first 24 hours of fermentation. A study by Khetarpaul and Chauhan (1990) reported that fermentation of pearl millet flour fermented by *S. cerevisiae* significantly increased the protein content to that of flour fermented by *S. diastaticus*, *L. brevis*, and *L. fermentum*. Additionally, Khetarpaul and Chauhan (1990) reported a decrease in the fat content of pearl millet flour fermented with *S. cerevisiae*, *L. brevis*, and *L. fermentum* whereas the flour fermented with *S. diastaticus* had no change in the fat content. Yeast fermentation enhances nutrient digestibility and availability in fermented food. An experiment performed by Khetarpaul and Chauhan (1990) reported that fermentation of pearl millet flour by *S. diastaticus* had the highest starch digestibility whereas fermentation by *S. cerevisiae* significantly increased the *in vitro* protein digestibility of the flour.

Yeast fermentation alters the flavor, aroma, texture, and appearance of fermented products. Many studies have reported the involvement of yeasts in different types of African indigenous fermented foods and beverages. The significance of yeast and moulds occurring in maize dough fermentation for kenkey production has been reported. Jespersen (2003) reported that the number of yeast present and the significant multiplication of the dominant species are likely to influence the organoleptic and structural quality of the dough for Kenkey production. Hamad et al. (1992) found that fermented sorghum with a high

number of yeasts has a more pleasant aroma than dough with less yeast. The presence of yeasts and LAB in the fermentation of other cereal products such as mawe and mahewu has also been reported (Hounhouigan et al., 1993).

**Table 2.6:** Yeast species involved in the different varieties of fermented foods

| Food product | Yeast species | References |
|---|---|---|
| **Cereals** | | |
| Ogi | *Pichia kudriavzevii* | (Lara-Hidalgo et al., 2017) |
| Sourdough | *Candida famata, Candida humilis, Kazanchastania exigua, Saccharomyces cerevisiae, Wickerhamomyces anomalus* | (Kline and Sugihara, 1971) |
| Pozol | *Candida guilliermondii, Debaryomyces hansenii, Geotrichum candidum, Kluyveromyces lactis, Rhodotorula minuta* | (Wacher et al., 2000) |
| **Dairy** | | |
| Kefir | *Kluyveromyces* spp., *Saccharomyces* spp., *Torula* spp., *Williopsis saturnus* var. *saturnus* | (Ahmed et al., 2013) |
| **Vegetables** | | |
| Cassava dough | *Candida ethanolica, Galactomyces geotrichum, Issatchenkia* spp. | (Lara-Hidalgo et al., 2017) |
| Kanji | *Rhodotorula glutinis* | (Lara-Hidalgo et al., 2017) |
| Pulque | *Candida diversa, Kluyveromyces marxianus, Pichia fermentans, Torulaspora delbrueckii* | (Escalante et al., 2004) |
| **Fruits** | | |
| Cocoa | *Hanseniaspora opuntiae, Saccharomyces cerevisiae* | (Papalexandratou and De Vuyst, 2011) |
| Olives | *Candida boidinii, Candida krusei, Candida parapsilosis, Candida rugosa, Debaryomyces hansenii, Kluyveromyces marxianus, Pichia anomala, Pichia membranifaciens, Rhodotorula glutinis, Saccharomyces cerevisiae, Torulaspora delbrueckii* | (Nychas et al., 2002) |
| Tepache | *Hanseniaspora uvarum, Pichia guilliermondii* | (Ahmed et al., 2013) |
| Masau | *Cryptococcus flavus, Hanseniaspora opuntiae, Issatchenkia orientalis, Kluyveromyces marxianus, Pichia fabianii, Rhodotorula mucilaginosa, Saccharomyces cerevisiae, Saccharomycopsis fibuligera* | (Lara-Hidalgo et al., 2017) |
| Chilli pepper | *Cryptococcus* spp., *Dekkera* spp., *Hanseniaspora guilliermondii, Kodamaea ohmeri, Rhodotorula* spp. | (Zhao et al., 2016) |

**Table 2.7:** Yeast species associated with the production of alcoholic beverages

| Food product | Yeast species | References |
|---|---|---|
| **Wine** | | |
| Grape | *Saccharomyces bayanus, Saccharomyces cerevisiae* | (Hutzler et al., 2012) |
| Apple cider | *Saccharomyces bayanus, Saccharomyces cerevisiae, Saccharomyces pastorianus* | (Hutzler et al., 2012) |
| Other fruits | *Saccharomyces cerevisiae* | (Hutzler et al., 2012) |
| **Indigenous fermented** | | |
| Kaffir/sorghum or millet beer | *Saccharomyces cerevisiae* | (Ahmed et al., 2013) |
| Fermented agave juice | *Saccharomyces cerevisiae* | (Lara-Hidalgo et al., 2017) |
| Sake (alcoholic rice beverage) | *Candida sake, Saccharomyces cerevisiae* var. *sake* | (Blandino et al., 2003) |
| Palm wine | *Candida tropicalis, Pichia fermentans, Pichia membranifaciens, Saccharomyces cerevisiae, Schizosaccharomyces pombe* | (Hutzler et al., 2012) |
| Tej (honey wine) | *Saccharomyces cerevisiae* | (Hutzler et al., 2012) |
| Cheese whey-derived beverages | *Kluyveromyces marxianus* | (Blandino et al., 2003) |
| Sugar cane wine | *Saccharomyces cerevisiae, Saccharomycopsis fibuligera* | (Hutzler et al., 2012) |
| **Beer** | | |
| Lager beer (bottom-fermented) | *Saccharomyces pastorianus* | (Walker and Stewart, 2016) |
| Ale (top-fermented) | *Saccharmyces cerevisiae* | (Walker and Stewart, 2016) |
| Lambic beer (top fermented, specialty beers) | *Brettanomyces bruxellensis, Dekkera bruxellensis, Saccharomyces cerevisiae* | (Walker and Stewart, 2016) |
| **Liquor** | | |
| Whisky | *Saccharomyces cerevisiae* | (Walker and Hill, 2016) |
| Rum | *Saccharomyces cerevisiae, Schizosaccharomyces pombe* | (Walker and Stewart, 2016) |
| Tequila, Mezcal, Bacanora | *Brettanomyces* spp., *Kluyveromyces marxianus, Pichia* spp., *Rhodotorula* spp., *Saccharomyces cerevisiae* | (Walker and Stewart, 2016) |
| Brandies, Gin, Vodka | *Saccharomyces cerevisiae* | (Pauley and Maskell, 2017) |

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Overview

This chapter discusses the research methods used for this study. It entails the collection and preparation of rice and wheat grains, isolation of microorganisms, preparation of culture medium, preparation of rice and wheat substrate for fermentation, preparation of inoculum suspension, fermentation treatments, proximate analysis, and data analysis.

## 3.2 Rice and Wheat Samples

Rice and wheat grains were purchased from Kotokuraba market in Cape Coast, Ghana, and brought to the Microbiology Laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast. The grains were pretreated by handpicking debris (stones, remnants of rice husk, dead insects) and were transferred into a well-cleaned tray, after which they were sun-dried for three days and then dried in the oven at 60 °C for 24 hours (Figures 3.1 and 3.2 (a)). The samples were then milled using a JK M20 IKA®-WERKE milling machine to obtain a fine powder and stored in an airtight container for future use (Figures 3.1 and 3.2 (b)).


**Figure 3.1:** Rice grains (a) and rice flour (b)

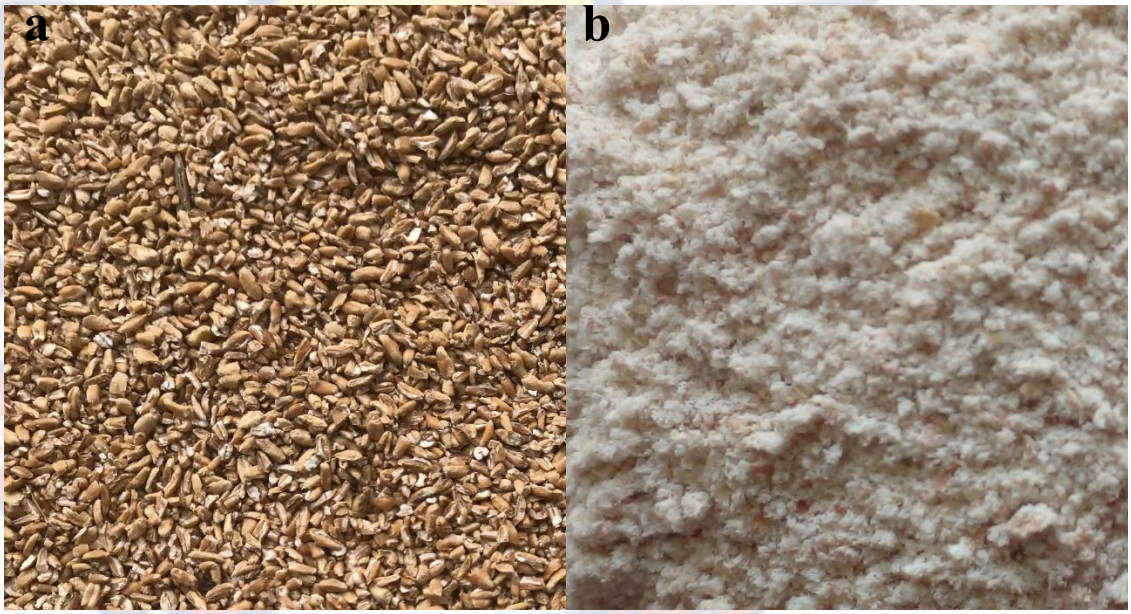


**Figure 3.2:** Wheat grains (a) and wheat flour (b)

### 3.3 *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*

#### 3.3.1 *Saccharomyces cerevisiae*

Commercial dry, saf-instant baker's yeast (S.I. Lesaffre, France) was purchased from a supermarket in Cape Coast, Ghana. The yeast was brought to the Microbiology Laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast, and stored at 25 °C for future use.

#### 3.3.2 *Lactobacillus bulgaricus*

*Lactobacillus bulgaricus* was isolated from a starter culture obtained from the Microbiology Laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast, Ghana. In obtaining pure cultures of *L. bulgaricus*, molten de Man Rogosa, and Sharpe (MRS) agar was poured into sterile Petri dishes and allowed to solidify, after which inoculating loop was flame sterilized by passing it through the flame of a Bunsen burner until was red hot and allowed to cool down. A loop full of *L. bulgaricus* was picked from the starter culture and streaked onto the solidified MRS agar plates, after which the plates were incubated at 25 °C for 24 to 48 hours to allow growth. After 48 hours, pure cultures of *L. bulgaricus* were obtained on MRS agar plates and stored in a refrigerator at + 4 °C for future use (Figure 3.3).

**Figure 3.3:** A pure culture of *Lactobacillus bulgaricus*

### 3.4 Microbiological Media Preparation

#### 3.4.1 de Man Rogosa and Sharpe (MRS) Agar

Sixty-two (62) grams of MRS agar (Oxoid Ltd., England) dehydrated powder (10.0 g Peptone; 8.0 g 'Lab-lemco' powder; 4.0 g Yeast extract; 20.0 g Glucose; 1 ml Sorbitan mono-oleate; 2.0 g Dipotassium hydrogen phosphate; 5.0 g Sodium acetate; 2.0 g Triammonium citrate; 0.2 g Magnesium sulphate $7H_2O$; 0.05 g Magnesium sulphate $4H_2O$; 10.0 g Agar; adjusted to pH 6.2 ± 0.2 at 25 °C) was weighed and transferred into 1000 ml distilled water in a 1 L round bottom flask,

and gently boiled to dissolve completely. The MRS agar medium obtained was dispensed in aliquots of 250 ml into four separate 500 ml Erlenmeyer flasks and sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes. The MRS agar medium was stored for future use.

### 3.4.2 de Man Rogosa and Sharpe (MRS) Broth

Fifty-two (52) grams of MRS broth (Oxoid Ltd., England) dehydrated powder (10.0 g Peptone; 8.0 g 'Lab-lemco' powder; 4.0 g Yeast extract; 20.0 g Glucose; 1 ml Sorbitan mono-oleate; 2.0 g Dipotassium hydrogen phosphate; 5.0 g Sodium acetate; 2.0 g Triammonium citrate; 0.2 g Magnesium sulphate $7H_2O$; 0.05 g Magnesium sulphate $4H_2O$; adjusted to pH 6.2 ± 0.2 at 25 °C) was weighed and transferred into 1000 ml distilled water in 1 L round bottom flask, and gently boiled to dissolve completely. The MRS broth medium was dispensed in aliquots of 250 ml into four separate 500 ml Erlenmeyer flasks and sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes. The MRS broth medium was stored until use.

### 3.4.3 Yeast-Extract Peptone Dextrose (YPD) Broth

Ten (10) grams of yeast extract, 20.0 g peptone, and 20.0 g dextrose (adjusted to pH 6.5 ± 0.2 at 25 °C) was weighed and transferred into 1000 ml of distilled water in a 1 L round bottom flask, and gently boiled to dissolve completely. The YPD broth was dispensed in aliquots of 250 ml into four separate 500 ml Erlenmeyer flasks and sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes. The YPD broth medium was stored for future use.

### 3.5 Preparation of rice or wheat flour for fermentation with monocultures of *S. cerevisiae* and *L. bulgaricus*

**Rice or wheat substrates**

Sixty (60) grams each of milled rice or wheat was weighed and transferred into five separate 500 ml Erlenmeyer flasks and plugged with cotton and aluminium foil. The samples were then labeled appropriately and sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes.

**Formulation of rice and wheat flour blends**

To prepare a 1:1 blend of rice and wheat flour, thirty (30) grams of rice flour and 30 g of wheat flour were weighed and transferred into five separate 500 ml Erlenmeyer flasks. Similarly, 1:3 blend of rice and wheat flour was prepared by transferring fifteen (15) grams of rice flour and 45 g of wheat flour into five separate 500 ml Erlenmeyer. Finally, the 3:1 blend of rice and wheat flour was made by weighing forty-five (45) grams of rice flour and mixing with 15 g of wheat flour transferring into five separate 500 ml Erlenmeyer flasks. After plugging the samples with cotton and aluminium foil, they were shaken for 2 minutes to mix thoroughly and sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes.

### 3.6 Preparation of rice or wheat flour for two-step fermentation treatment

**Rice or wheat substrates**

One hundred and twenty (120) grams each of milled rice or wheat was weighed and transferred into two separate 500 ml Erlenmeyer flasks and plugged with cotton and aluminium foil. The samples were labeled appropriately and left unsterilized.

**Formulation of rice and wheat flour blends**

To prepare a 1:1 blend of rice and wheat flour, sixty (60) grams of rice flour and 60 g of wheat flour were weighed and transferred into two separate 500 ml Erlenmeyer flasks. Likewise, 1:3 blend of rice and wheat flour was prepared by transferring thirty (30) grams of rice flour and 90 g of wheat flour into two separate 500 ml Erlenmeyer flasks. Finally, the 3:1 blend of rice flour and wheat flour was made by weighing ninety (90) grams of rice flour and 30 g of wheat flour into two separate 500 ml Erlenmeyer flasks. The samples were plugged with cotton and aluminium foil and left unsterilized.

### 3.7 Preparation of Inoculum Suspension

#### 3.7.1 *Lactobacillus bulgaricus* inoculum suspension

MRS agar plates with pure cultures of *L. bulgaricus* stored in a refrigerator at +4 °C were obtained from the Microbiology Laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast. The culture plates were allowed to stand for 10 minutes before use. A sterile cork

borer was used to create MRS agar discs. Four discs of MRS agar with *L. bulgaricus* were picked with a sterile inoculating loop and transferred gently into a 250 ml sterilized MRS broth medium. The inoculated MRS broth medium was incubated on a flask shaker at a speed of 150 rpm at 25 °C for 24 hours to allow proliferation and even distribution of bacterium in the MRS broth medium to obtain *L. bulgaricus* inoculum suspension. The *L. bulgaricus* inoculum suspension was stored and used when needed for fermentation experiments.

### 3.7.2 *Saccharomyces cerevisiae* inoculum suspension

Ten (10) grams of commercial dry yeast was weighed and transferred into 1 L sterilized Yeast-Extract Peptone Dextrose (YPD) broth and was swirled gently to dissolve completely. The inoculated YPD broth was incubated on a flask shaker at a speed of 150 rpm at 25 °C for 24 hours to allow proliferation and even distribution of *S. cerevisiae* in the YPD broth medium to obtain inoculum suspension of *S. cerevisiae*. The *S. cerevisiae* inoculum suspension was stored and used for fermentation studies.

## 3.8 Fermentation of rice and wheat substrates and their formulated blends using monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*

This aspect of the research involves the fermentation of rice and wheat substrates and their formulated blends using the following ratios: 1:1, 1:3, and 3:1 (rice: wheat). The substrates were fermented with monocultures of *S. cerevisiae* and *L. bulgaricus* as follows:

## EXPERIMENTAL DESIGN

The methods and procedures employed in these studies was adapted from Hongzhang et al. (2012) and Yafetto et al. (2022).


**Figure 3.4**: Flow diagram of the fermentation treatments of rice and wheat substrate and their formulated blends. The initial % crude protein content and nutritional composition of unfermented substrate were determined at day 0 using the procedures of AOAC, (2005)

Exactly 60 g of milled rice or wheat, 30 g of milled rice and 30 g of milled wheat substrates (1:1 blend), 15 g of milled rice and 45 g of milled wheat substrates (1:3 blend) or 45 g of milled rice and 15 g of milled wheat substrates (3:1 blend), were separately inoculated with 50 ml each of *S. cerevisiae* inoculum suspension.

The processes outlined for *S. cerevisiae* above were repeated for the *Lactobacillus bulgaricus* treatment by inoculating the samples with 50 ml *L. bulgaricus* suspension. All samples were incubated at 25 °C to allow fermentation for 10 days.

### 3.9 Two-step fermentation of rice and wheat substrates and their formulated blends

Two-step fermentation is a process that involves the sequential use of two separate microorganisms in a continuous fermentation process (Hongzhang et al., 2012). The two-step fermentation treatment was carried out as follows:

In the first set of the experiments, *S. cerevisiae* was inoculated onto the substrate to ferment for 5 days after which *L. bulgaricus* was inoculated onto the fermented substrate to ferment for 10 days. This was repeated in the second set of experiments beginning with *L. bulgaricus* followed by *S. cerevisiae*.

**Two-step fermentation treatment using *S. cerevisiae* followed by *L. bulgaricus***

The substrates corresponding to 100 % rice or wheat flour, 50 % rice flour: 50 % wheat flour, 33.3 % rice flour: 66.6 % wheat flour or 66.6 % rice flour: 33.3 %

wheat flour were separately inoculated with 100 ml of inoculum suspension of *S. cerevisiae* only to obtain 50 % moisture and fermented at 25 °C for 5 days. On the 5$^{th}$ day, 60 g of the corresponding fermented substrate was weighed and dried in an oven for three days at 60 °C. The crude protein and the nutritional composition were determined. Then the remaining 60 g of the substrate was sterilized by autoclaving at a pressure of 1.1 kg/cm$^2$ at 121 °C for 15 minutes after which the substrate was allowed to cool down and then inoculated with 50 ml of inoculum suspension of *L. bulgaricus* only to continue fermentation till 10 days.

The processes outlined for *S. cerevisiae* followed by *L bulgaricus* above were repeated for the second set of experiments using *L. bulgaricus* followed by *S. cerevisiae*.

