NUTRITIONAL COMPOSITION AND *IN VITRO* STARCH DIGESTIBILITY OF CRACKERS FROM PEARL MILLET AND WHEAT COMPOSITED WITH MUSHROOMS

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE (FOOD SCIENCE AND TECHNOLOGY)

OF

THE UNIVERSITY OF NAMIBIA

BY

ERICK N. UUKULE

201146924

MARCH 2020

SUPERVISOR: DR. KOMEINE NANTANGA (UNIVERSITY OF NAMIBIA)

ABSTRACT

Type 2 diabetes is a global problem that can be controlled by a diet that consists of carbohydrates with substantial amounts of slowly digestible starch amongst others. Pearl millet (*Pennisetum glaucum* (L) R. Br.) is an underutilised cereal crop that in recent times, raised interest due to its health benefits. The low starch hydrolysis rate of pearl millet is particularly of interest in type 2 diabetes modulation. To contribute to the value-addition and food diversification using pearl millet, this project investigated the nutritional content and starch digestibility of composite flours and crackers made of pearl millet-wheat, truffle *(Kalaharituber pfeilii)* and Oyster mushroom (*Pleurotus ostreatus)* composite flours and crackers. Equal amounts of fermented whole pearl millet grain and bread wheat flour were mixed. Of this mixture, 5%, 10% and 15% were substituted with Oyster mushroom and Kalahari truffle powders. The moisture, fat, protein, ash and minerals contents, water absorption (WAI) and water solubility (WSI) indices, total starch and starch digestibility of the flour formulations and the resultant crackers were determined. The consumer acceptance of the composite crackers was also assessed. The ash content, WSI and WAI were higher in the mushroom flours than in the cereals composite flours. Contrary, mushroom containing flours had the lowest starch content of all the flours. For crackers, ash content, WAI and WSI were directly proportional to the levels of mushroom incorporation. In terms of consumer acceptance, 5% oyster mushroom and 5% Kalahari truffle incorporated crackers scored the highest on the 9 point hedonic scale. Starch digestibility of mushroom incorporated flours and crackers were lower than those of cereal composite products. The higher the mushroom incorporation level, the slower the glucose release rate, showing that mushroom incorporation may be beneficial to people with type 2 diabetes.

LIST OF PUBLICATIONS OF CONFERENCE PROCEEDINGS

Uukule, E.N., Embashu, W., Nantanga, K.K.M. and Kadhila-Muandingi N.P. *Towards improved control of Type 2 diabetes: use of pearl millet and Kalahari truffle*. PowerPoint presentation at the second annual International Conference on Agriculture and Natural Resources (ICANR), 15-16 October 2018, Neudamm Campus, University of Namibia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AMTA	AgroMarketing and Trade Agency
ANOVA	Analysis of Variance
GI	Glycaemic Index
IBM	International Business Machines
KTC	Kalahari Truffle Cracker
KTF	Kalahari Truffle Flour
LSD	Least Significant Differences
NAB	Namibia Agronomic Board
NCRST	Namibia Commission on Research Science and Technology
OMC	Oyster Mushroom Cracker
OMF	Oyster Mushroom Flour
RAG	Rapidly Available Glucose
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SAG	Slowly Available Glucose
SDS	Slowly Digestible Starch
USA	United States of America
WAI	Water Absorption Index
WSI	Water Solubility Index
ZERI	Zero Emissions Research Initiatives

ACKNOWLEDGMENTS

I would like to acknowledge the following individuals and organisations for their roles in making this work a reality.

I would like to convey my sincere gratitude to my supervisor Dr. Komeine Nantanga for his unwavering support, mentorship and constant encouragement. I would also like to use this platform to thank Mr Werner Embashu from the University of Namibia's Multidisciplinary Research Centre for his inputs and support throughout this journey. I would also like to convey my gratitude to Dr Pauline Kadhila and everyone at the University of Namibia's Multidisciplinary Research Centre's Zero Emissions Research Initiatives (ZERI) division for availing the oyster mushroom samples.