### 3.10 Proximate analysis of fermented rice and wheat substrate and their formulated blends

Fermented rice and wheat substrates and their formulated blends (1:1, 1:3, and 3:1) were transferred from an Erlenmeyer flask onto glass plates and dried in an oven at 60 °C for three days, after which the fermented substrates were milled using a JK M20 IKA®-WERKE milling machine to obtain a fine powder and kept in airtight ziplock bags, and then labeled appropriately. The nutrient composition of unfermented and fermented rice and wheat substrates and their formulated blends were determined in triplicates using analytical procedures by the AOAC, (2005) as follows:

### 3.10.1 Protein determination

The micro-Kjeldahl method AOAC, (2005) was used to determine the crude protein of the substrates. The method involves three steps; digestion, distillation, and titration.

**Digestion process**

Zero-point two (0.2) gram of the sample was weighed and carefully transferred into a 100 ml Kjeldahl flask, 4.5 ml digestion mixture (Selenium powder, Lithium sulphate, 30 % Hydrogen peroxide, and Conc. $H_2SO_4$) was measured using a measuring cylinder, and dispensed into the Kjeldahl flask containing the sample. A blank (digestion mixture without sample) was also prepared. The sample and blank were placed in a digestion chamber to allow digestion at 360 °C for 2 hours, after digestion, the digest was carefully transferred into a 100 ml volumetric flask and made up to the volume by diluting with distilled water.

**Distillation process**

The distillation process was conducted using the steam distillation method. A steam distillation apparatus was set up and the apparatus was flushed with distilled water for 20 minutes. After flushing out the apparatus, 5 ml of boric acid indicator (wine red) was measured into a volumetric flask and placed under the condenser of the distillation apparatus with the tip of the condenser completely immersed in the boric acid solution. Twenty (20) milliliters of the sample digest was introduced into the reaction chamber through the trap funnel. Ten (10) milliliters of NaOH (sodium hydroxide) which served as an alkali

mixture was added to start distillation immediately. Fifty (50) milliliters of the distillate was collected.

**Titration process**

Fifty (50) milliliters of the distillate was titrated against 0.1 N HCl until the solution changed from green to wine red. The % $N_2$ of the substrates was determined by using the following formula:

$$\textbf{\% Nitrogen (\% N)} = \frac{(T-B)\times M \times 14.007 \times 100}{Weight\ of\ sample\ (mg)} \times dilution\ factor$$

Where;

T = sample titre value

B = blank titre value

M = molarity of HCl

The % crude protein of the substrates was determined by using the following formula.

**% Crude Protein** = % N X Conversion Factor

The conversion factor of 5.75 and 5.81 were respectively used for rice and wheat.

The percentage increase in the protein contents of all substrates was calculated using the following formula:

$$\textbf{\% Increase in Protein Content} = \frac{Final-Initial}{Initial} \times 100\ \%$$

### 3.10.2 Moisture determination

Porcelain crucibles were washed, oven-dried, and weighed. Twelve (12) grams of the sample (unfermented and fermented rice and wheat substrate and

their formulated blends) was weighed and transferred into the weighed crucibles. The crucibles with the sample were weighed and the sample was spread to the uniformity over the base of the crucibles to ensure even distribution of heat and placed in a thermostatically controlled oven at 105 °C for 48 hours. After 48 hours of drying the samples were carefully removed from the oven and cooled in a desiccator. After it has cooled, the crucibles with the dried sample were reweighed and the moisture content was determined as the percentage of water loss using the following formula:

$$\textbf{\% Moisture} = \frac{(W1-W2)}{W1} \times 100$$

Where; *w1*= weight of the sample before drying

*w2*= weight of the sample after drying

### 3.10.3 Ash determination

Porcelain crucibles were washed and placed in a furnace at 550 °C overnight to ensure that impurities on the surface of the crucibles are burned off. The crucibles were cooled in the desiccator for 30 minutes and weighed. Twelve (12) grams of the sample was weighed into the crucibles and was heated over a low Bunsen burner flame till there were no fumes produced. The crucibles with samples were then placed in a furnace and heated at 550 °C overnight, the heating continued till all the carbon particles were burnt away. The crucible was removed from the furnace and placed in the desiccator to cool. The crucible with the burnt sample (ash) was weighed and the ash content was determined using the following formula:

$$\textbf{\% Ash} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

### 3.10.4 Fibre determination

One (1) gram of the sample was weighed and transferred into a boiling flask. A 100 ml of 1.25 % $H_2SO_4$ (Sulphuric acid) was measured using a measuring cylinder and transferred into the boiling flask containing the sample and boiled for 30 minutes. After 30 minutes of boiling, the sample was filtered into a labeled glass crucible and the residue was transferred back into the boiling flask. A 100 ml of 1.25 % NaOH (Sodium hydroxide) was measured and transferred into the boiling flask containing the residue and boiled for additional 30 minutes. After boiling, the sample was filtered into a glass crucible and the residue was washed with boiling water and methanol. After washing, the crucible was dried in an oven at 105 °C overnight and weighed afterward. After drying in an oven, the crucible was placed in a furnace at 500 °C for 4 hours. The crucible was then transferred into a desiccator to slowly cool 25 °C and was weighed. The fiber content of the substrates was determined using the following formula:

$$\textbf{\% Fibre} = \frac{Weight\ loss\ through\ ashing}{Weight\ of\ Sample\ (g)} \times 100$$

### 3.10.5 Fat determination

Ten (10) grams of the sample was weighed and transferred into a 50 × 10 mm Soxhlet extraction thimble and placed in a 50 ml capacity Soxhlet extractor. A 250 ml round bottom flask was washed, dried, and weighed, after which 150 ml of Petroleum ether (spirit) was weighed and transferred into the flask. The flask

with Petroleum ether was connected to the Soxhlet extractor and extraction was done for 6 hours using a heating mantle as a source of heat. After 6 hours the flask was removed and placed in an oven at 60 °C for 2 hours. After 2 hours, the round bottom flask was removed and placed in a desiccator to cool. The flask was reweighed and the percentage of fat was determined using the following formula:

$$\textbf{\% Fat} = \frac{W\,(g)}{Weight\ of\ Sample\ (g)} \times 100$$

Where; w = weight of oil

### 3.10.6 Carbohydrate determination

% CHO (percentage carbohydrate) was determined by:

**% Carbohydrate** = 100 - % (Protein + Ash + Moisture + Fibre + Dry Matter + Fat)

### 3.11 Data analysis

All analyses were performed in triplicates. Data obtained were subjected to One-Way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS Statistics, USA). Also, Tukey's multiple comparison test with a confidence interval of 95 % ($P \leq 0.05$) was used to measure the significant difference between the data obtained. Results obtained were presented in means ± standard deviation (SD).

# CHAPTER FOUR

# RESULTS

## 4.1 Overview

This chapter summarizes the key findings of the study. It entails summaries of proximate analyses (protein, ash, carbohydrate, fiber, and fat content) conducted before and after fermentation of rice and wheat substrate and their formulated blend with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process. It also summarizes the percentage increase in the protein contents of all the substrates with their treatments.

## 4.2 Proximate analyses of rice substrate fermented with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) a two-step fermentation process

### 4.2.1 Protein content

Table 4.1 summarizes the data obtained. The initial % crude protein content of unfermented rice substrates was 8.28 ± 0.31 %. Rice substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in % crude protein content at 11.53 ± 0.20 % and

10.78 ± 0.06 %, respectively, after 10 days of fermentation (Table 4.1). Two-step fermentation of the rice substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus*, showed a significant ($p \leq 0.05$) increase in % crude protein content at 14.42 ± 0.31 % after 10 days of fermentation. Similarly, two-step fermentation of rice substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae*, showed a significant ($p \leq 0.05$) increase in % crude protein content at 15.19 ± 0.15 % after 10 days of fermentation.

### 4.2.2 Carbohydrate content

Table 4.1 shows a summary of results obtained. The initial carbohydrate content of unfermented rice substrates was 86.75 ± 0.16 %. Fermentation with *S. cerevisiae* showed a significant ($p \leq 0.05$) decrease to 82.93 ± 0.15 % after 10 days of fermentation. However, the carbohydrate content in the rice substrate fermented with *L. bulgaricus* showed an insignificant ($p \geq 0.05$) decrease at 86.40 ± 0.26 % after 5 days of fermentation and then further significant ($p \leq 0.05$) decrease to 83.69 ± 0.07 % after 10 days fermentation. Two-step fermentation of the rice substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) decrease in carbohydrate content to 79.93 ± 0.25 % after 10 days of fermentation. Similarly, two-step fermentation of the rice substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* showed a significant ($p \leq 0.05$) decrease in carbohydrate content to 78.44 ± 0.20 % after 10 days of fermentation.

### 4.2.3 Ash content

Results are presented in Table 4.1. The initial ash content of unfermented rice substrates was 0.34 ± 0.01 %. Rice substrate fermented with *S. cerevisiae* increased significantly ($p \leq 0.05$) to 0.90 ± 0.02 % after 10 days of fermentation. On the contrary, there was no significant ($p \geq 0.05$) increase in the rice substrate fermented with *L. bulgaricus* that is 0.36 ± 0.04 % after 5 days of fermentation followed by a significant ($p \leq 0.05$) increase to 0.93 ± 0.02 % after 10 days of fermentation. Two-step fermentation of the rice substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in ash content to 1.25 ± 0.02 % after 10 days of fermentation. Similarly, two-step fermentation of the rice substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* showed a significant ($p \leq 0.05$) increase in ash content to 1.15 ± 0.04 % after 10 days of fermentation.

### 4.2.4 Fat content

Table 4.1 summarizes the data obtained. The initial fat content of unfermented rice substrates was 0.18 ± 0.00 %. There was a significant ($p \leq 0.05$) decrease in fat content of the rice substrate fermented with *L. bulgaricus* to 0.11 ± 0.00 % after 10 days of fermentation. But fermentation of rice substrate with *S. cerevisiae* showed a significant ($p \leq 0.05$) decrease in the fat content at 0.11 ± 0.00 % after 5 days of fermentation and then increased significantly ($p \leq 0.05$) to 0.17 ± 0.00 % after 10 days of fermentation. Two-step fermentation of the rice substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* gave a

significant ($p \leq 0.05$) increase in the fat content to 0.20 ± 0.01 % after 5 days of fermentation and then declined significantly ($p \leq 0.05$) to 0.17 ± 0.00 % after 10 days of fermentation. However, two-step fermentation of rice substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* increased insignificantly ($p \geq 0.05$) in fat content to 0.19 ± 0.00 % after 10 days of fermentation.

### 4.2.5 Fibre content

Table 4.1 shows a summary of results obtained. The initial fibre content of unfermented rice substrate was 4.45 ± 0.04 %. There was no significant ($p \geq 0.05$) increase in the rice substrate fermented with *S. cerevisiae* that is 4.64 ± 0.17 % after 5 days of fermentation followed by a marginal decrease ($p \leq 0.05$) to 4.47 ± 0.07 % after 10 days of fermentation. However, the fiber content in the rice substrate fermented with *L. bulgaricus* showed an insignificant ($p \geq 0.05$) increase in the fibre content (4.49 ± 0.05 %) after 10 days of fermentation. Two-step fermentation of the rice substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* did not increase significantly ($p \geq 0.05$) in fibre content (4.52 ± 0.16%) after 5 days of fermentation and then declined marginally ($p \geq 0.05$) to 4.24 ± 0.12 % after 10 days of fermentation. On the other hand, the two-step fermentation of the rice substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* showed a significant ($p \leq 0.05$) decrease in the fibre content (3.40 ± 0.17 %) after 5 days of fermentation and then increased significantly ($p \leq 0.05$) to 5.03 ± 0.05 % after 10 days of fermentation (Table 4.1)

**Table 4.1:** Proximate composition of rice substrate fermented with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) two-step fermentation process

| Fermentation treatment | Day | Moisture (%) | Ash (%) | Fat (%) | Fibre (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | 0 | 9.98 ± 0.01$^{a}$ | 0.34 ± 0.01$^{a}$ | 0.18 ± 0.00$^{a}$ | 4.45 ± 0.04$^{a}$ | 8.28 ± 0.13$^{a}$ | 86.75 ± 0.16$^{a}$ |
| | 5 | 49.15 ± 0.08$^{b}$ | 0.66 ± 0.01$^{b}$ | 0.11 ± 0.00$^{b}$ | 4.64 ± 0.17$^{a}$ | 10.89 ± 0.09$^{b}$ | 83.70 ± 0.23$^{b}$ |
| | 10 | 49.88 ± 0.09$^{c}$ | 0.90 ± 0.02$^{c}$ | 0.17 ± 0.00$^{c}$ | 4.47 ± 0.07$^{a}$ | 11.53 ± 0.20$^{c}$ | 82.93 ± 0.15$^{c}$ |
| *L. bulgaricus* | 0 | 9.98 ± 0.01$^{a}$ | 0.34 ± 0.01$^{a}$ | 0.18 ± 0.00$^{a}$ | 4.45 ± 0.04$^{a}$ | 8.28 ± 0.13$^{a}$ | 86.75 ± 0.16$^{a}$ |
| | 5 | 48.24 ± 0.05$^{b}$ | 0.36 ± 0.04$^{a}$ | 0.11 ± 0.00$^{b}$ | 3.49 ± 0.21$^{b}$ | 9.64 ± 0.25$^{b}$ | 86.40 ± 0.26$^{a}$ |
| | 10 | 48.52 ± 0.22$^{b}$ | 0.93 ± 0.02$^{b}$ | 0.11 ± 0.00$^{b}$ | 4.49 ± 0.05$^{a}$ | 10.78 ± 0.06$^{c}$ | 83.69 ± 0.07$^{b}$ |
| *S. cerevisiae* followed by *L. bulgaricus* | 0 | 9.98 ± 0.01$^{a}$ | 0.34 ± 0.01$^{a}$ | 0 18 ± 0.00$^{a}$ | 4.45 ± 0.04$^{a}$ | 8.28 ± 0.13$^{a}$ | 86.75 ± 0.16$^{a}$ |
| | 5 | 48.95 ± 0.18$^{b}$ | 0.84 ± 0.01$^{b}$ | 0.20 ± 0.01$^{b}$ | 4.52 ± 0.16$^{a}$ | 10.58 ± 0.19$^{b}$ | 83.86 ± 0.04$^{b}$ |
| | 10 | 59.59 ± 0.27$^{c}$ | 1.25 ± 0.02$^{c}$ | 0.17 ± 0.00$^{c}$ | 4.24 ± 0.12$^{a}$ | 14.42 ± 0.31$^{c}$ | 79.93 ± 0.25$^{c}$ |
| *L. bulgaricus* followed by *S. cerevisiae* | 0 | 9.98 ± 0.01$^{a}$ | 0.34 ± 0.01$^{a}$ | 0.18 ± 0.00$^{a}$ | 4.45 ± 0.04$^{a}$ | 8.28 ± 0.13$^{a}$ | 86.75 ± 0.16$^{a}$ |
| | 5 | 47.79 ± 0.29$^{b}$ | 0.73 ± 0.01$^{b}$ | 0.16 ± 0.01$^{b}$ | 3.40 ± 0.17$^{b}$ | 10.13 ± 0.13$^{b}$ | 85.60 ± 0.06$^{b}$ |
| | 10 | 60.45 ± 0.32$^{c}$ | 1.15 ± 0.04$^{c}$ | 0.19 ± 0.00$^{a}$ | 5.03 ± 0.05$^{c}$ | 15.19 ± 0.15$^{c}$ | 78.44 ± 0.20$^{c}$ |

$^{a\text{-}c}$Means within a column with different superscripts are significantly different ($p \leq 0.05$)

## 4.3 Proximate analyses of wheat substrate fermented with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process

### 4.3.1 Protein content

Table 4.2 summarizes the data obtained. The initial % crude protein content of unfermented wheat substrates was 10.80 ± 0.20 %. Fermentation of wheat substrate with mono-cultures of *S. cerevisiae* and *L. bulgaricus* increased significantly ($p \leq 0.05$) in % crude protein to 19.17 ± 0.09 % and 17.41 ± 0.30 %, respectively, after 10 days of fermentation. Two-step fermentation of the wheat substrate with *S. cerevisiae* followed by *L. bulgaricus* significantly ($p \leq 0.05$) increased the % crude protein content to 20.16 ± 0.13 % after 10 days of fermentation. Similarly, two-step fermentation of the wheat substrate with *L. bulgaricus* followed by *S. cerevisiae* showed a significant ($p \leq 0.05$) increase in the % crude protein content that is 19.84 ± 0.04 % after 10 days of fermentation.

### 4.3.2 Carbohydrate content

The initial carbohydrate content of wheat substrates was 78.21 ± 0.43 % (Table 4.2). There was a significant ($p \leq 0.05$) decrease in the carbohydrate content of the wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* (Table 4.2) yielding 73.37 ± 0.19 % and 75.19 ± 0.25 %, respectively, after 10 days of fermentation. Two-step fermentation of the wheat substrate with *S. cerevisiae* followed by *L. bulgaricus* showed a significant ($p \leq$

0.05) decrease in the carbohydrate content to 72.91 ± 0.27 % after 10 days of fermentation. Similarly, two-step fermentation of the wheat substrate with *L. bulgaricus* followed by *S. cerevisiae* significantly ($p \leq 0.05$) decreased the carbohydrate content to 73.21 ± 0.12 % after 10 days of fermentation (Table 4.2).

### 4.3.3 Ash content

Table 4.2 summarizes the results obtained. The initial ash content of unfermented wheat substrates was 1.67 ± 0.06 %. Fermentation of the wheat substrate with mono-cultures of *S. cerevisiae* and *L bulgaricus* showed a significant ($p \leq 0.05$) increase in the ash content to 2.27 ± 0.21 % and 2.40 ± 0.05 %, respectively, after 10 days of fermentation. Two-step fermentation of wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus*, gave an insignificant ($p \geq 0.05$) marginal decrease in the ash content to 1.53 ± 0.08 % after 5 days of fermentation followed by significant ($p \leq 0.05$) increase to 2.38 ± 0.27 % after 10 days of fermentation. However, in the case where *L. bulgaricus* was the first fermenter before *S. cerevisiae* in a two-step fermentation, the ash content of the wheat substrate increased significantly ($p \leq 0.05$) to 2.26 ± 0.23 % after 10 days of fermentation (Table 4.2).

### 4.3.4 Fat content

Table 4.2 shows a summary of results obtained. The initial fat content of unfermented wheat substrates was 1.66 ± 0.03 %. There was a significant ($p \leq 0.05$) decrease in the wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* to 1.18 ± 0.00 % and 1.10 ± 0.02 %, respectively,

after 10 days of fermentation. Two-step fermentation of wheat substrate with *S. cerevisiae* followed by *L. bulgaricus* showed a significant ($p \leq 0.05$) decrease in fat content (0.73 ± 0.02 %) after 10 days of fermentation. Correspondingly, two-step fermentation of wheat substrate with *L. bulgaricus* followed by *S. cerevisiae* significantly ($p \leq 0.05$) decreased the fat content to 0.72 ± 0.00 % after 10 days of fermentation (Table 4.2).

### 4.3.5 Fibre content

Table 4.2 summarizes the data obtained. The initial fibre content of unfermented wheat substrates was 7.66 ± 0.32 %. There was a significant ($p \leq 0.05$) decrease in the fibre content in the wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* to 4.01 ± 0.01 % and 3.89 ± 0.07 %, respectively, after 10 days of fermentation. Two-step fermentation of wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) decrease to 3.82 ± 0.09 % after 10 days of fermentation. Likewise, the two-step fermentation of wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* significantly ($p \leq 0.05$) decreased the fibre content to 3.97 ± 0.07 % after 10 days of fermentation (Table 4.2).

**Table 4.2:** Proximate composition of wheat substrate fermented with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) two-step fermentation process

| Fermentation treatment | Day | Moisture (%) | Ash (%) | Fat (%) | Fibre (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | 0 | 9.76 ± 0.03$^{a}$ | 1.67 ± 0.06$^{a}$ | 1.66 ± 0.03$^{a}$ | 7.66 ± 0.32$^{a}$ | 10.80 ± 0.20$^{a}$ | 78.21 ± 0.43$^{a}$ |
| | 5 | 50.17 ± 0.19$^{b}$ | 1.88 ± 0.02$^{a}$ | 1.31 ± 0.02$^{b}$ | 4.20 ± 0.12$^{b}$ | 18.33 ± 0.07$^{b}$ | 74.29 ± 0.10$^{b}$ |
| | 10 | 50.60 ± 0.61$^{b}$ | 2.27 ± 0.21$^{b}$ | 1.18 ± 0.00$^{c}$ | 4.01 ± 0.01$^{b}$ | 19.17 ± 0.09$^{c}$ | 73.37 ± 0.19$^{c}$ |
| *L. bulgaricus* | 0 | 9.76 ± 0.03$^{a}$ | 1.67 ± 0.06$^{a}$ | 1.66 ± 0.03$^{a}$ | 7.66 ± 0.32$^{a}$ | 10.80 ± 0.20$^{a}$ | 78.21 ± 0.43$^{a}$ |
| | 5 | 48.85 ± 0.24$^{b}$ | 2.12 ± 0.09$^{b}$ | 1.25 ± 0.00$^{b}$ | 3.74 ± 0.28$^{b}$ | 16.52 ± 0.41$^{b}$ | 76.14 ± 0.27$^{b}$ |
| | 10 | 48.41 ± 0.11$^{c}$ | 2.40 ± 0.05$^{c}$ | 1.10 ± 0.02$^{c}$ | 3.89 ± 0.07$^{b}$ | 17.41 ± 0.30$^{c}$ | 75.19 ± 0.25$^{c}$ |
| *S. cerevisiae* followed by *L. bulgaricus* | 0 | 9.76 ± 0.03$^{a}$ | 1.67 ± 0.06$^{a}$ | 1.66 ± 0.03$^{a}$ | 7.66 ± 0.32$^{a}$ | 10.80 ± 0.20$^{a}$ | 78.21 ± 0.43$^{a}$ |
| | 5 | 51.22 ± 0.16$^{b}$ | 1.53 ± 0.08$^{a}$ | 1.30 ± 0.00$^{b}$ | 3.98 ± 0.13$^{b}$ | 19.07 ± 0.60$^{b}$ | 74.12 ± 0.11$^{b}$ |
| | 10 | 61.37 ± 0.41$^{c}$ | 2.38 ± 0.27$^{b}$ | 0.73 ± 0.02$^{c}$ | 3.82 ± 0.09$^{b}$ | 20.16 ± 0.13$^{c}$ | 72.91 ± 0.27$^{c}$ |
| *L. bulgaricus* followed by *S. cerevisiae* | 0 | 9.76 ± 0.03$^{a}$ | 1.67 ± 0.06$^{a}$ | 1.66 ± 0.03$^{a}$ | 7.66 ± 0.32$^{a}$ | 10.80 ± 0.20$^{a}$ | 78.21 ± 0.43$^{a}$ |
| | 5 | 49.70 ± 0.43$^{b}$ | 2.26 ± 0.04$^{b}$ | 1.08 ± 0.01$^{b}$ | 3.39 ± 0.26$^{b}$ | 17.12 ± 0.66$^{b}$ | 76.14 ± 0.25$^{b}$ |
| | 10 | 60.48 ± 0.39$^{c}$ | 2.26 ± 0.23$^{b}$ | 0.72 ± 0.00$^{c}$ | 3.97 ± 0.07$^{b}$ | 19.84 ± 0.04$^{c}$ | 73.21 ± 0.12$^{c}$ |

$^{a\text{-}c}$Means within a column with different superscripts are significantly different ($p \leq 0.05$)

## 4.4 Proximate analyses of 1:1 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process

### 4.4.1 Protein content

Table 4.3 summarizes the data obtained. The initial % crude protein content of the unfermented 1:1 formulated blend of rice and wheat substrates was 11.66 ± 0.25 %. Fermentation of the 1:1 formulated blend of rice and wheat substrate with monocultures of *S. cerevisiae* and *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in the % crude protein content to 17.07 ± 0.32 % and 14.29 ± 0.39 %, respectively, after 10 days of fermentation. Two-step fermentation of the 1:1 formulated blend of rice and wheat substrate with *S. cerevisiae* followed by *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in the % crude protein content to 17.75 ± 0.14 % after 10 days of fermentation. Similarly, two-step fermentation of the 1:1 formulated blend of rice and wheat substrate with *L. bulgaricus* followed by *S. cerevisiae* increased significantly ($p \leq 0.05$) in the % crude protein content to 16.73 ± 0.35 % after 10 days of fermentation.

### 4.4.2 Carbohydrate content

The initial carbohydrate content of the unfermented 1:1 formulated blend of rice and wheat substrates was 82.73 ± 0.35 % (Table 4.3). Fermentation with monocultures of *S. cerevisiae* and *L. bulgaricus* significantly ($p \leq 0.05$) decreased

the carbohydrate content of the 1:1 formulated blend of rice and wheat substrate to 75.97 ± 0.19 % and 79.23 ± 0.49 %, respectively, after 10 days of fermentation. Two-step fermentation of the 1:1 formulated blend of rice and wheat substrate with *S. cerevisiae* followed by *L. bulgaricus* significantly decreased ($p \leq 0.05$) the carbohydrate content to 77.55 ± 1.62 % after 10 days of fermentation. Similarly, two-step fermentation of the 1:1 formulated blend of rice and wheat substrate with *L. bulgaricus* followed by *S. cerevisiae* showed a significant ($p \leq 0.05$) decrease in the carbohydrate content to 76.90 ± 0.14 % after 10 days of fermentation (Table 4.3).

### 4.4.3 Ash content

The initial ash content of the 1:1 formulated blend of rice and wheat was 1.17 ± 0.04 %. There was a significant ($p \leq 0.05$) increase in ash content in the 1:1 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* to 1.54 ± 0.06 % after 10 days of fermentation. However, there was a significant ($p \leq 0.05$) increase in ash content in the 1:1 formulated blend of rice and wheat substrate fermented with *L. bulgaricus* to 1.62 ± 0.04 % after 5 days of fermentation followed by a significant ($p \leq 0.05$) decrease to 1.44 ± 0.16 % after day 10. Two-step fermentation of the 1:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in the ash content to 1.83 ± 0.02 % after 10 days of fermentation. Similarly, two-step fermentation of the 1:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S.*

*cerevisiae* significantly ($p \leq 0.05$) increased in the ash content to 1.70 ± 0.09 % after 10 days of fermentation. (Table 4.3).

### 4.4.4 Fat content

Table 4.3 summarizes data obtained. The initial fat content of the unfermented 1:1 formulated blend of rice and wheat was 0.86 ± 0.00 %. The fat content decreased significantly ($p \leq 0.05$) in the 1:1 formulated blend of rice and wheat substrate fermented with *L. bulgaricus* to 0.63 ± 0.01 % after 10 days of fermentation. However, there was a significant decrease in fat content in the 1:1 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* to 0.71 ± 0.01 % after 5 days of fermentation followed by a significantly ($p \leq 0.05$) increase to same level 0.84 ± 0.01 % after 10 days of fermentation. Two-step fermentation of the 1:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) decrease in the fat content to 0.29 ± 0.01 % after 10 days of fermentation. Similarly, two-step fermentation of the 1:1 formulated blend of rice and wheat, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* decreased significantly ($p \leq 0.05$) in the fat content to 0.24 ± 0.02 % after 10 days of fermentation.

### 4.4.5 Fibre content

The initial fiber content of the unfermented 1:1 formulated blend of rice and wheat was 3.58 ± 0.10 %. The fibre content increased significantly ($p \leq 0.05$) in the 1:1 formulated blend of rice and wheat substrate fermented with *S.*

*cerevisiae* to 4.58 ± 0.20 % after 10 days of fermentation. However, fermentation of the 1:1 formulated blend of rice and wheat substrate with *L. bulgaricus* also significantly ($p \leq 0.05$) increased the fibre content to 5.49 ± 0.36 % after 5 days and then followed by a significant ($p \leq 0.05$) decrease after 10 days of fermentation to 4.41 ± 0.22 %. Two-step fermentation of 1:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* marginally increased the fibre content to 4.36 ± 0.09 % after 5 days and then followed by a decline to 2.58 ± 1.75 % after 10 days of fermentation. On the contrary, two-step fermentation of a 1:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* showed a marginal decline in the fibre content to 3.28 ± 0.27 % after 5 days and then increased to 4.43 ± 0.22 after 10 days of fermentation (Table 4.3).

**Table 4.3:** Proximate composition of 1:1 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) two-step fermentation process

| Fermentation treatment | Day | Moisture (%) | Ash (%) | Fat (%) | Fibre (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | 0 | 8.03 ± 0.12$^{a}$ | 1.17 ± 0.04$^{a}$ | 0.86 ± 0.00$^{a}$ | 3.58 ± 0.10$^{a}$ | 11.66 ± 0.25$^{a}$ | 82.73 ± 0.35$^{a}$ |
| | 5 | 47.70 ± 0.31$^{b}$ | 1.45 ± 0.12$^{b}$ | 0.71 ± 0.01$^{b}$ | 4.53 ± 0.32$^{b}$ | 14.48 ± 0.42$^{b}$ | 78.83 ± 0.15$^{b}$ |
| | 10 | 51.13 ± 0.10$^{c}$ | 1.54 ± 0.06$^{b}$ | 0.84 ± 0.01$^{c}$ | 4.58 ± 0.20$^{b}$ | 17.07 ± 0.32$^{c}$ | 75.97 ± 0.19$^{c}$ |
| *L. bulgaricus* | 0 | 8.03 ± 0.12$^{a}$ | 1.17 ± 0.04$^{a}$ | 0.86 ± 0.00$^{a}$ | 3.58 ± 0.10$^{a}$ | 11.66 ± 0.25$^{a}$ | 82.73 ± 0.35$^{a}$ |
| | 5 | 46.63 ± 0.16$^{b}$ | 1.62 ± 0.04$^{b}$ | 0.73 ± 0.01$^{b}$ | 5.49 ± 0.36$^{b}$ | 13.41 ± 0.12$^{b}$ | 78.74 ± 0.50$^{b}$ |
| | 10 | 49.09 ± 0.20$^{c}$ | 1.44 ± 0.16$^{b}$ | 0.63 ± 0.01$^{c}$ | 4.41 ± 0.22$^{c}$ | 14.29 ± 0.39$^{c}$ | 79.23 ± 0.49$^{b}$ |
| *S. cerevisiae* followed by *L. bulgaricus* | 0 | 8.03 ± 0.12$^{a}$ | 1.17 ± 0.04$^{a}$ | 0.86 ± 0.00$^{a}$ | 3.58 ± 0.10$^{a}$ | 11.66 ± 0.25$^{a}$ | 82.73 ± 0.35$^{a}$ |
| | 5 | 52.25 ± 0.11$^{b}$ | 1.59 ± 0.12$^{b}$ | 0.78 ± 0.01$^{b}$ | 4.36 ± 0.09$^{a}$ | 16.50 ± 0.20$^{b}$ | 76.78 ± 0.20$^{b}$ |
| | 10 | 62.90 ± 0.08$^{c}$ | 1.83 ± 0.02$^{c}$ | 0.29 ± 0.01$^{c}$ | 2.58 ± 1.75$^{a}$ | 17.75 ± 0.14$^{c}$ | 77.55 ± 1.62$^{b}$ |
| *L. bulgaricus* followed by *S. cerevisiae* | 0 | 8.03 ± 0.12$^{a}$ | 1.17 ± 0.04$^{a}$ | 0.86 ± 0.00$^{a}$ | 3.58 ± 0.10$^{a}$ | 11.66 ± 0.25$^{a}$ | 82.73 ± 0.35$^{a}$ |
| | 5 | 48.20 ± 0.60$^{b}$ | 1.57 ± 0.04$^{b}$ | 0.67 ± 0.01$^{b}$ | 3.28 ± 0.27$^{a}$ | 14.40 ± 0.09$^{b}$ | 80.09 ± 0.32$^{b}$ |
| | 10 | 61.02 ± 0.09$^{c}$ | 1.70 ± 0.09$^{b}$ | 0.24 ± 0.02$^{c}$ | 4.43 ± 0.22$^{b}$ | 16.73 ± 0.35$^{c}$ | 76.90 ± 0.14$^{c}$ |

$^{a\text{-}c}$Means within a column with different superscripts are significantly different ($p \leq 0.05$)

## 4.5 Proximate analyses of 1:3 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process

### 4.5.1 Protein content

Table 4.4 summarizes the data obtained. The initial % crude protein content of the unfermented 1:3 formulated blend of rice and wheat substrates was 12.91 ± 0.13 %. Fermentation of the 1:3 formulated blend of rice and wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in protein content to 18.53 ± 0.22 % and 16.46 ± 0.03 %, respectively, after 10 days of fermentation. Two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* significantly ($p \leq 0.05$) increased the % crude protein content to 20.15 ± 0.10 % after 10 days of fermentation. Likewise, two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* significantly ($p \leq 0.05$) increased the % crude protein content to 21.45 ± 0.41 % after 10 days of fermentation.

### 4.5.2 Carbohydrate content

The initial carbohydrate content of the unfermented 1:3 formulated blend of rice and wheat was 80.03 ± 0.25 %. There was a significant ($p \leq 0.05$) decrease in carbohydrate content in the 1:3 formulated blend of rice and wheat substrate

inoculated with mono-cultures of *S. cerevisiae* and *L. bulgaricus* to 73.87 ± 0.64 % and 76.67 ± 0.31 %, respectively, after 10 days of fermentation. Two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* decreased significantly ($p \leq 0.05$) in carbohydrate content to 73.46 ± 0.06 % after 10 days. Similarly, two-step fermentation of a 1:3 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* decreased significantly ($p \leq 0.05$) in carbohydrate content to 71.89 ± 0.57 % after 10 days of fermentation (Table 4.4).

### 4.5.3 Ash content

The initial ash content of the unfermented 1:3 formulated blend of rice and wheat substrates was 1.50 ± 0.09 %. The ash content increased significantly ($p \leq 0.05$) in the 1:3 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* to 2.25 ± 0.05 % after 10 days of fermentation. However, the ash content marginally increased in the 1:3 formulated blend of rice and wheat substrate fermented with *L. bulgaricus* to 2.20 ± 0.30 % after 10 days of fermentation. Two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* produced a significant ($p \leq 0.05$) increase in ash content to 2.12 ± 0.04 % after 10 days of fermentation. Similarly, two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S.*

*cerevisiae* produced a significant ($p \leq 0.05$) increase in ash content to 2.09 ± 0.07 % after 10 days of fermentation (Table 4.4).

### 4.5.4 Fat content

The initial fat content of the unfermented 1:3 formulated blend of rice and wheat at day 0 is 0.65 ± 0.01. There was a significant ($p \leq 0.05$) decrease in the fat content in the 1:3 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* to 0.35 ± 0.01 % after 5 days and thereafter increased marginally to 0.37 ± 0.01 % after 10 days of fermentation. Similarly, fermentation of the 1:3 formulated blend of rice and wheat substrate with *L. bulgaricus* significantly ($p \leq 0.05$) lowered the fat content to 0.22 ± 0.01 after 5 days and then increased marginally to 0.42 ± 0.01 % after 10 days of fermentation. Two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* resulted in a significant ($p \leq 0.05$) decrease in the fat content to 0.33 ± 0.01 % after 10 days of fermentation. The two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* also significantly ($p \leq 0.05$) decreased the fat content to 0.27 ± 0.01 % after 5 days and then increased marginally to 0.38 ± 0.02 % after 10 days of fermentation (Table 4.4).

### 4.5.5 Fibre content

The initial fibre content of the unfermented 1:3 formulated blend of rice and wheat was 4.91 ± 0.11 %. The fibre content decreased marginally in the 1:3 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* to 4.39

± 0.20 % after 5 days and then increased slightly after 10 days of fermentation to approximate the initial (4.98 ± 0.65 %). Fermentation of a 1:3 formulated blend of rice and wheat substrate fermented with *L. bulgaricus* decreased insignificantly ($p \geq 0.05$) in the fibre content to 4.06 ± 0.60 % after 5 days and then increased marginally after 10 days of fermentation to 4.25 ± 0.10 %. Two-step fermentation of the 1:3 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* increased marginally in the fibre content to 4.98 ± 0.66 % after 5 days and thereafter decreased significantly to 3.89 ± 0.10 % after 10 days of fermentation. However, two-step fermentation of 1:3 formulated blend rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* significantly increased ($p \leq 0.05$) in the fibre content to 6.64 ± 0.29 % after 5 days and thereafter declined after 10 days of fermentation to 4.23 ± 0.57 (Table 4.4).

**Table 4.4:** Proximate composition of 1:3 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) two-step fermentation process

| Fermentation treatment | Day | Moisture (%) | Ash (%) | Fat (%) | Fibre (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| | 0 | 8.61 ± 0.09$^{a}$ | 1.50 ± 0.09$^{a}$ | 0.65 ± 0.01$^{a}$ | 4.91 ± 0.11$^{a}$ | 12.91 ± 0.13$^{a}$ | 80.03 ± 0.25$^{a}$ |
| *S. cerevisiae* | 5 | 50.02 ± 0.10$^{b}$ | 1.81 ± 0.06$^{b}$ | 0.35 ± 0.01$^{b}$ | 4.39 ± 0.20$^{a}$ | 16.45 ± 0.30$^{b}$ | 77.00 ± 0.38$^{b}$ |
| | 10 | 50.46 ± 0.40$^{b}$ | 2.25 ± 0.05$^{c}$ | 0.37 ± 0.01$^{b}$ | 4.98 ± 0.65$^{a}$ | 18.53 ± 0.22$^{c}$ | 73.87 ± 0.64$^{c}$ |
| | 0 | 8.61 ± 0.09$^{a}$ | 1.50 ± 0.09$^{a}$ | 0.65 ± 0.01$^{a}$ | 4.91 ± 0.11$^{a}$ | 12.91 ± 0.13$^{a}$ | 80.03 ± 0.25$^{a}$ |
| *L. bulgaricus* | 5 | 49.05 ± 0.54$^{b}$ | 1.66 ± 0.37$^{a}$ | 0.22 ± 0.01$^{b}$ | 4.06 ± 0.60$^{a}$ | 15.05 ± 0.04$^{b}$ | 79.01 ± 0.22$^{b}$ |
| | 10 | 48.84 ± 0.69$^{b}$ | 2.20 ± 0.30$^{a}$ | 0.42 ± 0.01$^{c}$ | 4.25 ± 0.10$^{a}$ | 16.46 ± 0.03$^{c}$ | 76.67 ± 0.31$^{c}$ |
| | 0 | 8.61 ± 0.09$^{a}$ | 1.50 ± 0.09$^{a}$ | 0.65 ± 0.01$^{a}$ | 4.91 ± 0.11$^{a}$ | 12.91 ± 0.13$^{a}$ | 80.03 ± 0.25$^{a}$ |
| *S. cerevisiae* followed by *L. bulgaricus* | 5 | 47.27 ± 0.18$^{b}$ | 1.72 ± 0.03$^{b}$ | 0.27 ± 0.01$^{b}$ | 4.98 ± 0.66$^{a}$ | 19.61 ± 0.25$^{b}$ | 73.42 ± 0.91$^{b}$ |
| | 10 | 62.31 ± 0.81$^{c}$ | 2.12 ± 0.04$^{c}$ | 0.38 ± 0.02$^{c}$ | 3.89 ± 0.10$^{b}$ | 20.15 ± 0.10$^{c}$ | 73.46 ± 0.06$^{b}$ |
| | 0 | 8.61 ± 0.09$^{a}$ | 1.50 ± 0.09$^{a}$ | 0.65 ± 0.01$^{a}$ | 4.91 ± 0.11$^{a}$ | 12.91 ± 0.13$^{a}$ | 80.03 ± 0.25$^{a}$ |
| *L. bulgaricus* followed by *S. cerevisiae* | 5 | 49.44 ± 0.14$^{b}$ | 1.91 ± 0.05$^{b}$ | 0.40 ± 0.00$^{b}$ | 6.64 ± 0.29$^{b}$ | 17.20 ± 0.04$^{b}$ | 73.85 ± 0.32$^{b}$ |
| | 10 | 60.64 ± 0.53$^{c}$ | 2.09 ± 0.07$^{b}$ | 0.33 ± 0.01$^{c}$ | 4.23 ± 0.57$^{a}$ | 21.45 ± 0.41$^{c}$ | 71.89 ± 0.57$^{c}$ |

$^{a\text{-}c}$Means within a column with different superscripts are significantly different ($p \leq 0.05$)

## 4.6 Proximate analyses of 3:1 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process

### 4.6.1 Protein content

Table 4.5 summarizes the data obtained. The initial % crude protein content of the unfermented 3:1 formulated blend of rice and wheat substrates was 10.52 ± 0.37 %. Fermentation (of the 3:1 formulated blend of rice and wheat substrate fermented) with mono-cultures of *S. cerevisiae* and *L. bulgaricus* produced a significant ($p \leq 0.05$) increase in the protein content to 16.30 ± 0.21 % and 15.61 ± 0.17 %, respectively, after 10 days of fermentation. Two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* significantly ($p \leq 0.05$) increased the % crude protein content to 16.48 ± 0.16 % after 10 days of fermentation. Likewise, two-step fermentation of a 3:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* significantly ($p \leq 0.05$) increased the % crude protein content to 17.23 ± 0.12 % after 10 days of fermentation.