Dr Stephen Barrion for his constant encouragement and for always pushing me to do better, his contributions are highly appreciated. Most of all, I thank him for recommending me for this study opportunity.

Mr Joel Muzanima's recommendation and endorsement for this study opportunity will always be appreciated.

The entire Food Science and Technology Department staff members, especially Dr Penny Hiwilepo-van Hal, Mr Sakeus Emvula and Mr Chinemba Samundengu, for the opportunities provided.

My fellow postgraduate colleagues at the University of Namibia's Food Science and Technology Department for fostering a resilient attitude, despite the known hardships and challenges we faced since the commencement of this study programme.

My friends and family, for standing by my side and keeping me sane enough to see this through. Meme Nally Shaimemanya, for always being supportive, whether emotionally or financially. I will forever be indebted to you.

Last but not least, the National Commission on Research Science and Technology and the University of Namibia for availing this study opportunity and the funding provided.

DEDICATION

This work is dedicated to Dr Hilka Litha Stefanus. Your resilience and strength has been a great source of inspiration throughout this study period. Thank you for your unwavering support, I will forever be indebted to you. Love you always!

DECLARATIONS

I, Erick Natangwe Uukule, hereby declare that this study is my own work and a true reflection of my research results, and that this work, or any part thereof has not been submitted for a degree at any other institution.

I, Erick Natangwe Uukule, grant The University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which The University of Namibia may deem fit.

Erick Natangwe Uukule	**……………………….**	**………………..**
Name of Student	**Signature**	**Date**

CHAPTER 1

This chapter is composed of the introduction and background of the study. Study questions, hypotheses and significance of the study are also stated in this chapter.

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Snacks are smaller portions of foods that are usually eaten between meals, they may be fresh, packaged or processed (Tumuluru, 2015). They offer convenience as they require no or minimal cooking. Processed snacks may be manufactured through extrusion cooking (Ajitaa & Jha, 2017) or baking. Traditionally baked goods such as biscuits and crackers are made from wheat. To improve these products nutritional content, the use of composite flours in the production of baked snacks is increasing (Chandra et al., 2015).

Apart from wheat, there are underutilised cereals such as pearl millet. Compared to other cereals, pearl millet has a lower glycaemic response (Annor et al., 2017). It is grown in arid and semi-arid regions in countries such as India, Niger, Mozambique and Namibia. In Namibia, it is commonly known as *Mahangu* and is a major staple food that has seen little value addition. It is mostly utilised at a household level to prepare porridges, traditional brews and it is rarely used for commercial products.

Namibia is also home to indigenous and or endemic species of edible mushrooms such as Kalahari truffles (*Kalaharituber pfeilii*), (*Termitomyces schimperi*), *Termitomyces sagittiformis,* Kakalahambo and *Ganoderma* mushroom species. They are mostly seasonal and are rarely preserved, thus leading to their general underutilisation.

1.2 Statement of the problem

The World Health Organisation has classified diabetes as a global problem that was estimated to have affected 422 million adults in 2014 (WHO, 2019). A diet that entails low GI foods plays an important role in diabetes management. Pearl millet's slowly digestible starch makes it an ideal candidate for the management of type 2 diabetes (Annor *et al*., 2017).

Although pearl millet may contain relatively higher amounts of protein (12.4%) compared to other cereals, its lysine content is limited (0.5 – 3.2 g/100 g of protein) (Amadou et al., 2013; Obilana, 2003). To improve protein content and quality, mushrooms such as Kalahari truffle and oyster mushrooms may be used. Kalahari truffle (100 g) contains 20 – 27 g of crude protein with 85% digestibility (Kagan-zur & Roth-bejerano, 2008). On the other hand, oyster mushroom is relatively rich in, lysine 22.9 g/100g on dry basis (Deepalakshmi & Mirunalini, 2014).