### 4.6.2 Carbohydrate content

The initial carbohydrate content of the unfermented 3:1 formulated blend of rice and wheat substrates was 84.30 ± 0.36 % (Table 4.5). The carbohydrate content decreased significantly ($p \leq 0.05$) in the 3:1 formulated blend of rice and

wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* namely to 77.21 ± 0.15 % and 78.20 ± 0.15 % (Table 4.5), respectively, after 10 days of fermentation. The two-step fermentation of a 3:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* decreased significantly ($p \leq 0.05$) in the carbohydrate content to 78.22 ± 0.20 % after 10 days of fermentation. Similarly, two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* produced a significant ($p \leq 0.05$) decrease in the carbohydrate content to 77.51 ± 0.17 % after 10 days of fermentation (Table 4.5).

### 4.6.3 Ash content

Table 4.5 shows the results obtained. The initial ash content of the unfermented 3:1 formulated blend of rice and wheat substrates was 0.89 ± 0.03 %. There was a significant ($p \leq 0.05$) increase in ash content in the 3:1 formulated blend of rice and wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* namely to 1.14 ± 0.04 % and 1.04 ± 0.04 %, respectively, after 10 days of fermentation. The two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in the ash content to 1.26 ± 0.05 % after 10 days of fermentation. However, the two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the

first fermenter before *S. cerevisiae* also increased marginally in the ash content to 1.10 ± 0.14 % after 10 days of fermentation (Table 4.5).

### 4.6.4 Fat content

The initial fat content of the unfermented 3:1 formulated blend of rice and wheat substrates was 1.05 ± 0.02 %. The fat content decreased significantly ($p \leq 0.05$) in the 3:1 formulated blend of rice and wheat substrate fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus* namely to 0.74 ± 0.00 % and 0.72 ± 0.00 %, respectively, after 10 days of fermentation. The two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) decrease in the fat content to 0.54 ± 0.00 % after 10 days of fermentation. Similarly, two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *L. bulgaricus* was the first fermenter before *S. cerevisiae* significantly ($p \leq 0.05$) decreased the fat content to 0.54 ± 0.00 % after 10 days of fermentation (Table 4.5).

### 4.6.5 Fibre content

The initial fibre content of the unfermented 3:1 formulated blend of rice and wheat substrates was 3.25 ± 0.03 %. There was a significant ($p \leq 0.05$) increase in the fibre content of the 3:1 formulated blend of rice and wheat substrate fermented with *L. bulgaricus* to 4.44 ± 0.05 % after 10 days of fermentation. There was also a significant ($p \leq 0.05$) increase in fibre content of the 3:1 formulated blend of rice and wheat substrate fermented with *S. cerevisiae*

to 4.57 ± 0.24 % after 5 days of fermentation and thereafter decreased marginally after 10 days to 4.55 ± 0.08 %. Two-step fermentation of the 3:1 formulated blend of rice and wheat substrate, where *S. cerevisiae* was the first fermenter before *L. bulgaricus* showed a significant ($p \leq 0.05$) increase in the fibre content to 5.69 ± 0.15 % after 5 days of fermentation and then decreased after 10 days to 3.50 ± 0.09 %. On the contrary, two-step fermentation of a 3:1 formulated blend of rice and wheat where *L. bulgaricus* was the first fermenter before *S. cerevisiae* marginally decreased the fibre content to 3.13 ± 0.13 % after 5 days of fermentation and thereafter increased significantly after 10 days to 3.62 ± 0.12 % (Table 4.5).

**Table 4.5:** Proximate composition of 3:1 (w/w) formulated blend of rice and wheat substrate fermented with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) two-step fermentation process

| Fermentation treatment | Day | Moisture (%) | Ash (%) | Fat (%) | Fibre (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | 0 | 10.24 ± 0.06$^{a}$ | 0.89 ± 0.03$^{a}$ | 1.05 ± 0.02$^{a}$ | 3.25 ± 0.03$^{a}$ | 10.52 ± 0.37$^{a}$ | 84.30 ± 0.36$^{a}$ |
| | 5 | 49.28 ± 0.32$^{b}$ | 1.03 ± 0.05$^{b}$ | 0.99 ± 0.01$^{b}$ | 4.57 ± 0.24$^{b}$ | 14.06 ± 0.10$^{b}$ | 79.35 ± 0.37$^{b}$ |
| | 10 | 50.11 ± 0.09$^{c}$ | 1.14 ± 0.04$^{c}$ | 0.74 ± 0.00$^{c}$ | 4.55 ± 0.08$^{b}$ | 16.36 ± 0.21$^{c}$ | 77.21 ± 0.15$^{c}$ |
| *L. bulgaricus* | 0 | 10.24 ± 0.06$^{a}$ | 0.89 ± 0.03$^{a}$ | 1.05 ± 0.02$^{a}$ | 3.25 ± 0.03$^{a}$ | 10.52 ± 0.37$^{a}$ | 84.30 ± 0.36$^{a}$ |
| | 5 | 49.18 ± 0.13$^{b}$ | 1.08 ± 0.08$^{b}$ | 0.89 ± 0.01$^{b}$ | 3.46 ± 0.04$^{b}$ | 11.60 ± 0.21$^{b}$ | 82.98 ± 0.19$^{b}$ |
| | 10 | 49.05 ± 0.17$^{b}$ | 1.04 ± 0.04$^{b}$ | 0.72 ± 0.00$^{c}$ | 4.44 ± 0.05$^{c}$ | 15.61 ± 0.17$^{c}$ | 78.20 ± 0.15$^{c}$ |
| *S. cerevisiae* followed by *L. bulgaricus* | 0 | 10.24 ± 0.06$^{a}$ | 0.89 ± 0.03$^{a}$ | 1.05 ± 0.02$^{a}$ | 3.25 ± 0.03$^{a}$ | 10.52 ± 0.37$^{a}$ | 84.30 ± 0.36$^{a}$ |
| | 5 | 53.76 ± 0.93$^{b}$ | 0.94 ± 0.05$^{a}$ | 0.81 ± 0.00$^{b}$ | 5.69 ± 0.15$^{b}$ | 15.48 ± 0.22$^{b}$ | 77.08 ± 0.17$^{b}$ |
| | 10 | 62.99 ± 0.03$^{c}$ | 1.26 ± 0.05$^{b}$ | 0.54 ± 0.00$^{c}$ | 3.50 ± 0.09$^{c}$ | 16.48 ± 0.16$^{c}$ | 78.22 ± 0.20$^{c}$ |
| *L. bulgaricus* followed by *S. cerevisiae* | 0 | 10.24 ± 0.06$^{a}$ | 0.89 ± 0.03$^{a}$ | 1.05 ± 0.02$^{a}$ | 3.25 ± 0.03$^{a}$ | 10.52 ± 0.37$^{a}$ | 84.30 ± 0.36$^{a}$ |
| | 5 | 48.28 ± 0.49$^{b}$ | 1.11 ± 0.07$^{a}$ | 0.72 ± 0.00$^{b}$ | 3.13 ± 0.13$^{a}$ | 13.16 ± 0.10$^{b}$ | 81.90 ± 0.27$^{b}$ |
| | 10 | 60.28 ± 0.62$^{c}$ | 1.10 ± 0.14$^{a}$ | 0.54 ± 0.00$^{c}$ | 3.62 ± 0.12$^{b}$ | 17.23 ± 0.12$^{c}$ | 77.51 ± 0.17$^{c}$ |

$^{a\text{-}c}$Means within a column with different superscripts are significantly different ($p \leq 0.05$)

# CHAPTER FIVE

# DISCUSSION

## 5.1 Introduction

Fermented food products are eaten all around the world and are sometimes categorized as "functional foods" due to their purported health benefits (Luan et al., 2021). These food products have been in existence since human civilization and are going to be with us far into the future. Fermented food products, especially those derived from cereals are important contributors to the variety of diets and are vital to food security, and significantly contribute to nutrition in many sub-Saharan African countries (Obafemi et al., 2022). Microbial fermentation, with either natural or pure cultures, of cereals, legumes, oil seed meals, and their blends is known to improve their nutritional, sensory, and functional qualities of the final product (Pswarayi and Ganzle, 2022). Numerous fermented foods are traditionally prepared and consumed all over the world without much knowledge about their nutritional quality (Verni et al., 2019). This study was conducted to assess the use of microbial biotechnology using solid-state fermentation for the enhancement of protein contents of rice and wheat substrate and their formulated blends and to also evaluate the nutritional composition of the substrate after fermentation. The outcome of this study revealed that (a) the protein content of rice and wheat substrates and their formulated blends could be improved using microbial biotechnology, (b) fermentation with *S. cerevisiae* was more effective in increasing the protein

content of rice and wheat substrate and their formulated blends, compared to *L. bulgaricus*, (c) fermentation with *L. bulgaricus* before *S. cerevisiae* in the two-step fermentation process was more effective in increasing the protein content of rice, 1:3 blend and 3:1 blend (ratios represent rice: wheat in that order) whereas fermentation with *S. cerevisiae* before *L. bulgaricus* in the two-step fermentation process was more effective in increasing the protein content of wheat and 1:1 blend and (d) the protein content in wheat was significantly ($p \leq 0.05$) higher, compared to rice.

## 5.2 Percentage increase in protein contents of rice and wheat and their formulated blends after 10 days of fermentation with (i) monocultures of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus*, and (ii) two-step fermentation process

### 5.2.1 Percentage increase in protein contents of rice substrate

The % increase in protein content of the rice substrate inoculated with S. *cerevisiae* only was 39.25 %; it was 30.19 % for *L. bulgaricus* only and 74.15 % for two-step fermentation with *S. cerevisiae* before *L. bulgaricus*. Finally, the % protein increase was 83.57 % for two-step fermentation with *L. bulgaricus* before *S. cerevisiae*. Therefore, the fermentation of rice substrate with *S. cerevisiae* only (39.25 %) recorded a higher % increase in protein content than same substrate fermented with *L. bulgaricus* only (30.19 %). Rice substrate fermented with *L. bulgaricus* first followed by *S. cerevisiae* (83.57 %) in a two-step fermentation process recorded a higher % increase in protein content than same substrate

fermented with *S. cerevisiae* first followed by *L. bulgaricus* (74.15 %). Generally, among the fermentation treatments of rice substrate, the two-step fermentation process with *L. bulgaricus* followed by *S. cerevisiae* produced the highest % increase of protein (83.57 %) in rice whereas treatment with *L. bulgaricus* only resulted in the least % increase of protein (30.19 %) after 10 days of fermentation.

### 5.2.2 Percentage increase in protein contents of wheat substrate

The % increase in protein content in the wheat substrate fermented with *S. cerevisiae* alone was 77.50 %; it was 61.20 % for *L. bulgaricus*, 86.67 % for two-step fermentation with *S. cerevisiae* first followed by *L. bulgaricus*, and 83.70 % for two-step fermentation with *L. bulgaricus* first followed by *S. cerevisiae*. Therefore, the fermentation of wheat substrate with *S. cerevisiae* (77.50 %) recorded a higher increase in protein content than same substrate fermented with *L. bulgaricus* (61.20 %). Wheat substrate fermented with *S. cerevisiae* followed by *L. bulgaricus* (86.67 %) in a two-step fermentation process recorded a higher increase in protein content compared to wheat substrate fermented with *L. bulgaricus* followed by *S. cerevisiae* (83.70 %). In general, between the fermentation treatments of wheat substrates, the two-step fermentation with *S. cerevisiae* before *L. bulgaricus* produced the highest % increase of protein (86.67 %) in the wheat substrate while the fermentation treatment with single-culture, *L. bulgaricus* produced the lowest % increase of protein (61.20 %) after 10 days of fermentation.

### 5.2.3 Percentage increase in protein contents of 1:1 (w/w) formulated blend of rice and wheat

The % increase in protein content of the 1:1 formulated blend of rice and wheat substrate was 46.40 % for single inoculation with *S. cerevisiae*; 22.56 % for *L. bulgaricus* alone; 52.23 % for two-step fermentation with *S. cerevisiae* before *L. bulgaricus* and 43.48 % for two-step fermentation with *L. bulgaricus* before *S. cerevisiae*. Thus, *S. cerevisiae* used alone recorded a higher % increase of protein (46.40 %) in the 1:1 formulated blend of rice and wheat than in *L. bulgaricus* alone (22.56 %) after fermentation. Fermentation with *S. cerevisiae* followed by *L. bulgaricus* in a two-step fermentation process recorded a higher increase of protein (52.23 %) in the 1:1 formulated blend of rice and wheat substrate as compared to *L. bulgaricus* followed by *S. cerevisiae* (43.48 %). Of all the fermentation treatments of the 1:1 formulated blend of rice and wheat substrate, treatment with mono-culture, *L. bulgaricus* produced the lowest % increase of protein (22.56 %) in the 1:1 formulated blend of rice and wheat substrate whereas the two-step fermentation process with *S. cerevisiae* before *L. bulgaricus* produced the highest % increase of protein (52.23 %) in the 1:1 formulated blend of rice and wheat substrate after 10 days of fermentation.

### 5.2.4 Percentage increase in protein contents of 1:3 (w/w) formulated blend of rice and wheat

The % increase in protein content of the 1:3 formulated blend of rice and wheat substrate inoculated with *S. cerevisiae* only was 43.53 %; 27.50 % for *L.*

*bulgaricus* alone; 56.08 % for two-step fermentation with *S. cerevisiae* before *L. bulgaricus* and 66.15 % for two-step fermentation with *L. bulgaricus* before *S. cerevisiae*. Fermentation of the 1:3 formulated blend of rice and wheat fermented with *S. cerevisiae* only recorded a higher % increase in protein content (43.53 %) than in fermentation with *L. bulgaricus* only (27.50 %). The two-step fermentation with *L. bulgaricus* before *S. cerevisiae* produced a higher % increase in protein content (66.15 %) in the 1:3 formulated blend of rice and wheat than in *S. cerevisiae* before *L. bulgaricus* (56.08 %). Generally, among the fermentation treatments of the 1:3 formulated blend of rice and wheat, fermentation with *L. bulgaricus* obtained the lowest % increase in protein (27.50 %) whilst two-step fermentation with *L. bulgaricus* before *S. cerevisiae* obtained the highest % increase in protein (66.15 %) after 10 days of fermentation.

### 5.2.5 Percentage increase in protein contents of 3:1 (w/w) formulated blend of rice and wheat

The % increase in protein content of the 3:1 formulated blend of rice and wheat substrate fermented with *S. cerevisiae* only was 55.51 %; it was 48.38 % for *L. bulgaricus* alone and 56.65 % for two-step fermentation with *S. cerevisiae* before *L. bulgaricus*. Finally, the % protein increase was 63.78 % for two-step fermentation with *L. bulgaricus* before *S. cerevisiae*. After the fermentation of the 3:1 formulated blend of rice and wheat with mono-cultures of *S. cerevisiae* and *L. bulgaricus*, the highest increase in protein content was obtained by *S. cerevisiae*. In the two-step fermentation process, *L. bulgaricus* followed by *S. cerevisiae*

recorded a higher increase of protein content (63.78 %) in the 3:1 formulated blend of rice and wheat as compared to *S. cerevisiae* followed by *L. bulgaricus* (56.65 %). Of all the fermentation treatment of the 3:1 formulated blend of rice and wheat, treatment with *L. bulgaricus* produced the lowest % increase in protein (48.38 %) whereas two-step fermentation with *L. bulgaricus* followed by *S. cerevisiae* showed the highest % increase in protein content (63.78 %) after 10 days of fermentation.

In general, wheat and 3:1 blend substrate obtained the highest % increase in protein whilst rice substrate obtained the lowest % increase in protein after fermentation with S. *cerevisiae* only. On the other hand, the lowest % increase in protein was recorded in the 1:1 blend substrate after fermentation with *L. bulgaricus* whereas the highest % increase in protein content was observed in wheat and the 3:1 blend substrate. In the two-step fermentation treatment, rice and wheat substrate recorded the highest % increase in protein content after fermentation with *S. cerevisiae* before *L. bulgaricus* while the least % increase in protein was obtained in the 1:1 blend. The same observation was seen in the *L. bulgaricus* before *S. cerevisiae* fermentation, rice, and wheat recorded the % highest increase in protein whereas the 1:1 blend obtained the lowest % increase in protein content after 10 days of fermentation.