Classified as a subsistence crop, pearl millet is a staple to many individuals, especially in Africa and Asia (Dias-Martins et al., 2018). Minerals such as iron and zinc are present in lower quantities (Dias-Martins et al., 2018). Additionally, these pearl millet's minerals are located in the pericarp, eleurone layer and germ (Taylor, 2016). Their location makes them susceptible to loss during milling (decortication) (Taylor, 2016). On the other hand, edible mushrooms are generally rich in minerals such as copper, iron and zinc (Gençcelep et al., 2009), thus making them ideal for use in fortifying pearl millet and wheat based food products. Incorporation of mushrooms can potentially enhance the presence of these micro-elements (Cu, Fe, Zn) in pearl millet and wheat based food products. The enhancement of micro-minerals in food is necessary in the fight against minerals deficiencies, with over 2 billion people estimated to be deficient in minerals such as iron and zinc (WHO, 2007).

To enhance antioxidant activities, modulate glycaemic response, improve nutritional value, sensory and rheological properties of various baked products, several studies have incorporated mushroom powder into different cereal based foods. Lu *et al.*, (2018) investigated the effect of mushroom powder addition on the antioxidant and predictive glycaemic response of pasta. Regula & Gramza-Michalowska (2010) incorporated mushrooms in maize flour cookies and investigated the chemical composition of the resultant cookies. Eissa, Hussein, & Mostafa, (2007) investigated the rheological properties of oyster mushroom enriched biscuits and Egyptian Balady bread. Farzana & Mohajan, (2015) investigated the sensory and nutritional properties of biscuits fortified with mushroom. Additionally, Ng, Robert, Ahmad, & Ishak, (2017) investigated the effect of mushroom powder incorporation in biscuits on their postprandial glycaemic response. The nutritional and sensory quality of bread incorporated with Nigerian oyster mushroom was also investigated by Okafor, Okafor, Ozumba, & Elemo (2012), whereas Seguchi, Morimoto, Abe, & Yoshino, (2001) investigated the effect of Maitake (*Grifola frondosa*) on bread properties. Gadallah & (Ashoush, 2016) developed wheat-truffle (*Terfezia claveryi*) composite biscuits and found that the protein, ash and crude fibre of the biscuits increased with an increase in the amount of truffle powder added.

Considering these studies on cereal-mushroom composite products, the following questions arise: Can mushrooms be used to improve the nutrient content of pearl millet via the development of a mushroom-pearl millet crackers? How does this compositing, and baking affect the *in vitro* starch digestibility of the developed crackers? So far, no mushroom, pearl millet-wheat composite flours and crackers have been found in literature. Therefore, this project aims at investigating the effect of mixing wheat-pearl millet with indigenous Namibian truffle and Oyster mushroom on nutrient content and starch digestibility on derived flours and crackers.

1.3 Research questions

This aim of this study was to answer the following questions:

1. Does Kalahari truffle and Oyster mushroom incorporation affect the nutritional (moisture, protein, crude fibre, fat, starch) content of pearl millet-wheat composite flour and crackers?
2. Does Kalahari truffle and Oyster mushroom incorporation influence the starch digestibility of pearl millet-wheat composite flours and crackers?
3. Does Kalahari truffle and Oyster mushroom incorporation affect the functional (Water absorption Index (WAI) and Water solubility Index (WSI)) properties of pearl millet-wheat composite flour and crackers?
4. Are oyster mushroom and Kalahari truffle incorporated crackers acceptable by consumers?

1.4 Hypotheses

1. Kalahari truffle and Oyster mushroom incorporation has no effect on the nutritional (moisture, protein, crude fibre, fat, starch) content of pearl millet-wheat composite flour and crackers.
2. Kalahari truffle and Oyster mushroom incorporation has no effect on starch digestibility of pearl millet-wheat composite flour and crackers.
3. Kalahari truffle and Oyster mushroom incorporation has no effect on the functional (WAI and WAI) properties of pearl millet-wheat composite flour and crackers.