### 5.3 Fermentation of rice and wheat and their formulated blends with single cultures of *S. cerevisiae* and *L. bulgaricus*

The protein content of all substrates increased after fermentation, however, the % increase in protein content varied among the substrates and their respective fermentation treatments. In the fermentation treatment where mono-cultures of *S. cerevisiae* and *L. bulgaricus* were used, *S. cerevisiae* was more effective compared to *L. bulgaricus*. Fermentation of rice and wheat and their formulated blends with *S. cerevisiae* recorded a % increase in protein varying from 39.25 % to 77.50 %, whereas fermentation with *L. bulgaricus* showed a % increase in protein varying from 22.56 % to 61.20 %. This increase in protein content could be attributed to the utilization of carbohydrates by microorganisms as an energy source and producing carbon dioxide as a by-product. This caused the nitrogen in the fermented substrate to increase leading to increment of protein content in the substrate (Christ-Ribeiro et al., 2021). Data from this thesis are in agreement with that of other studies in the pertinent literature showing a significant increase in the protein content in cereals and legumes. For example, Chis et al. (2020) recorded an increase in the protein content of rice flour after 24 hours of fermentation with *Lactobacillus spicheri* DSM 15429. Teniola and Odunfa (2001) observed an increment of protein content in a cereal porridge after 10 days of fermentation with mixed starter cultures of *S. cerevisiae* and *Lactobacillus brevis*. Khetarpaul and Chauhan (1990) also reported a significant ($p \leq 0.05$) increase in protein content in pearl millet flour after 72 hours of fermentation with *Saccharomyces diastatics*, *L. brevis*, and *Lactobacillus*

*fermentum*. The increase in protein could be attributed to the metabolic activities of *S. cerevisiae* which involved the secretion of cellular enzymes that hydrolyzed the substrate into composite amino acids and other metabolic products. Nicolau et al. (2011) reported that protein content doubled in rice flour after 3 days of fermentation with yeasts at 30 °C, where the % increase in protein content varied from 35.3 to 69.64 %. Likewise, Wang and Fields (1978) showed that 3 days of fermentation of corn meal with *S. cerevisiae* and *Candida tropicalis* increased the protein content from 7.6 to 16.4 % and 14.5 %, respectively. Katina et al. (2012) also found an increase in the protein content of wheat bran after 20 hours of fermentation with yeast, not excepting Flander et al. (2011) who also reported an increase in oat-wheat sourdough after 20 hours of fermentation with *L. plantarum*. Recently, the effectiveness of *S. cerevisiae* in increasing protein content after fermentation was also demonstrated by Yafetto et al. (2022) in the 10 days of fermentation of malted and unmalted sorghum with *S. cerevisiae* and *L. bulgaricus*. Fermentation of malted and unmalted sorghum with *S. cerevisiae* recorded an increase by 68.40 % and 58.20 %, respectively, whereas *L. bulgaricus* recorded an increase by 34.98 % for malted and 39.36 % for unmalted, respectively.

Data from this thesis show a considerably higher increase in protein content varying from 22.56 to 77.5 % in comparison to the aforementioned instances by other workers. The differences observed to be attributed to differences in grain varieties and variation in the initial protein, as well as fermentation conditions employed in this present study. Factors such as

temperature, moisture content, choice of microorganisms, and duration of fermentation, could have played a role in the differences observed in protein content. Results obtained in this current study as well as reports by other authors clearly show that fermentation with *S. cerevisiae* can effectively increase the protein content in cereals and legumes.

Results show that rice and wheat substrates and their formulated blends fermented singly with *S. cerevisiae* and *L. bulgaricus* decreased carbohydrate content after 10 days of fermentation (Tables 4.1-4.5). Other studies have also recorded a decrease in carbohydrate content after fermentation. Ojokoh et al. (2019) reported a carbohydrate decrease in the formulated blend of rice and mucuna beans after 72 hours of fermentation *Aspergillus flavus*, *Aspergillus fumigatus*, *Geotrichum candida,* and *Rhizopus stolonifera*. Balogun and Olatidoye (2012) attributed the reduction in carbohydrate content during fermentation to the hydrolysis of the cellulose in the substrate by microorganisms. El-Tinay et al. (1979) studied the changes in starch and total sugar in sorghum during 18 hours of fermentation with *Lactobacillus confusus*, *L. brevis*, *L. fermentum*, and *Lactobacillus amylovorous*, and they reported a significant decrease in starch during the first 6 hours and a decrease in the total sugar during the first 3 to 6 hours of fermentation. This decrease could be due to the microbial utilization of released sugars as a ready source of energy. Ogodo et al. (2019) also reported a decrease in carbohydrate content in sorghum after 48 hours of fermentation with lactic acid bacteria. Likewise, Ojokoh et al. (2014) observed a decrease in carbohydrate content in breadfruit and cowpea flour blends after 72 hours of

fermentation with *Lactobacillus plantarum*. Ojokoh and Bello (2014) also made similar observations in blends of millet and soybean flour after 3 days of fermentation with the LAB. The metabolic activities of yeasts and bacteria during fermentation hydrolyses the carbohydrate content in the substrate into simple compounds and other products, thus, the reduction in carbohydrate levels.

The ash content reported in this current study increased in the rice and wheat substrate and their formulated blends after 10 days of fermentation with single cultures of *S. cerevisiae* and *L. bulgaricus* (Tables 4.1-4.5). Ash content increment in this study is an indication of the level of mineral composition of the substrate. The result obtained in this present study is in agreement with other studies. For example, Ilowefah et al. (2018) investigated the effect of solid-state yeast fermentation on the nutritional and antioxidant properties of brown rice flour for 12 hours and reported an increase in ash content; Chinma et al. (2014) found an increase in ash content of rice bran after 20 hours of fermentation with *S. cerevisiae*. The increase in ash content could be attributed to the degradation of antinutritional substances or could be a result of the rearrangement of fiber composition (Ilowefah et al., 2018). Ojokoh and Bello (2014) reported an increment of ash content in the formulated blend of millet and soybean flour after 72 hours of natural fermentation with the LAB. Ash content is a measure of the total amount of minerals present within the substrate, therefore, an increase in ash content after fermentation could be attributed to the incomplete utilization of available minerals by fermenting microorganisms during metabolism (Balogun and Olatidoye, 2012). Terefe et al. (2021), showed that the ash content slightly

increased in maize flour after 48 hours of fermentation with mono-cultures of *L. plantarum* and *S. cerevisiae* and their co-culture. This increment in ash content could be attributed to the loss of organic matter and accumulation of inorganic matter caused by the activities of enzymes produced by microorganisms during fermentation (Obadina et al., 2013).

The results obtained in this study showed an inconsistent change in the fat content. The fat content decreased in all substrates fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus*, except rice substrate where the fat content either increased or decreased erratically after fermentation (Tables 4.1-4.5). The decrease in fat contents in the substrates after fermentation can be attributed to the metabolic activities of the lipolytic enzymes produced by the fermenting microorganism which were able to hydrolyze the lipids into fatty acids and glycerol during fermentation thereby causing a drop in the fat content (Onweluzo and Nnamuchi, 2009). Becila et al. (2022) reported a decrease in fat content after 12 months of fermentation of durum wheat with LAB. On the other hand, the reported erratic increase or decrease in rice substrate after fermentation in the current study has also been reported by Kazanas and Fields (1981); they observed an inconsistent change in fat content after 4 days of fermentation of sorghum with LAB. The same trend was also observed in the 24-hour fermentation of pearl millet (Osman, 2011). The decline in fat content could also be attributed to the production of aromatic compounds through the breakdown of fatty acids and glycerol by the microorganisms during fermentation (Ojokoh and Bello, 2014). Terefe et al. (2021) observed a decrease in fat content in maize flour after 48

hours of fermentation with *L. plantarum* and *S. cerevisiae* and their co-culture and attributed the decrease in fat content to the utilization of fat as an energy source for microbial metabolic activities during fermentation. Studies indicate that high levels of fat in the substrate causes rancidity during storage, giving rise to an unpleasant aroma. Reduction in the fat content also increases the shelf life of the substrate. Therefore, fermentation with microorganisms could serve as an effective way of reducing the fat content in cereals (Olatubi and Ojokoh, 2015). Information on the changes of fat in cereals during fermentation is scanty as compared to protein and carbohydrates. This could be attributed to the fact that cereals are generally low in fat content (Osman, 2011).

The results obtained in this present study showed an inconsistent change in fiber content. The fiber content decreased in all substrates fermented with mono-cultures of *S. cerevisiae* and *L. bulgaricus*, except rice, 1:1, 1:3, and 3:1 blends where the fiber content either increased or decreased erratically after fermentation (Tables 4.1-4.5). The decrease in fiber content could be attributed to the ability of the enzymes produced by fermentation microorganisms to hydrolyze some components of the fiber. This observation agrees with the report of Ogodo et al. (2019) who reported a decrease in fiber content in sorghum after 48 hours of fermentation with LAB. Low fiber content was also observed in the formulated blend of corn and groundnut after 3 days of natural fermentation with LAB (Olatubi and Ojokoh, 2015). Indeed, Terefe et al. (2021) also observed a decrease in fiber content in maize flour after 48 hours of fermentation with *L. plantarum* and *S. cerevisiae* and their co-cultures. The decrease in fiber contents after

fermentation is an indication of softening of fibrous tissues and increased digestibility of the substrate due to the activities of microorganisms which are known for the bioconversion of carbohydrates and lignocellulose into protein (Adegunloye and Oparinde, 2017).

### 5.4 Two-step fermentation of rice and wheat and their formulated blends

Results show an increase in protein content in rice and wheat and their formulated blends after two-step fermentation with *S. cerevisiae* and *L. bulgaricus*. There were two treatments involved in the two-step fermentation process, (a) fermentation of substrates with *S. cerevisiae* as the first fermenter before *L. bulgaricus* and (b) fermentation of substrates with *L. bulgaricus* as the first fermenter followed by *S. cerevisiae*. Each treatment resulted in a higher % increase in protein. Fermentation with *L. bulgaricus* before *S. cerevisiae* increased the protein content of rice by 83.57 %, wheat by 83.70 %, 1:1 blend by 43.48 %, 1:3 blend by 66.15 % and 3:1 blend by 63.78 %, whereas fermentation with *S. cerevisiae* before *L bulgaricus* showed an increase in protein content to 74.17 % for rice, 86.67 % for wheat, 52.23 % for 1:1 blend, 56.08 % for 1:3 blend and 56.65 % for 3:1 blend (ratios represent rice: wheat in that order). However, fermentation with *L. bulgaricus* as the first fermenter before *S. cerevisiae* was more effective in increasing the protein content in rice, 1:3 blend and 3:1 blend, whereas, fermentation with *S. cerevisiae* before *L. bulgaricus* was more effective in increasing the protein content in wheat and 1:1 blend. Sindhu and Khetarpaul (2003) recorded percentage protein increase in indigenously developed food

containing rice flour, milk, sprouted green gram paste, and tomato pulp after 24 hours of fermentation with *Saccharomyces boulardii* and *L. plantarum* in a two-step fermentation process. Data from this thesis agree with the findings of Sindhu and Khetarpaul (2003) although their fermenting organisms were different. Protein increment after fermentation with *L. bulgaricus* as the first fermenter before *S. cerevisiae* could be attributed to the production of lactic acid by *L. bulgaricus* which provided an acidic substrate for *S. cerevisiae* to effectively utilize. In the case of fermentation of substrate with *S. cerevisiae* as the first fermenter before *L. bulgaricus*, protein increase could be as a result of metabolic activities of *S. cerevisiae* which secreted a miscellany of metabolites, such as amino acids, vitamins, and pyruvate, that stimulated the growth and metabolic activities of *L. bulgaricus* (Ponomarova et al., 2017).

In general, protein content for all substrates (rice and wheat and their formulated blends) increased after 10 days of fermentation with (i) monocultures of *S. cerevisiae* and *L. bulgaricus*, and (ii) a two-step fermentation process. Compared to single-cultured fermentation, the two-step fermentation method produced a synergistic effect in increasing the protein content of rice and wheat substrate and their formulated blends. The percentage increase in protein content of two-step fermentation varied from 43.48 to 86.67 %, whereas, the percentage increase in protein content of single-culture fermentation varied from 22.56 to 77.50 % (Figure 4.6). The variation in fermentation processes and the metabolic activities of the microorganisms employed can account for this outcome. Presumably, in single-culture fermentation, the involvement of only one

microorganism limits metabolic activities. Conversely, in the two-step fermentation process, the continuous and unlimited metabolic activities of microorganisms enabled the breakdown of a greater number of complex compounds into simpler compounds. This led to the concentration of nitrogen in the fermented substrate, thereby increasing the protein content. These findings agree with previous studies that utilized a co-culture fermentation technique. For example, Teniola and Odunfa (2001) reported a protein increase in cereal porridge after 10 days of fermentation with co-cultures of *S. cerevisiae* and *L. brevis*. Wang and Fields (1978) obtained protein increment after 3 days of fermentation of corn meal with co-cultures of *S. cerevisiae* and *C. tropicalis*. Yafetto et al. (2022) also observed a protein increase in malted and unmalted sorghum after 10 days of fermentation with *S. cerevisiae*, *L. bulgaricus* and co-cultures of *S. cerevisiae* and *L. bulgaricus*.

The carbohydrate content of rice and wheat substrate and their formulated blends decreased significantly ($p \leq 0.05$) after 10 days of fermentation after inoculation with *S. cerevisiae* and *L. bulgaricus* in a two-step fermentation process (Tables 4.1-4.5). The finding of this current study agrees with that of Khetarpaul and Chauhan (1991) who reported a decrease in carbohydrate content in pearl millet fermented with yeast and *Lactobacilli* and attributed the changes to the utilization of sugars by the microorganisms after fermentation.

The ash content recorded in this present study increased in the rice and wheat substrate and their formulated blends after 10 days of fermentation in the two-step fermentation process with *S. cerevisiae* followed by *L. bulgaricus* and *L.*

*bulgaricus* followed by *S. cerevisiae* (Tables 4.1-4.5). A decrease in the ash content of rice and wheat substrates and their formulated blends could generally be attributed to a concurrent decrease in the nutritional and mineral content of the substrates after fermentation because the ash content of substrates is an indicator of the mineral components and content.

The fat content in this current study decreased in all substrates after 10 days of fermentation with *S. cerevisiae* followed by *L. bulgaricus* and *L. bulgaricus* followed by *S. cerevisiae* in a two-step fermentation process. However, the fat content in rice substrate after fermentation either increased or decreased erratically after 10 days of fermentation (Tables 4.1-4.5). The decrease in fat content could be attributed to the hydrolysis of fat by lipase into fatty acids and glycerol. The increase in fat content could have been a result of the shift in metabolic pathway from glycolysis to fatty acid synthesis, where excess carbohydrate or glucose in the substrate could be converted into fat.

The fibre content either increased or decreased in the rice and formulated blend substrate after 10 days of fermentation. However, wheat substrate showed a significant ($p \leq 0.05$) decrease in the fibre content after 10 days of fermentation (Tables 4.1-4.5). The decrease in fibre content could be a result of the enzymatic activities of microorganisms that hydrolyzed the fibre content during the fermentation process into beneficial microbial metabolites such as short chain fatty acids.

Studies on the two-step fermentation process in cereals and legumes are few, however, the two-step fermentation process has been used extensively in the nutritional enhancement of agro-industrial waste for animal feed, wine production, and enzyme production (Hongzhang et al., 2012; Sugiharto et al., 2018). Hongzhang et al. (2012) reported an increment in the protein content of sweet sorghum stalk after two-step fermentation with *Candida tropicalis* followed by *Lactobacillus rhamnosus*. Sugiharto et al. (2018) also investigated the effect of two-step fermentation by *Chrysonilia crassa* and *Bacillus subtilis* on the nutritional values and antioxidant properties of agro-industrial by-products as poultry feed ingredients and reported an increase in protein and ash content with decrease in fiber. However, the fat content increased after the fermentation process. Finally, Opoku and Adoga (1979) also studied the effect of the two-step fermentation method on the production of protein-enriched feed from cassava after inoculation with *Trichoderma reesei* and yeast and reported an increment in the protein content.

### 5.5 Nutritional Compositions and their health implications

In general, protein content increased in the rice and wheat substrate and their formulated blends after 10 days of fermentation. Rice and wheat are good sources of essential amino acids and fermentation can enhance the availability and digestibility of these amino acids (Samtiya et al., 2021). An increase in protein content in the fermented substrates (rice and wheat and their formulated blends) in this present study could provide pregnant women and lactating mothers with

essential amino acids significant for their growth and development, and the developing foetus or breastfed baby. For infants, high protein intake is important as it supports their rapid growth and development, especially in terms of building muscle, bones, and other tissues (Tang, 2018). High protein content allows efficient nutrient absorption and utilization in infants (Patel and Rouster, 2022). In diabetics, a high protein intake can improve blood sugar level control by reducing post-meal blood sugar spikes (Gannon et al., 2003). Intake of a protein-rich diet also improves insulin sensitivity in individuals with type 1 & 2 diabetes potentially leading to better blood sugar control and reduced insulin resistance (Paterson et al., 2015). Incorporating a high-protein fermented substrate could help maintain blood sugar levels and reduce insulin resistance in diabetic individuals. For hypertensive individuals, a high protein intake, including fermented rice and wheat substrate and their formulated blends, could help in the modest reduction in blood pressure levels (Vasdev and Stuckless, 2010). This can contribute to better blood pressure control. Protein-rich diets also promote satiety and can aid in weight management for hypertensive patients (Te Morenga and Mann, 2012). A high protein content in the fermented rice and wheat substrate and their formulated blend could help maintain a healthy weight important for managing blood pressure and overall cardiovascular health.

Generally, rice and wheat substrate and their formulated blends obtained a decrease in carbohydrate content after 10 days of fermentation. Carbohydrates are the primary source of energy for the body during pregnancy and lactation, however, maintaining a stable blood level is important (Jouanne et al., 2021). Low

carbohydrate content in the fermented substrates can help pregnant women and lactating mothers in managing their blood sugar levels, reducing the risk of excessive sugar in the blood that can negatively affect energy levels and overall well-being. Some pregnant women may develop gestational diabetes, a condition characterized by elevated blood sugar levels during pregnancy (Mustad et al., 2020). As such, a low carbohydrate diet is recommended to help manage blood sugar levels. Incorporating fermented foods (such as fermented rice and wheat and their formulated blends) obtained from this study, with low carbohydrate content into their diet can contribute to better glycemic control in these individuals. In infant nutrition, carbohydrates provide energy and are essential for healthy growth and development, but in some cases such as blood sugar level maintenance, carbohydrate is needed at low level (Stephen et al., 2012). So, low carbohydrate content in fermented rice and wheat substrate and their formulated blends could help in maintaining and managing blood sugar levels in infants. Individuals with diabetes and hypertension, also need a diet with low carbohydrate content to help maintain blood sugar levels and improve blood pressure control, respectively (Arora and McFarlane, 2005; Wheatley et al., 2021). Additionally, low carbohydrate content is needed for weight management in such individuals (Unwin et al., 2019). Low carbohydrate content in fermented rice and wheat and their formulated blends after 10 days of fermentation with *S. cerevisiae* and *L. bulgaricus* and in a two-step fermentation process could help maintain blood sugar levels, leading to weight loss, and improved blood pressure control.

Generally, the ash content increased in the rice and wheat substrate and their formulated blends after 10 days of fermentation. Ash content in food refers to the inorganic mineral content including essential minerals such as calcium, magnesium, potassium, and phosphorus (Wada et al., 2019). Pregnant women and lactating mothers have increased nutritional needs, including a higher requirement for minerals to support their health and the development of the foetus or the production of breast milk (Marshall et al., 2022). A high ash content in the fermented substrates can contribute to meeting their increased mineral needs. For infants, specific minerals are required for their healthy growth and development (Farias et al., 2020). A high ash content in fermented rice and wheat and their formulated blends can provide essential minerals such as calcium, phosphorus, magnesium, and potassium, which are important for bone development, nerve function, and overall health and well-being. Diabetic individuals are at risk of electrolyte imbalances due to fluctuations in blood sugar levels and medication usage (Khan et al., 2019). Maintaining proper electrolyte levels is important for various bodily functions, including nerve function, muscle contraction, and fluid balance (Khan et al., 2019). A high ash content in fermented rice and wheat and their formulated blends can contribute to improved electrolyte balance by providing minerals such as potassium, sodium, calcium, and magnesium. In high blood pressure individuals, adequate intake of certain minerals, such as potassium and magnesium, is important for maintaining healthy blood pressure levels (Houston and Harper, 2008). These minerals can help counteract the effects of sodium on the heart and support cardiovascular health (Aaron and Sanders, 2013).

A high ash content in the fermented rice and wheat substrate and their formulated blends may contribute to higher mineral content and potentially provide benefits for blood pressure regulation.

Wheat substrate and the formulated blends after 10 days of fermentation with *S. cerevisiae* and *L. bulgaricus* and in a two-step fermentation process obtained a decrease in fat content. Low-fat content in wheat substrate could help reduce the risk of diseases in pregnant women and lactating mothers (Marshall et al., 2022). Rice substrate showed erratic levels in the results; fat content either increased or decreased after 10 days of fermentation. During pregnancy and lactation, women have increased energy and nutrient requirements (Jouanne et al., 2021). Fat is a concentrated source of energy and provides essential fatty acids necessary for the development of the foetus and the production of breast milk (Khor et al., 2021). An increase in fat content in the fermented substrate could provide an adequate source of energy and essential fatty acids. Fats play a crucial role in hormone production and regulation, and absorption of fat-soluble vitamins such as vitamins A, D, E, and K in pregnant women and lactating mothers (Gannon et al., 2020; Jouanne et al., 2021). A high level of essential fatty acids in the fermented substrate could provide the necessary essential fatty acids for hormone synthesis and absorption of important vitamins. Hypertensive individuals are at a higher risk of cardiovascular problems, and reducing fat intake, particularly unhealthy saturated fats, can be beneficial for heart health (Hooper et al., 2022). Lowering fat intake can assist in the weight management for hypertensive patients as excess body weight is often associated with increased

blood pressure (Cohen, 2017). So, a low-fat content in fermented rice and wheat substrate and their formulated blends can contribute to the maintenance of cardiovascular health and weight management. For individuals with diabetes, a low-fat content in the fermented substrate may be beneficial for diabetics who need to manage their blood sugar levels (Paterson et al., 2015). Diabetics are at an increased risk of cardiovascular complications, and a healthy fat intake is important for cardiovascular health and also helps manage weight (Garonzi et al., 2021). Low-fat content in the fermented substrate rice and wheat substrate and their formulated blends may not provide sufficient energy and essential fatty acids for infants, however, infants have higher energy needs and require an adequate intake of healthy fats for optimal growth and development (Huffman et al., 2011). Fats provide essential fatty acids that support brain development, nutrient absorption, and overall energy supply.

After 10 days of fermentation wheat substrate obtained a decrease in fibre content, whereas, the fibre content in rice and the formulated blend substrates either increased or decreased erratically after fermentation with *S. cerevisiae* and *L. bulgaricus* and in the two-step fermentation process. Fibre is known for its role in promoting healthy digestion and preventing constipation (Barber et al., 2020). During pregnancy, hormonal changes can affect bowel movements leading to constipation. Adequate fibre intake helps regulate bowel movements and maintain optimal digestive health (Zerfu and Mekuria, 2019). Dietary fiber can also help regulate blood sugar levels by slowing down the absorption of sugars from the diet (Jouanne et al., 2021). This can be particularly important for pregnant women

and lactating mothers who may be at risk of gestational diabetes or have difficulty managing their blood sugar levels (Marshall et al., 2022). However, decrease in fibre content in the substrate may not provide the same level of digestive support and maintain blood sugar levels. For hypertensive patients, fibre, especially soluble fibre, has been associated with a lower risk of cardiovascular diseases (Reynolds et al., 2022). Fibre can help reduce cholesterol levels and regulate blood pressure (Soliman, 2019). The low fibre content in the fermented substrate may limit the potential cardiovascular benefits associated with fibre. In diabetic individuals, fibre has a beneficial impact on blood sugar control by slowing down the absorption of glucose and reducing post-meal blood sugar (Giuntini et al., 2022). The low fibre content in the fermented rice and wheat substrate and their formulated blends may not provide sufficient fibre for optimal blood sugar control. For infants, fiber plays a crucial role in promoting healthy digestion and preventing constipation and also helps regulate the absorption of nutrients and promote a healthy gut environment (Guan et al., 2021).

# CHAPTER SIX

# SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

## 6.1 Overview

Chapter Six presents the summary of the results and the conclusions derived from the findings of this work. Recommendations based on the findings from this study are also provided.

## 6.2 Summary

This study was carried out in the Microbiology Laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast, Ghana. Solid-state fermentation was the main technique used for this study (it involved the use of fermentation with mono-cultures and a two-step fermentation process). This study involved the use of rice and wheat grains as the main substrates for fermentation, and *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus* as the main microorganisms for the fermentation processes. The data

obtained were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS Statistics, USA) to find the mean and standard deviation. Also, Tukey's multiple comparison test with a confidence interval of 95 % ($P \leq 0.05$) was used to measure the significant difference between the data obtained. After the study, the results obtained showed four key findings (a) the protein content increased significantly ($p \leq 0.05$) in all substrates, (b) wheat substrate obtained a higher % increase in protein in all fermentation treatments, (c) two-step fermentation with *S. cerevisiae* as the first fermenter before *L. bulgaricus* was more effective in increasing the protein content in wheat and 1:1 blend whereas two-step fermentation with *L. bulgaricus* followed by *S. cerevisiae* was more effective in increasing the protein content in rice, 1:3 blend and 3:1 blend and (d) fermentation with *S. cerevisiae* was more effective in increasing the protein content in rice and wheat substrate and their formulated blends, compared to *L. bulgaricus*.

### 6.3 Conclusions

The protein content increased significantly in the rice and wheat substrate and their formulated blends. The ash content increased and carbohydrate content decreased in all fermentation treatments with rice and wheat and their formulated blends. The fat content in wheat and the formulated blends decreased whereas the fat content in rice either increased or decreased erratically after fermentation. The fibre content decreased in the wheat substrate, however, the fibre content in rice and the formulated blends either increased or decreased erratically after

fermentation. Fermentation of rice and wheat substrate and their formulated blends with *S. cerevisiae* only obtained a higher % increase in protein content than in fermentation with *L. bulgaricus*. Fermentation with *L. bulgaricus* before *S. cerevisiae* in the two-step fermentation process was more effective in increasing the protein content in rice, 1:3 blend and 3:1 blend whilst fermentation with *S. cerevisiae* before *L. bulgaricus* was more effective in increasing the protein in wheat and 1:1 blend. In all the fermentation treatments, wheat substrate recorded the % highest protein content. Fermentation with *S. cerevisiae* only was effective in increasing the protein content in all substrate, compared to *L. bulgaricus*. Two-step fermentation treatment produced a synergistic effect in increasing the protein content of rice and wheat substrate and their formulated blends, compared to mono-culture fermentation treatment.

### 6.4 Recommendations

Based on findings from this study, it is recommended that:

i. Effects on the physicochemical properties should be determined.

ii. Further studies should be carried out to identify the specific amino acids formed in the substrates in the after fermentation.

iii. Animal models should be used to evaluate the suitability of the fermented product to the balance of their gut microbiota ecosystem.

iv. Further studies are required on the functional properties and the health benefits of the fermented product for the management of chronic diseases such as diabetes, cardiovascular disease, and hypertension.

v. Clinical studies are required to know the presented level of proportions of the fermented product in foods to understand its nutritional and health benefit to infants, pregnant women, and aged alike.

vi. Individuals and food industries should consider incorporating fermentation as a viable step in the process to enhance the protein quality and other nutritional composition of cereals, as it could offer cost-effective benefits and viable economic implications.

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# APPENDICES

## APPENDIX A: Proximate analyses

**Appendix A1: Proximate analyses (%) for unfermented and fermented rice flour**

| Sample | Dry Matter | Moisture | Ash | Protein | Fat | Fibre | CHO |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D0-RF | 90.03047 | 9.96953 | 0.32698 | 8.34035 | 0.18729 | 4.49475 | 86.65063 |
| D0-RF | 90.01505 | 9.98495 | 0.35547 | 8.36303 | 0.18569 | 4.42846 | 86.66735 |
| D0-RF | 90.00877 | 9.99123 | 0.34804 | 8.13337 | 0.17897 | 4.41215 | 86.92747 |
| D5-RFsc | 50.92845 | 49.07155 | 0.66495 | 10.89510 | 0.11124 | 4.48270 | 83.84602 |
| D5-RFsc | 50.77823 | 49.22177 | 0.65814 | 10.97635 | 0.11254 | 4.82221 | 83.43076 |
| D5-RFsc | 50.85070 | 49.14930 | 0.65457 | 10.79276 | 0.11022 | 4.62359 | 83.81887 |
| D10-RFsc | 50.16655 | 49.83345 | 0.88088 | 11.63316 | 0.17439 | 4.39818 | 82.91338 |
| D10-RFsc | 50.17841 | 49.82159 | 0.89808 | 11.30472 | 0.16959 | 4.53413 | 83.09348 |
| D10-RFsc | 50.02481 | 49.97519 | 0.91219 | 11.66374 | 0.17326 | 4.46321 | 82.78759 |
| D5-RFlb | 51.71508 | 48.28492 | 0.39736 | 9.35027 | 0.11091 | 3.56251 | 86.57895 |
| D5-RFlb | 51.76015 | 48.23985 | 0.36366 | 9.75166 | 0.10987 | 3.25337 | 86.52144 |
| D5-RFlb | 51.81627 | 48.18373 | 0.32109 | 9.80927 | 0.11022 | 3.65875 | 86.10069 |
| D10-RFlb | 51.28614 | 48.71386 | 0.92702 | 10.75159 | 0.10656 | 4.54475 | 83.67008 |
| D10-RFlb | 51.44053 | 48.55947 | 0.95686 | 10.84423 | 0.11021 | 4.45239 | 83.63631 |
| D10-RFlb | 51.72429 | 48.27571 | 0.91710 | 10.73408 | 0.10897 | 4.46321 | 83.77665 |
| D5-RFsc(TSF) | 51.07398 | 48.92602 | 0.84338 | 10.78125 | 0.20650 | 4.35120 | 83.81767 |
| D5-RFsc(TSF) | 51.22398 | 48.77602 | 0.82835 | 10.41837 | 0.19659 | 4.67470 | 83.88200 |
| D5-RFsc(TSF) | 50.86147 | 49.13853 | 0.85368 | 10.53438 | 0.20024 | 4.52189 | 83.88981 |
| D10-RFlb(TSF) | 40.71561 | 59.28439 | 1.25945 | 14.68161 | 0.16599 | 4.21547 | 79.67748 |
| D10-RFlb(TSF) | 40.19728 | 59.80272 | 1.22056 | 14.07007 | 0.17021 | 4.36587 | 80.17329 |
| D10-RFlb(TSF) | 40.32221 | 59.67779 | 1.25534 | 14.49905 | 0.16959 | 4.12549 | 79.95053 |
| D5-RFlb(TSF) | 51.97855 | 48.02145 | 0.72504 | 10.28551 | 0.15072 | 3.20050 | 85.63822 |
| D5-RFlb(TSF) | 52.12141 | 47.87859 | 0.74560 | 10.05289 | 0.16525 | 3.46878 | 85.56748 |
| D5-RFlb(TSF) | 52.54410 | 47.45590 | 0.72397 | 10.06380 | 0.15988 | 3.52855 | 85.52381 |
| D10-RFsc(TSF) | 39.48212 | 60.51788 | 1.15418 | 15.35572 | 0.18261 | 5.03645 | 78.27105 |
| D10-RFsc(TSF) | 39.25761 | 60.74239 | 1.18818 | 15.15974 | 0.18550 | 5.08235 | 78.38423 |
| D10-RFsc(TSF) | 39.89766 | 60.10234 | 1.10997 | 15.06031 | 0.18966 | 4.98574 | 78.65432 |

**D0**- Day 0, **D5**- Day 5, **D10**- Day 10, **RF**- Rice flour, **sc**- *Saccharomyces cerevisiae*, **lb**- *Lactobacillus bulgaricus*, **TSF**- Two-step fermentation, **CHO**- Carbohydrate

**Appendix A2: Proximate analyses (%) for unfermented and fermented wheat flour**

| Sample | Dry Matter | Moisture | Ash | Protein | Fat | Fibre | CHO |
|---|---|---|---|---|---|---|---|
| D0-WF | 90.20108 | 9.79892 | 1.73208 | 10.88796 | 1.68141 | 7.98600 | 77.71256 |
| D0-WF | 90.25867 | 9.74133 | 1.66202 | 10.94688 | 1.62366 | 7.35639 | 78.41105 |
| D0-WF | 90.24894 | 9.75106 | 1.61270 | 10.57351 | 1.68585 | 7.63255 | 78.49539 |
| D5-WFsc | 49.90948 | 50.09052 | 1.85628 | 18.29928 | 1.31726 | 4.12274 | 74.40445 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D5-WFsc | 49.97078 | 50.02922 | 1.90167 | 18.40542 | 1.32655 | 4.12274 | 74.24362 |
| D5-WFsc | 49.61248 | 50.38752 | 1.87928 | 18.28399 | 1.29659 | 4.32658 | 74.21356 |
| D10-WFsc | 49.04506 | 50.95494 | 2.29047 | 19.08123 | 1.18839 | 4.01215 | 73.42776 |
| D10-WFsc | 49.08574 | 50.91426 | 2.47012 | 19.17179 | 1.18125 | 4.02658 | 73.15025 |
| D10-WFsc | 50.12800 | 49.87200 | 2.04276 | 19.25580 | 1.17897 | 4.00542 | 73.51706 |
| D5-WFlb | 51.41704 | 48.58296 | 2.21726 | 16.06215 | 1.25259 | 3.41649 | 76.32154 |
| D5-WFlb | 50.94516 | 49.05484 | 2.10522 | 16.87242 | 1.25125 | 3.93895 | 75.83217 |
| D5-WFlb | 51.07655 | 48.92345 | 2.02878 | 16.61458 | 1.24597 | 3.85480 | 76.25588 |
| D10-WFlb | 51.72213 | 48.27787 | 2.34742 | 17.29409 | 1.10907 | 3.89657 | 75.35285 |
| D10-WFlb | 51.51861 | 48.48139 | 2.42407 | 17.18642 | 1.11255 | 3.96587 | 75.31108 |
| D10-WFlb | 51.54241 | 48.45759 | 2.44173 | 17.74857 | 1.08458 | 3.82155 | 74.90357 |
| D5-WFsc(TSF) | 48.60646 | 51.39354 | 1.44472 | 19.13143 | 1.30239 | 4.08031 | 74.04114 |
| D5-WFsc(TSF) | 48.91799 | 51.08201 | 1.61186 | 19.01059 | 1.29659 | 3.83331 | 74.24766 |
| D5-WFsc(TSF) | 48.81224 | 51.18776 | 1.52985 | 19.07340 | 1.30022 | 4.01254 | 74.08400 |
| D10-WFlb(TSF) | 38.51209 | 61.48791 | 2.14195 | 20.21056 | 0.74514 | 3.82365 | 73.07869 |
| D10-WFlb(TSF) | 38.29064 | 61.70936 | 2.67750 | 20.26049 | 0.73266 | 3.72654 | 72.60280 |
| D10-WFlb(TSF) | 39.07629 | 60.92371 | 2.32916 | 20.01528 | 0.71210 | 3.89659 | 73.04687 |
| D5-WFlb(TSF) | 50.01445 | 49.98555 | 2.22112 | 17.14303 | 1.08481 | 3.13931 | 76.41173 |
| D5-WFlb(TSF) | 50.09407 | 49.90593 | 2.25344 | 17.17283 | 1.08966 | 3.38316 | 76.10091 |
| D5-WFlb(TSF) | 50.79907 | 49.20093 | 2.30093 | 17.04613 | 1.07870 | 3.65260 | 75.92165 |
| D10-WFsc(TSF) | 39.86754 | 60.13246 | 2.52934 | 19.80230 | 0.71426 | 3.88548 | 73.06862 |
| D10-WFsc(TSF) | 39.60785 | 60.39215 | 2.12086 | 19.84268 | 0.72155 | 4.00215 | 73.31276 |
| D10-WFsc(TSF) | 39.09586 | 60.90414 | 2.12675 | 19.88706 | 0.71966 | 4.01021 | 73.25632 |

**D0**- Day 0, **D5**- Day 5, **D10**- Day 10, **WF**- Wheat flour, **sc**- *Saccharomyces cerevisiae*, **lb**- *Lactobacillus bulgaricus*, **TSF**- Two-step fermentation, **CHO**- Carbohydrate

## Appendix A3: Proximate analyses (%) for 1:1 formulated blend of unfermented and fermented rice and wheat flour

| Sample | Dry Matter | Moisture | Ash | Protein | Fat | Fibre | CHO |
|---|---|---|---|---|---|---|---|
| D0-RF+WF | 91.82915 | 8.17085 | 1.12548 | 11.40073 | 0.86592 | 3.47687 | 83.13101 |
| D0-RF+WF | 92.02335 | 7.97665 | 1.19659 | 11.69319 | 0.85697 | 3.68342 | 82.56983 |
| D0-RF+WF | 92.04340 | 7.95660 | 1.17891 | 11.89097 | 0.86125 | 3.56985 | 82.49901 |
| D5-RF+WFsc | 52.08148 | 47.91852 | 1.45287 | 14.94816 | 0.70264 | 4.19525 | 78.70108 |
| D5-RF+WFsc | 52.65883 | 47.34117 | 1.32968 | 14.32174 | 0.71543 | 4.83923 | 78.79392 |
| D5-RF+WFsc | 52.17277 | 47.82723 | 1.57240 | 14.15882 | 0.70966 | 4.56232 | 78.99680 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D10-RF+WFsc | 48.97811 | 51.02189 | 1.61680 | 17.40453 | 0.84922 | 4.36759 | 75.76186 |
| D10-RF+WFsc | 48.78104 | 51.21896 | 1.51028 | 16.76705 | 0.82636 | 4.75320 | 76.14310 |
| D10-RF+WFsc | 48.86308 | 51.13692 | 1.50536 | 17.03016 | 0.83655 | 4.63296 | 75.99497 |
| D5-RF+WFlb | 53.54800 | 46.45200 | 1.63515 | 13.32527 | 0.73063 | 5.27188 | 79.03706 |
| D5-RF+WFlb | 53.29496 | 46.70504 | 1.65750 | 13.54067 | 0.72563 | 5.91041 | 78.16579 |
| D5-RF+WFlb | 53.25938 | 46.74062 | 1.57271 | 13.35383 | 0.74597 | 5.29659 | 79.03090 |
| D10-RF+WFlb | 50.68880 | 49.31120 | 1.61170 | 13.99168 | 0.62066 | 4.62813 | 79.14783 |
| D10-RF+WFlb | 51.04773 | 48.95227 | 1.28732 | 14.15446 | 0.62363 | 4.18513 | 79.74946 |
| D10-RF+ WFlb | 51.00210 | 48.99790 | 1.41585 | 14.72852 | 0.64251 | 4.42518 | 78.78793 |
| D5-RF+WFsc (TSF) | 47.87365 | 52.12635 | 1.47769 | 16.29513 | 0.77853 | 4.45167 | 76.99699 |
| D5-RF+WFsc (TSF) | 47.70195 | 52.29805 | 1.71341 | 16.50507 | 0.78451 | 4.26540 | 76.73160 |
| D5-RF+WFsc (TSF) | 47.65969 | 52.34031 | 1.56966 | 16.69320 | 0.77215 | 4.36259 | 76.60240 |
| D10-RF+WFlb (TSF) | 37.02726 | 62.97274 | 1.81355 | 17.59270 | 0.27947 | 3.76797 | 76.54631 |
| D10-RF+WFlb (TSF) | 37.07947 | 62.92053 | 1.84247 | 17.78906 | 0.30125 | 3.39344 | 76.67377 |
| D10-RF+WFlb (TSF) | 37.18574 | 62.81426 | 1.84680 | 17.86607 | 0.29855 | 0.56963 | 79.41895 |
| D5-RF+WFlb (TSF) | 51.76635 | 48.23365 | 1.55569 | 14.45745 | 0.67629 | 3.56800 | 79.74257 |
| D5-RF+WFlb (TSF) | 51.75709 | 48.24291 | 1.60641 | 14.29222 | 0.67459 | 3.04442 | 80.38236 |
| D5-RF+WFlb (TSF) | 51.86598 | 48.13402 | 1.53625 | 14.44216 | 0.65987 | 3.21548 | 80.14624 |
| D10-RF+WFsc (TSF) | 38.96840 | 61.03160 | 1.75049 | 16.32725 | 0.22371 | 4.86423 | 76.83432 |
| D10-RF+WFsc (TSF) | 39.08220 | 60.91780 | 1.59349 | 16.96540 | 0.25487 | 4.12950 | 77.05714 |
| D10-RF+WFsc (TSF) | 38.89916 | 61.10084 | 1.75085 | 16.90329 | 0.24966 | 4.29658 | 76.79963 |