1.5 Significance of the study

Sorghum and millets are often underutilised (Rooney, 2010). To change this perspective, there is a need to add value to pearl millet. Traditionally, crackers are made with wheat flour, therefore the use of pearl millet as an ingredient in crackers' baking could contribute to the diversification of pearl millet usage. Mushrooms are well known for their desirable organoleptic properties. Mushrooms such as Kalahari truffle are seasonal. The use of Kalahari truffle in alternative food products could lead to the employment of preservation methods such as drying to ensure that truffle products are available even in seasons when fresh truffles are not. Furthermore, these additional value addition streams could create a bigger market for Kalahari truffles and in return generate an income for local Kalahari truffle hunters. Compositing cereal with mushrooms such as oyster mushroom and Kalahari truffle can enhance the nutrient content of the cereal and improve the nutrition status of communities, especially those suffering from protein and mineral malnutrition. Finally, nutritional information on the Kalahari truffle is limited or non-existent, thus this study could help fill that gap in literature. This document adds to the knowledge on pearl millet, wheat, oyster mushroom and Kalahari truffle composite flours and crackers.

CHAPTER 2

LITERATURE REVIEW

This chapter looks at the production, distribution, structure and composition of the raw materials (Pearl millet, Kalahari truffle and Oyster mushroom). The raw materials' uses, processing and health benefits or lack thereof are also discussed.

2.1 Pearl millet

2.1.1 Production and distribution

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is a resilient and water efficient cereal crop that can grow in areas with an annual rainfall that is as low as 250 – 400 mm (Taylor, 2017). However, an even rainfall distribution during the growing season is vital for the crop's growth and development (Taylor, 2016). The main locations for pearl millet production are India, West and Central Africa (Taylor, 2016). Pearl millet is also cultivated in Southern Africa. Its cultivation spreads from Angola, northern Namibia, Zimbabwe and Mozambique (Taylor, 2016).

In Namibia over 50% of the population consume pearl millet. In Namibia, pearl millet is the third major cereal crop grown in the country, after white maize and wheat. In the 2017/2018 financial year, local farmers produced 76 660 tonnes of white maize and 6 863 tonnes of wheat, respectively (Agro Marketing and Trade Agency, 2019). In the same period, pearl millet trading done in the formal domestic markets showed that 2 344 tonnes of pearl millet were sourced from local producers in the formal domestic market, whereas 3 469 tonnes of pearl millet were imported. Pearl millet is however the most consumed cereal crop in the country.

2.1.2 Grain structure

Pearl millet are small tear shaped (Figure 1) (Delcour & Hoseney, 2010) naked (Taylor & Kruger, 2016) kernels of up to 2 mm in length (Taylor, 2016). Kernel colour varies between yellow, white, brown and slate grey, with the latter being the most common (Delcour & Hoseney, 2010).

The grain is composed of the pericarp, the aleurone layer and the starchy endosperm (McDonough & Rooney, 1989). Pearl millet's pericarp does not contain starch granules (Delcour & Hosney, 2010), and is made up of the epicarp, mesocarp and endocarp layers (McDonough & Rooney, 1989). The mesocarp may contain some starch granules (Taylor, 2016).

The aleurone layer is part of the endosperm (Taylor, 2016) and is one cell layer thick (McDonough & Rooney, 1989). Lipids are found in this layer (McDonough & Rooney, 1989). Furthermore, McDonough & Rooney (1989) found the starch endosperm to consist of the peripheral, corneous and floury layers. The peripheral layer consists of tightly packed starch granules enclosed in a protein matrix (McDonough & Rooney, 1989). The corneous and floury layers made up most of the starch endosperm. The cell contents of the corneous and floury layers are not as tightly packed as those found in the peripheral layer (McDonough & Rooney, 1989).

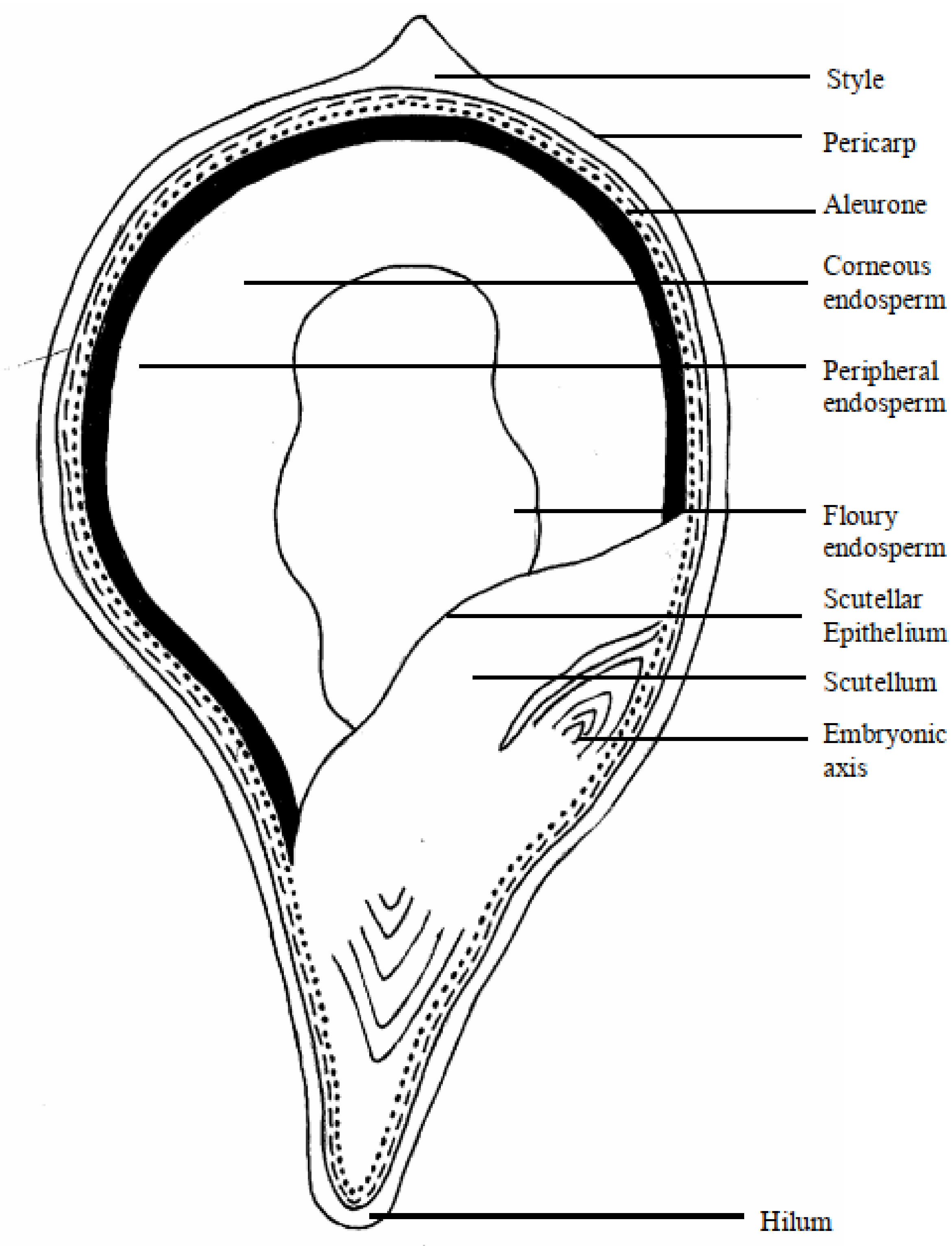

Figure 1 Schematic diagram of a longitudinal section through a pearl millet kernel (Barrion, 2008)

2.1.3 Nutrient composition

Millets are rich in vitamins and minerals. They also contain 60-70% carbohydrates, 7-11% proteins, 1.5-5% fat and 2-7% crude fibre (Sarita & Singh, 2016; Vinoth & Ravindhran, 2017). Millets contain phenolic acids, phenols and carotenoids (Sarita & Singh, 2016; Vinoth & Ravindhran, 2017). Compared to many other cereals, pearl millet has similar or higher nutritional quality in terms of proteins (19-33% higher) minerals and macronutrients (Mweu, Akuja, & Mburu, 2016; Taylor & Kruger, 2016).