**D0**- Day 0, **D5**- Day 5, **D10**- Day 10, **RF**- Rice flour, **WF**- Wheat flour, **sc**- *Saccharomyces cerevisiae*, **lb**- *Lactobacillus bulgaricus*, **TSF**- Two-step fermentation, **CHO**- Carbohydrate

## Appendix A4: Proximate analyses (%) for 1:3 formulated blend of unfermented and fermented rice and wheat flour

| Sample | Dry Matter | Moisture | Ash | Protein | Fat | Fibre | CHO |
|---|---|---|---|---|---|---|---|
| D0-RF+WF 1:3 | 91.32520 | 8.67480 | 1.39556 | 12.75707 | 0.64811 | 4.91864 | 80.28062 |
| D0-RF+WF 1:3 | 91.34706 | 8.65294 | 1.52855 | 12.97975 | 0.65214 | 4.80160 | 80.03796 |
| D0-RF+WF 1:3 | 91.48403 | 8.51597 | 1.56361 | 12.99270 | 0.63599 | 5.02155 | 79.78616 |
| D5-RF+WFsc 1:3 | 50.05588 | 49.94412 | 1.87121 | 16.18601 | 0.35333 | 4.43418 | 77.15527 |
| D5-RF+WFsc 1:3 | 50.00711 | 49.99289 | 1.81777 | 16.37935 | 0.36255 | 4.16733 | 77.27300 |
| D5-RF+WFsc 1:3 | 49.86261 | 50.13739 | 1.74283 | 16.77126 | 0.34587 | 4.56933 | 76.57071 |
| D10-RF+WFsc 1:3 | 49.99229 | 50.00771 | 2.29628 | 18.35344 | 0.36698 | 5.63589 | 73.34741 |
| D10-RF+WFsc 1:3 | 49.26530 | 50.73470 | 2.26227 | 18.45827 | 0.35965 | 4.33026 | 74.58954 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D10-RF+WFsc 1:3 | 49.35874 | 50.64126 | 2.20575 | 18.77597 | 0.37855 | 4.96587 | 73.67385 |
| D5-RF+WFlb 1:3 | 51.50795 | 48.49205 | 1.87920 | 15.08477 | 0.20873 | 3.55913 | 79.26817 |
| D5-RF+WFlb 1:3 | 50.90541 | 49.09459 | 1.22587 | 15.00171 | 0.21327 | 4.66945 | 78.88971 |
| D5-RF+WFlb 1:3 | 50.43977 | 49.56023 | 1.87094 | 15.07026 | 0.22342 | 3.96599 | 78.86940 |
| D10-RF+WFlb 1:3 | 50.86234 | 49.13766 | 2.01796 | 16.45559 | 0.42862 | 4.16062 | 76.93721 |
| D10-RF+WFlb 1:3 | 50.66748 | 49.33252 | 2.54229 | 16.48793 | 0.41257 | 4.21927 | 76.33793 |
| D10-RF+WFlb 1:3 | 51.94343 | 48.05657 | 2.04050 | 16.42318 | 0.43266 | 4.36266 | 76.74100 |
| D5-RF+WFsc 1:3/TSF | 52.52223 | 47.47777 | 1.68869 | 19.34420 | 0.26286 | 4.23587 | 74.46838 |
| D5-RF+WFsc 1:3/TSF | 52.81546 | 47.18454 | 1.72766 | 19.64838 | 0.27854 | 5.50162 | 72.84380 |
| D5-RF+WFsc 1:3/TSF | 52.85478 | 47.14522 | 1.74588 | 19.84103 | 0.26959 | 5.20215 | 72.94136 |
| D10-RF+WFlb 1:3/TSF | 37.24272 | 62.75728 | 2.08137 | 20.04074 | 0.38608 | 3.98574 | 73.50608 |
| D10-RF+WFlb 1:3/TSF | 38.62550 | 61.37450 | 2.12684 | 20.21893 | 0.39563 | 3.78542 | 73.47319 |
| D10-RF+WFlb 1:3/TSF | 37.19170 | 62.80830 | 2.16436 | 20.18842 | 0.36260 | 3.88966 | 73.39497 |
| D5-RF+WFlb 1:3/TSF | 50.72011 | 49.27989 | 1.84618 | 17.18897 | 0.39761 | 6.45312 | 74.11412 |
| D5-RF+WFlb 1:3/TSF | 50.50764 | 49.49236 | 1.94931 | 17.17891 | 0.40215 | 6.97108 | 73.49854 |
| D5-RF+WFlb 1:3/TSF | 50.45897 | 49.54103 | 1.92657 | 17.24695 | 0.39569 | 6.48597 | 73.94482 |
| D10-RF+WFsc 1:3/TSF | 38.98860 | 61.01140 | 2.14342 | 21.33586 | 0.32192 | 4.88353 | 71.31527 |
| D10-RF+WFsc 1:3/TSF | 39.96189 | 60.03811 | 2.00806 | 21.91210 | 0.33263 | 3.82461 | 71.92260 |
| D10-RF+WFsc 1:3/TSF | 39.13001 | 60.86999 | 2.11638 | 21.11219 | 0.34125 | 3.98548 | 72.44470 |

**D0**- Day 0, **D5**- Day 5, **D10**- Day 10, **RF**- Rice flour, **WF**- Wheat flour, **sc**- *Saccharomyces cerevisiae*, **lb**- *Lactobacillus bulgaricus*, **TSF**- Two-step fermentation, **CHO**- Carbohydrate

## Appendix A5: Proximate analysis (%) for 3:1 formulated blend of unfermented and fermented rice and wheat flour

| Sample | Dry Matter | Moisture | Ash | Protein | Fat | Fibre | CHO |
|---|---|---|---|---|---|---|---|
| D0-RF+WF 3:1 | 89.72759 | 10.27241 | 0.86352 | 10.09642 | 1.06751 | 3.26584 | 84.70671 |
| D0-RF+WF 3:1 | 89.72834 | 10.27166 | 0.92510 | 10.65039 | 1.03266 | 3.26514 | 84.12671 |
| D0-RF+WF 3:1 | 89.83362 | 10.16638 | 0.86817 | 10.81090 | 1.04860 | 3.21549 | 84.05684 |
| D5-RF+WFsc 3:1 | 51.07986 | 48.92014 | 1.08062 | 14.12082 | 0.99942 | 4.80754 | 78.99160 |
| D5-RF+WFsc 3:1 | 50.55257 | 49.44743 | 1.00800 | 13.94392 | 0.98570 | 4.32660 | 79.73579 |
| D5-RF+WFsc 3:1 | 50.51720 | 49.48280 | 0.99383 | 14.13000 | 0.97854 | 4.56237 | 79.33526 |
| D10-RF+WFsc 3:1 | 49.97008 | 50.02992 | 1.15772 | 16.20999 | 0.74215 | 4.57917 | 77.31098 |
| D10-RF+WFsc 3:1 | 49.78763 | 50.21237 | 1.16940 | 16.60320 | 0.73970 | 4.45215 | 77.03554 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D10-RF+WFsc 3:1 | 49.91517 | 50.08483 | 1.09449 | 16.25948 | 0.74123 | 4.61202 | 77.29277 |
| D5-RF+WFlb 3:1 | 50.97380 | 49.02620 | 1.09992 | 11.37322 | 0.88065 | 3.49162 | 83.15459 |
| D5-RF+WFlb 3:1 | 50.71831 | 49.28169 | 0.99159 | 11.64266 | 0.89657 | 3.47862 | 82.99056 |
| D5-RF+WFlb 3:1 | 50.77145 | 49.22855 | 1.13632 | 11.78776 | 0.88215 | 3.41255 | 82.78122 |
| D10-RF+WFlb 3:1 | 50.76111 | 49.23889 | 1.01709 | 15.70272 | 0.71125 | 4.48870 | 78.08024 |
| D10-RF+WFlb 3:1 | 51.00492 | 48.99508 | 1.01378 | 15.72331 | 0.71855 | 4.39659 | 78.14778 |
| D10-RF+WFlb 3:1 | 51.09331 | 48.90669 | 1.08418 | 15.41183 | 0.71625 | 4.42518 | 78.36255 |
| D5-RF+WFsc 3:1/TSF | 45.92248 | 54.07752 | 0.99715 | 15.36425 | 0.81761 | 5.85586 | 76.96514 |
| D5-RF+WFsc 3:1/TSF | 47.28496 | 52.71504 | 0.91361 | 15.35119 | 0.81255 | 5.65249 | 77.27016 |
| D5-RF+WFsc 3:1/TSF | 45.50397 | 54.49603 | 0.90002 | 15.73182 | 0.81003 | 5.56597 | 76.99216 |
| D10-RF+WFlb 3:1/TSF | 36.98000 | 63.02000 | 1.19786 | 16.48322 | 0.54275 | 3.58457 | 78.19160 |
| D10-RF+WFlb 3:1/TSF | 37.03317 | 62.96683 | 1.29821 | 16.32028 | 0.54269 | 3.41260 | 78.42622 |
| D10-RF+WFlb 3:1/TSF | 37.01638 | 62.98362 | 1.27463 | 16.64410 | 0.53969 | 3.51202 | 78.02956 |
| D5-RF+WFlb 3:1/TSF | 51.86054 | 48.13946 | 1.06651 | 13.04609 | 0.71388 | 3.00429 | 82.16923 |
| D5-RF+WFlb 3:1/TSF | 52.12390 | 47.87610 | 1.06743 | 13.22593 | 0.72155 | 3.12547 | 81.85962 |
| D5-RF+WFlb 3:1/TSF | 51.18153 | 48.81847 | 1.18156 | 13.19921 | 0.71985 | 3.26575 | 81.63362 |
| D10-RF+WFsc 3:1/TSF | 40.41888 | 59.58112 | 1.02850 | 17.31430 | 0.53447 | 3.73075 | 77.39198 |
| D10-RF+WFsc 3:1/TSF | 39.24551 | 60.75449 | 1.01861 | 17.08625 | 0.54120 | 3.65215 | 77.70179 |
| D10-RF+WFsc 3:1/TSF | 39.50529 | 60.49471 | 1.25966 | 17.27750 | 0.53887 | 3.48653 | 77.43744 |

**D0**- Day 0, **D5**- Day 5, **D10**- Day 10, **RF**- Rice flour, **WF**- Wheat flour, **sc**- *Saccharomyces cerevisiae*, **lb**- *Lactobacillus bulgaricus*, **TSF**- Two-step fermentation, **CHO**- Carbohydrate