Millet proteins (Table 1) are however incomplete as they contain less than the recommended lysine (Table 2) content, with pearl millet containing only 17% of the 1.93 g of lysine required by adults (Taylor & Kruger, 2016).

Pearl millet has a relatively low starch hydrolysis which is beneficial to individuals with celiac disease and type 2 diabetes (Rooney, 2010).

Table 1 Proximate composition of pearl millet grain

Source	**(Obilana, 2003)**	**(Taylor & Kruger, 2016)**
Protein (g100g)	11.0	12.4
Carbohydrate (g/100g)	70.0	70
Fat (g/100g)	4.8	5.1
Crude Fibre(g/100g)	2.3	13.8
Ash (g/100g)	1.9	2.8
Food Energy (kJ/100 g)	1483	1499

*All values are on a dry basis.

Table 2 Essential amino acid profile for pearl millet (g/100g of protein)

Source *Amino Acids*	**(Amadou *et al.*, 2013)**	(Pelembe et al., 2003)	**(Obilana, 2003)**
Isoleucine	5.1	3.6-5.9	3.9-4.6
Leucine	14.1	8.0-25.1	9.5-12.4
Lysine	0.5	1.7-6.5	2.8-3.2
Methionine	1.0	1.5-2.9	1.8-2.6
Phenylalanine	7.6	4.4-5.6	4.1
Threonine	3.3	1.2-4.8	3.3-4.1
Valine	4.2	4.8-7.0	4.9-6.0
Histidine	1.7	1.8-2.6	N/a
Tryptophan	1.2	N/R	1.4-1.51

***N/R = Not reported.**

2.1.4 Processing and uses of pearl millet

In general, grain processing includes wetting, decortication (dehulling), milling, fermentation, malting, extrusion, flaking, popping and roasting (Obilana, 2003).

2.1.4.1 Milling

Milling of pearl millet grain is a crucial processing step as it yields pearl millet flour which can be used in the cooking and brewing of pearl millet based products (Taylor, 2016). Decortication as a part of milling refers to the removal of the bran to improve the palatability of the millet flour after milling (Taylor, 2016) and is achieved either by traditional means (mortar and pestle) or by using mechanical decorticators (Taylor, 2017). However, due to the grain's small size, decortication may lead to loss of the grain (Obilana, 2003). Milling as a size reduction process results in flour. Although, mechanically milled pearl millet flour has a longer shelf life, it retains less nutrients compared to flour milled through traditional means (Obilana, 2003).

2.1.4.3 Grain fermentation

International Food Information Service, (2009) defines fermentation as an:

> Energy yielding process in which organic compounds are metabolised, usually under anaerobic or micro-aerobic conditions to simpler compounds without the involvement of an exogenous electron acceptor (p. 163).

The fermentation of cereal grains dates back to ancient Egypt where beer and bread production took place with the help of yeasts and lactic acid bacteria (Poutanen et al., 2009). Cereal fermentation normally involves fermenting cereal flour with water (ratio 1:2-3 w/v) either spontaneously or by the addition of a starter culture (Taylor & Kruger, 2019), at moderate temperatures (25-37°C) for up to 24 hours (Poutanen *et al.*, 2009). Fermented products are popular in India and many countries in central and Southern Africa (El Hag et al., 2002). In the early days, spontaneous fermentation was employed, whereby naturally occurring microbes present on kernels and milled grains were allowed to thrive (Taylor & Kruger, 2019), these microbes usually entailed mixed cultures of yeast and lactobacilli (El Hag *et al.*, 2002). In Africa, most fermented products still undergo spontaneous fermentation, especially at a household or small scale fermentation operations (Adebiyi, Obadina, Adebo, & Kayitesi, 2018). Advances in fermentation technology led to what is referred to as back-slopping, which is the development and maintenance of microbial cultures and it is achieved by saving part of a previously successful fermentation product for future use (Taylor & Kruger, 2019). In larger fermentation operations, conditions are more controlled and specific starter cultures are used, based on the desired end product (Adebiyi, Obadina, Adebo, & Kayitesi, 2018).