## APPENDIX B: Statistical analyses

### Appendix B1: ANOVA test for proximate analysis of rice flour fermented with *S. cerevisiae*

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3126.077 | 2 | 1563.039 | 358700.922 | .000 |
| | Within Groups | .026 | 6 | .004 | | |
| | Total | 3126.103 | 8 | | | |
| Moisture | Between Groups | 3126.077 | 2 | 1563.039 | 358700.922 | .000 |
| | Within Groups | .026 | 6 | .004 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | 3126.103 | 8 | | | |
| Ash | Between Groups | .463 | 2 | .231 | 1410.316 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .464 | 8 | | | |
| Protein | Between Groups | 17.819 | 2 | 8.910 | 417.012 | .000 |
| | Within Groups | .128 | 6 | .021 | | |
| | Total | 17.948 | 8 | | | |
| Fat | Between Groups | .009 | 2 | .005 | 505.307 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .009 | 8 | | | |
| Fibre | Between Groups | .071 | 2 | .036 | 2.991 | .126 |
| | Within Groups | .071 | 6 | .012 | | |
| | Total | .142 | 8 | | | |
| CHO | Between Groups | 24.460 | 2 | 12.230 | 360.756 | .000 |
| | Within Groups | .203 | 6 | .034 | | |
| | Total | 24.663 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B2: ANOVA test for proximate analysis of rice flour fermented with *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 2948.370 | 2 | 1474.185 | 84913.148 | .000 |
| | Within Groups | .104 | 6 | .017 | | |
| | Total | 2948.475 | 8 | | | |
| Moisture | Between Groups | 2948.370 | 2 | 1474.185 | 84913.148 | .000 |
| | Within Groups | .104 | 6 | .017 | | |
| | Total | 2948.475 | 8 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ash | Between Groups | .677 | 2 | .338 | 481.753 | .000 |
| | Within Groups | .004 | 6 | .001 | | |
| | Total | .681 | 8 | | | |
| Protein | Between Groups | 9.382 | 2 | 4.691 | 171.536 | .000 |
| | Within Groups | .164 | 6 | .027 | | |
| | Total | 9.546 | 8 | | | |
| Fat | Between Groups | .011 | 2 | .006 | 718.057 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .011 | 8 | | | |
| Fibre | Between Groups | 1.902 | 2 | .951 | 57.829 | .000 |
| | Within Groups | .099 | 6 | .016 | | |
| | Total | 2.000 | 8 | | | |
| CHO | Between Groups | 16.771 | 2 | 8.386 | 257.650 | .000 |
| | Within Groups | .195 | 6 | .033 | | |
| | Total | 16.967 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B3: ANOVA test for proximate analysis of rice flour fermented with *S. cerevisiae* followed by *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 4092.302 | 2 | 2046.151 | 57650.770 | .000 |
| | Within Groups | .213 | 6 | .035 | | |
| | Total | 4092.515 | 8 | | | |
| Moisture | Between Groups | 4092.302 | 2 | 2046.151 | 57650.770 | .000 |
| | Within Groups | .213 | 6 | .035 | | |
| | Total | 4092.515 | 8 | | | |
| Ash | Between Groups | 1.224 | 2 | .612 | 2192.923 | .000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | .002 | 6 | .000 | | |
| | Total | 1.226 | 8 | | | |
| Protein | Between Groups | 57.698 | 2 | 28.849 | 581.166 | .000 |
| | Within Groups | .298 | 6 | .050 | | |
| | Total | 57.996 | 8 | | | |
| Fat | Between Groups | .002 | 2 | .001 | 47.805 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .002 | 8 | | | |
| Fibre | Between Groups | .127 | 2 | .064 | 4.462 | .065 |
| | Within Groups | .086 | 6 | .014 | | |
| | Total | .213 | 8 | | | |
| CHO | Between Groups | 70.206 | 2 | 35.103 | 1205.840 | .000 |
| | Within Groups | .175 | 6 | .029 | | |
| | Total | 70.380 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B4: ANOVA test for proximate analysis of rice flour fermented with *L. bulgaricus* followed by *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 4137.052 | 2 | 2068.526 | 32309.134 | .000 |
| | Within Groups | .384 | 6 | .064 | | |
| | Total | 4137.436 | 8 | | | |
| Moisture | Between Groups | 4137.052 | 2 | 2068.526 | 32309.134 | .000 |
| | Within Groups | .384 | 6 | .064 | | |
| | Total | 4137.436 | 8 | | | |
| Ash | Between Groups | .978 | 2 | .489 | 770.146 | .000 |
| | Within Groups | .004 | 6 | .001 | | |
| | Total | .982 | 8 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Protein | Between Groups | 76.813 | 2 | 38.407 | 2063.349 | .000 |
| | Within Groups | .112 | 6 | .019 | | |
| | Total | 76.925 | 8 | | | |
| Fat | Between Groups | .001 | 2 | .001 | 24.287 | .001 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .002 | 8 | | | |
| Fibre | Between Groups | 4.117 | 2 | 2.058 | 177.562 | .000 |
| | Within Groups | .070 | 6 | .012 | | |
| | Total | 4.186 | 8 | | | |
| CHO | Between Groups | 121.441 | 2 | 60.721 | 2751.430 | .000 |
| | Within Groups | .132 | 6 | .022 | | |
| | Total | 121.574 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B5: ANOVA test for proximate analysis of wheat flour fermented with *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 3298.756 | 2 | 1649.378 | 11938.715 | .000 |
| | Within Groups | .829 | 6 | .138 | | |
| | Total | 3299.585 | 8 | | | |
| Moisture | Between Groups | 3298.756 | 2 | 1649.378 | 11938.715 | .000 |
| | Within Groups | .829 | 6 | .138 | | |
| | Total | 3299.585 | 8 | | | |
| Ash | Between Groups | .554 | 2 | .277 | 16.564 | .004 |
| | Within Groups | .100 | 6 | .017 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | .654 | 8 | | | |
| Protein | Between Groups | 127.362 | 2 | 63.681 | 3653.596 | .000 |
| | Within Groups | .105 | 6 | .017 | | |
| | Total | 127.466 | 8 | | | |
| Fat | Between Groups | .371 | 2 | .185 | 380.176 | .000 |
| | Within Groups | .003 | 6 | .000 | | |
| | Total | .374 | 8 | | | |
| Fibre | Between Groups | 25.331 | 2 | 12.666 | 334.576 | .000 |
| | Within Groups | .227 | 6 | .038 | | |
| | Total | 25.558 | 8 | | | |
| CHO | Between Groups | 39.648 | 2 | 19.824 | 256.610 | .000 |
| | Within Groups | .464 | 6 | .077 | | |
| | Total | 40.112 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B6: ANOVA test for proximate analysis of wheat flour fermented with *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 3021.420 | 2 | 1510.710 | 62389.435 | .000 |
| | Within Groups | .145 | 6 | .024 | | |
| | Total | 3021.565 | 8 | | | |
| Moisture | Between Groups | 3021.420 | 2 | 1510.710 | 62389.435 | .000 |
| | Within Groups | .145 | 6 | .024 | | |
| | Total | 3021.565 | 8 | | | |
| Ash | Between Groups | .824 | 2 | .412 | 81.890 | .000 |
| | Within Groups | .030 | 6 | .005 | | |
| | Total | .855 | 8 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Protein | Between Groups | 77.094 | 2 | 38.547 | 384.594 | .000 |
| | Within Groups | .601 | 6 | .100 | | |
| | Total | 77.696 | 8 | | | |
| Fat | Between Groups | .508 | 2 | .254 | 526.512 | .000 |
| | Within Groups | .003 | 6 | .000 | | |
| | Total | .511 | 8 | | | |
| Fibre | Between Groups | 29.569 | 2 | 14.784 | 241.701 | .000 |
| | Within Groups | .367 | 6 | .061 | | |
| | Total | 29.936 | 8 | | | |
| CHO | Between Groups | 14.285 | 2 | 7.142 | 67.636 | .000 |
| | Within Groups | .634 | 6 | .106 | | |
| | Total | 14.918 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B7: ANOVA test for proximate analysis of wheat flour fermented with *S. cerevisiae* followed by *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 4485.366 | 2 | 2242.683 | 35383.191 | .000 |
| | Within Groups | .380 | 6 | .063 | | |
| | Total | 4485.746 | 8 | | | |
| Moisture | Between Groups | 4485.366 | 2 | 2242.683 | 35383.191 | .000 |
| | Within Groups | .380 | 6 | .063 | | |
| | Total | 4485.746 | 8 | | | |
| Ash | Between Groups | 1.259 | 2 | .629 | 22.358 | .002 |
| | Within Groups | .169 | 6 | .028 | | |
| | Total | 1.428 | 8 | | | |
| Protein | Between Groups | 157.163 | 2 | 78.581 | 3881.348 | .000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | .121 | 6 | .020 | | |
| | Total | 157.284 | 8 | | | |
| Fat | Between Groups | 1.329 | 2 | .664 | 1337.127 | .000 |
| | Within Groups | .003 | 6 | .000 | | |
| | Total | 1.332 | 8 | | | |
| Fibre | Between Groups | 28.356 | 2 | 14.178 | 345.340 | .000 |
| | Within Groups | .246 | 6 | .041 | | |
| | Total | 28.602 | 8 | | | |
| CHO | Between Groups | 46.196 | 2 | 23.098 | 259.240 | .000 |
| | Within Groups | .535 | 6 | .089 | | |
| | Total | 46.731 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B8: ANOVA test for proximate analysis of wheat flour fermented with *L. bulgaricus* followed by *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 4282.638 | 2 | 2141.319 | 18804.051 | .000 |
| | Within Groups | .683 | 6 | .114 | | |
| | Total | 4283.321 | 8 | | | |
| Moisture | Between Groups | 4282.638 | 2 | 2141.319 | 18804.051 | .000 |
| | Within Groups | .683 | 6 | .114 | | |
| | Total | 4283.321 | 8 | | | |
| Ash | Between Groups | .696 | 2 | .348 | 17.382 | .003 |
| | Within Groups | .120 | 6 | .020 | | |
| | Total | .816 | 8 | | | |
| Protein | Between Groups | 129.076 | 2 | 64.538 | 4165.679 | .000 |
| | Within Groups | .093 | 6 | .015 | | |
| | Total | 129.169 | 8 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Fat | Between Groups | 1.363 | 2 | .681 | 1637.619 | .000 |
| | Within Groups | .002 | 6 | .000 | | |
| | Total | 1.365 | 8 | | | |
| Fibre | Between Groups | 32.167 | 2 | 16.084 | 283.174 | .000 |
| | Within Groups | .341 | 6 | .057 | | |
| | Total | 32.508 | 8 | | | |
| CHO | Between Groups | 37.786 | 2 | 18.893 | 215.949 | .000 |
| | Within Groups | .525 | 6 | .087 | | |
| | Total | 38.310 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B9: ANOVA test for proximate analysis of 1:1 formulated blend of rice and wheat flour fermented with *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3441.611 | 2 | 1720.805 | 42976.024 | .000 |
| | Within Groups | .240 | 6 | .040 | | |
| | Total | 3441.851 | 8 | | | |
| Moisture | Between Groups | 3441.611 | 2 | 1720.805 | 42976.024 | .000 |
| | Within Groups | .240 | 6 | .040 | | |
| | Total | 3441.851 | 8 | | | |
| Ash | Between Groups | .232 | 2 | .116 | 17.331 | .003 |
| | Within Groups | .040 | 6 | .007 | | |
| | Total | .272 | 8 | | | |
| Protein | Between Groups | 43.856 | 2 | 21.928 | 195.133 | .000 |
| | Within Groups | .674 | 6 | .112 | | |
| | Total | 44.530 | 8 | | | |
| Fat | Between Groups | .040 | 2 | .020 | 313.231 | .000 |
| | Within Groups | .000 | 6 | .000 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Total | .041 | 8 | | | |
| Fibre | Between Groups | 1.932 | 2 | .966 | 18.816 | .003 |
| | Within Groups | .308 | 6 | .051 | | |
| | Total | 2.240 | 8 | | | |
| CHO | Between Groups | 69.221 | 2 | 34.610 | 577.791 | .000 |
| | Within Groups | .359 | 6 | .060 | | |
| | Total | 69.580 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B10: ANOVA test for proximate analysis of 1:1 formulated blend of rice and wheat flour fermented with *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 3181.121 | 2 | 1590.560 | 62012.148 | .000 |
| | Within Groups | .154 | 6 | .026 | | |
| | Total | 3181.275 | 8 | | | |
| Moisture | Between Groups | 3181.121 | 2 | 1590.560 | 62012.148 | .000 |
| | Within Groups | .154 | 6 | .026 | | |
| | Total | 3181.275 | 8 | | | |
| Ash | Between Groups | .314 | 2 | .157 | 15.713 | .004 |
| | Within Groups | .060 | 6 | .010 | | |
| | Total | .374 | 8 | | | |
| Protein | Between Groups | 10.745 | 2 | 5.372 | 71.839 | .000 |
| | Within Groups | .449 | 6 | .075 | | |
| | Total | 11.193 | 8 | | | |
| Fat | Between Groups | .081 | 2 | .041 | 446.947 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .082 | 8 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Fibre | Between Groups | 5.538 | 2 | 2.769 | 43.552 | .000 |
| | Within Groups | .381 | 6 | .064 | | |
| | Total | 5.919 | 8 | | | |
| CHO | Between Groups | 28.428 | 2 | 14.214 | 70.232 | .000 |
| | Within Groups | 1.214 | 6 | .202 | | |
| | Total | 29.642 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B11: ANOVA test for proximate analysis of 1:1 formulated blend of rice and wheat flour fermented with *S. cerevisiae* followed by *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 5079.274 | 2 | 2539.637 | 228339.593 | .000 |
| | Within Groups | .067 | 6 | .011 | | |
| | Total | 5079.341 | 8 | | | |
| Moisture | Between Groups | 5079.274 | 2 | 2539.637 | 228339.593 | .000 |
| | Within Groups | .067 | 6 | .011 | | |
| | Total | 5079.341 | 8 | | | |
| Ash | Between Groups | .683 | 2 | .341 | 64.773 | .000 |
| | Within Groups | .032 | 6 | .005 | | |
| | Total | .714 | 8 | | | |
| Protein | Between Groups | 62.014 | 2 | 31.007 | 772.889 | .000 |
| | Within Groups | .241 | 6 | .040 | | |
| | Total | 62.255 | 8 | | | |
| Fat | Between Groups | .565 | 2 | .283 | 4257.357 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .566 | 8 | | | |
| Fibre | Between Groups | 4.791 | 2 | 2.396 | 2.336 | .178 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | 6.153 | 6 | 1.026 | | |
| | Total | 10.945 | 8 | | | |
| CHO | Between Groups | 62.974 | 2 | 31.487 | 33.803 | .001 |
| | Within Groups | 5.589 | 6 | .931 | | |
| | Total | 68.562 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B12: ANOVA test for proximate analysis of 1:1 formulated blend of rice and wheat flour fermented with *L. bulgaricus* followed by *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry matter | Between Groups | 4584.811 | 2 | 2292.405 | 262596.260 | .000 |
| | Within Groups | .052 | 6 | .009 | | |
| | Total | 4584.863 | 8 | | | |
| Moisture | Between Groups | 4584.811 | 2 | 2292.405 | 262596.260 | .000 |
| | Within Groups | .052 | 6 | .009 | | |
| | Total | 4584.863 | 8 | | | |
| Ash | Between Groups | .459 | 2 | .230 | 63.065 | .000 |
| | Within Groups | .022 | 6 | .004 | | |
| | Total | .481 | 8 | | | |
| Protein | Between Groups | 38.641 | 2 | 19.321 | 300.486 | .000 |
| | Within Groups | .386 | 6 | .064 | | |
| | Total | 39.027 | 8 | | | |
| Fat | Between Groups | .602 | 2 | .301 | 2375.359 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .603 | 8 | | | |
| Fibre | Between Groups | 2.151 | 2 | 1.075 | 14.008 | .005 |
| | Within Groups | .461 | 6 | .077 | | |
| | Total | 2.611 | 8 | | | |
| CHO | Between Groups | 51.244 | 2 | 25.622 | 314.906 | .000 |

| | | | | |
|---|---|---|---|---|
| | Within Groups | .488 | 6 | .081 |
| | Total | 51.732 | 8 | |

**CHO**- Carbohydrates

**Appendix B13: ANOVA test for proximate analysis of 1:3 formulated blend of rice and wheat flour fermented with *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3466.140 | 2 | 1733.070 | 29888.855 | .000 |
| | Within Groups | .348 | 6 | .058 | | |
| | Total | 3466.488 | 8 | | | |
| Moisture | Between Groups | 3466.140 | 2 | 1733.070 | 29888.855 | .000 |
| | Within Groups | .348 | 6 | .058 | | |
| | Total | 3466.488 | 8 | | | |
| Ash | Between Groups | .872 | 2 | .436 | 92.724 | .000 |
| | Within Groups | .028 | 6 | .005 | | |
| | Total | .900 | 8 | | | |
| Protein | Between Groups | 48.420 | 2 | 24.210 | 468.978 | .000 |
| | Within Groups | .310 | 6 | .052 | | |
| | Total | 48.730 | 8 | | | |
| Fat | Between Groups | .162 | 2 | .081 | 1050.183 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .162 | 8 | | | |
| Fibre | Between Groups | .623 | 2 | .311 | 1.946 | .223 |
| | Within Groups | .960 | 6 | .160 | | |
| | Total | 1.583 | 8 | | | |
| CHO | Between Groups | 57.009 | 2 | 28.504 | 138.539 | .000 |
| | Within Groups | 1.234 | 6 | .206 | | |
| | Total | 58.243 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B14: ANOVA test for proximate analysis of 1:3 formulated blend of rice and wheat flour fermented with *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3253.249 | 2 | 1626.624 | 6364.881 | .000 |
| | Within Groups | 1.533 | 6 | .256 | | |
| | Total | 3254.782 | 8 | | | |
| Moisture | Between Groups | 3253.249 | 2 | 1626.624 | 6364.881 | .000 |
| | Within Groups | 1.533 | 6 | .256 | | |
| | Total | 3254.782 | 8 | | | |
| Ash | Between Groups | .816 | 2 | .408 | 5.181 | .049 |
| | Within Groups | .472 | 6 | .079 | | |
| | Total | 1.288 | 8 | | | |
| Protein | Between Groups | 19.131 | 2 | 9.566 | 1395.625 | .000 |
| | Within Groups | .041 | 6 | .007 | | |
| | Total | 19.173 | 8 | | | |
| Fat | Between Groups | .278 | 2 | .139 | 1734.934 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .278 | 8 | | | |
| Fibre | Between Groups | 1.198 | 2 | .599 | 5.311 | .047 |
| | Within Groups | .677 | 6 | .113 | | |
| | Total | 1.875 | 8 | | | |
| CHO | Between Groups | 17.823 | 2 | 8.911 | 130.461 | .000 |
| | Within Groups | .410 | 6 | .068 | | |
| | Total | 18.233 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B15: ANOVA test for proximate analysis of 1:3 formulated blend of rice and wheat flour fermented with *S. cerevisiae* followed by *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 4604.066 | 2 | 2302.033 | 9835.294 | .000 |
| | Within Groups | 1.404 | 6 | .234 | | |
| | Total | 4605.470 | 8 | | | |
| Moisture | Between Groups | 4604.066 | 2 | 2302.033 | 9835.294 | .000 |
| | Within Groups | 1.404 | 6 | .234 | | |
| | Total | 4605.470 | 8 | | | |
| Ash | Between Groups | .608 | 2 | .304 | 87.365 | .000 |
| | Within Groups | .021 | 6 | .003 | | |
| | Total | .629 | 8 | | | |
| Protein | Between Groups | 97.609 | 2 | 48.804 | 1638.195 | .000 |
| | Within Groups | .179 | 6 | .030 | | |
| | Total | 97.787 | 8 | | | |
| Fat | Between Groups | .223 | 2 | .111 | 792.674 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .224 | 8 | | | |
| Fibre | Between Groups | 2.254 | 2 | 1.127 | 7.353 | .024 |
| | Within Groups | .919 | 6 | .153 | | |
| | Total | 3.173 | 8 | | | |
| CHO | Between Groups | 87.042 | 2 | 43.521 | 145.965 | .000 |
| | Within Groups | 1.789 | 6 | .298 | | |
| | Total | 88.831 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B16: ANOVA test for proximate analysis of 1:3 formulated blend of rice and wheat flour fermented with *L. bulgaricus* followed by *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 4498.647 | 2 | 2249.324 | 22252.336 | .000 |
| | Within Groups | .606 | 6 | .101 | | |
| | Total | 4499.254 | 8 | | | |
| Moisture | Between Groups | 4498.647 | 2 | 2249.324 | 22252.336 | .000 |
| | Within Groups | .606 | 6 | .101 | | |
| | Total | 4499.254 | 8 | | | |
| Ash | Between Groups | .554 | 2 | .277 | 52.223 | .000 |
| | Within Groups | .032 | 6 | .005 | | |
| | Total | .586 | 8 | | | |
| Protein | Between Groups | 109.489 | 2 | 54.745 | 867.964 | .000 |
| | Within Groups | .378 | 6 | .063 | | |
| | Total | 109.868 | 8 | | | |
| Fat | Between Groups | .164 | 2 | .082 | 1399.387 | .000 |
| | Within Groups | .000 | 6 | .000 | | |
| | Total | .164 | 8 | | | |
| Fibre | Between Groups | 9.221 | 2 | 4.610 | 32.787 | .001 |
| | Within Groups | .844 | 6 | .141 | | |
| | Total | 10.064 | 8 | | | |
| CHO | Between Groups | 108.329 | 2 | 54.164 | 337.286 | .000 |
| | Within Groups | .964 | 6 | .161 | | |
| | Total | 109.292 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B17: ANOVA test for proximate analysis of 3:1 formulated blend of rice and wheat flour fermented with *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3115.116 | 2 | 1557.558 | 41796.903 | .000 |
| | Within Groups | .224 | 6 | .037 | | |
| | Total | 3115.339 | 8 | | | |
| Moisture | Between Groups | 3115.116 | 2 | 1557.558 | 41796.903 | .000 |
| | Within Groups | .224 | 6 | .037 | | |
| | Total | 3115.339 | 8 | | | |
| Ash | Between Groups | .098 | 2 | .049 | 29.562 | .001 |
| | Within Groups | .010 | 6 | .002 | | |
| | Total | .108 | 8 | | | |
| Protein | Between Groups | 51.914 | 2 | 25.957 | 394.504 | .000 |
| | Within Groups | .395 | 6 | .066 | | |
| | Total | 52.309 | 8 | | | |
| Fat | Between Groups | .160 | 2 | .080 | 573.359 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .161 | 8 | | | |
| Fibre | Between Groups | 3.421 | 2 | 1.711 | 77.998 | .000 |
| | Within Groups | .132 | 6 | .022 | | |
| | Total | 3.553 | 8 | | | |
| CHO | Between Groups | 79.191 | 2 | 39.596 | 410.005 | .000 |
| | Within Groups | .579 | 6 | .097 | | |
| | Total | 79.771 | 8 | | | |

**CHO-** Carbohydrates

**Appendix B18: ANOVA test for proximate analysis of 3:1 formulated blend of rice and wheat flour fermented with *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 3022.718 | 2 | 1511.359 | 88044.813 | .000 |
| | Within Groups | .103 | 6 | .017 | | |
| | Total | 3022.821 | 8 | | | |
| Moisture | Between Groups | 3022.718 | 2 | 1511.359 | 88044.813 | .000 |
| | Within Groups | .103 | 6 | .017 | | |
| | Total | 3022.821 | 8 | | | |
| Ash | Between Groups | .061 | 2 | .030 | 10.861 | .010 |
| | Within Groups | .017 | 6 | .003 | | |
| | Total | .078 | 8 | | | |
| Protein | Between Groups | 43.205 | 2 | 21.602 | 301.268 | .000 |
| | Within Groups | .430 | 6 | .072 | | |
| | Total | 43.635 | 8 | | | |
| Fat | Between Groups | .168 | 2 | .084 | 635.566 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .168 | 8 | | | |
| Fibre | Between Groups | 2.409 | 2 | 1.204 | 744.359 | .000 |
| | Within Groups | .010 | 6 | .002 | | |
| | Total | 2.418 | 8 | | | |
| CHO | Between Groups | 61.790 | 2 | 30.895 | 503.658 | .000 |
| | Within Groups | .368 | 6 | .061 | | |
| | Total | 62.158 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B19: ANOVA test for proximate analysis of 3:1 formulated blend of rice and wheat flour fermented with *S. cerevisiae* followed by *L. bulgaricus***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 4762.574 | 2 | 2381.287 | 8195.341 | .000 |
| | Within Groups | 1.743 | 6 | .291 | | |
| | Total | 4764.317 | 8 | | | |
| Moisture | Between Groups | 4762.574 | 2 | 2381.287 | 8195.341 | .000 |
| | Within Groups | 1.743 | 6 | .291 | | |
| | Total | 4764.317 | 8 | | | |
| Ash | Between Groups | .243 | 2 | .121 | 54.414 | .000 |
| | Within Groups | .013 | 6 | .002 | | |
| | Total | .256 | 8 | | | |
| Protein | Between Groups | 61.194 | 2 | 30.597 | 430.079 | .000 |
| | Within Groups | .427 | 6 | .071 | | |
| | Total | 61.621 | 8 | | | |
| Fat | Between Groups | .388 | 2 | .194 | 1803.484 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .388 | 8 | | | |
| Fibre | Between Groups | 10.820 | 2 | 5.410 | 533.287 | .000 |
| | Within Groups | .061 | 6 | .010 | | |
| | Total | 10.881 | 8 | | | |
| CHO | Between Groups | 90.419 | 2 | 45.210 | 693.574 | .000 |
| | Within Groups | .391 | 6 | .065 | | |
| | Total | 90.811 | 8 | | | |

**CHO**- Carbohydrates

**Appendix B20: ANOVA test for proximate analysis of 3:1 formulated blend of rice and wheat flour fermented with *L. bulgaricus* followed by *S. cerevisiae***

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Dry Matter | Between Groups | 4095.100 | 2 | 2047.550 | 9908.218 | .000 |
| | Within Groups | 1.240 | 6 | .207 | | |
| | Total | 4096.340 | 8 | | | |
| Moisture | Between Groups | 4095.100 | 2 | 2047.550 | 9908.218 | .000 |
| | Within Groups | 1.240 | 6 | .207 | | |
| | Total | 4096.340 | 8 | | | |
| Ash | Between Groups | .095 | 2 | .048 | 5.908 | .038 |
| | Within Groups | .048 | 6 | .008 | | |
| | Total | .143 | 8 | | | |
| Protein | Between Groups | 68.495 | 2 | 34.248 | 622.957 | .000 |
| | Within Groups | .330 | 6 | .055 | | |
| | Total | 68.825 | 8 | | | |
| Fat | Betwe-en Groups | .404 | 2 | .202 | 1822.441 | .000 |
| | Within Groups | .001 | 6 | .000 | | |
| | Total | .404 | 8 | | | |
| Fibre | Between Groups | .395 | 2 | .19+8 | 17.697 | .003 |
| | Within Groups | .067 | 6 | .011 | | |
| | Total | .462 | 8 | | | |
| CHO | Between Groups | 71.018 | 2 | 35.509 | 468.129 | .000 |
| | Within Groups | .455 | 6 | .076 | | |
| | Total | 71.473 | 8 | | | |

**CHO**- Carbohydrates