Some benefits of fermentation include food preservation and an increase in foods' organoleptic properties (Guyot, 2012), improved food safety and nutritional value (Singh, Rehal, Kaur, & Jyot, 2015). During fermentation, lactic acid bacteria may produce lactic and acetic acids, resulting in products with a pH below 5 (Poutanen *et al*., 2009). The low pH inhibits the growth of pathogenic microbes resulting in an improved shelf life (Singh *et al*., 2015).

Fermentation increased pearl millet grains' protein content from 10.99% to 13.65% (Akinola et al., 2017). Similarly, Adebiyi *et al*., (2017) recorded a significant increase in the crude protein content of fermented pearl millet flour. Biscuits prepared using fermented pearl millet flour also followed a similar trend. These authors further recorded an increase in the protein quality of fermented pearl millet flour. Essential amino acids content increased from 0.22 g/100g to 0.31 g/100g for lysine and from 0.78 g/100g to 0.86 g/100g for leucine (Adebiyi *et al*., 2017). As far as protein quality goes, fermentation was also found to increase in *in vitro* protein digestibility of fermented pearl millet flour from 61.9% to 86.2% after 28 hours of fermentation (Elyas et al., 2002). El Hag *et al*., (2002) also reported similar results, where the *in vitro* protein digestibility of fermented pearl millet dough increased from 72.7% to 83.6%.

Fermentation of pearl millet grains at ratios (1:3 w/v) (Akinola *et al*., 2017) and (1:4 w/v) (Adebiyi, Obadina, Adebo, & Kayitesi, 2017) reduced the grains' ash content after three days. A reduction in polyphenol (304 to 122 mg/100) and phytic acid (943 to 380 mg/100g) content was observed after fermenting pearl millet dough for 14 hours (El Hag *et al*., 2002). Equally so, Elyas *et al*., (2002) reported a 50% decrease in the phytic acid content of fermented pearl millet flour. Authors Adebiyi *et al*., (2017) reported a significant decrease in the crude fat content of biscuits made from fermented pearl millet flour. Fermentation was also found to decrease the total phenolic content of pearl millet flour (Adebiyi *et al*., 2017). However, Salar, Purewal, & Sandhu, (2017) recorded an initial increase in the total phenolic content of pearl millet grain, it later declined after the 6th day of fermentation.

2.1.5 Effect of pearl millet on health

Millets in general have been found to have a positive role in reducing oxidative stress and post-prandial glucose modulation (Kaur et al., 2018). As gluten free cereal (Moreno et al., 2014), pearl millet may be employed as a cheaper alternative for people suffering from celiac diseases (Dias-Martins *et al*., 2018). Additionally, phytochemicals found in pearl millet apparently lower cholesterol (phytic acid) and reduce the risk of cancer (Amadou *et al*., 2013).

2.1.4.4.1 Pearl millet and Diabetes

Saleh, Zhang, Chen, & Shen, (2013) define diabetes mellitus as "a chronic metabolic disorder characterised by hyperglycaemia with alterations in carbohydrate and lipid metabolism.' According to the World Health Organisation, diabetes is a global problem and an estimated 422 million adults were reported to be suffering from the disease in 2014. Currently, diabetes is classified into type 1 and type 2 diabetes and the distinction between the two is based on the age at which the victim develops the symptoms (WHO, 2019). As a disease linked to carbohydrate metabolism, the restriction of carbohydrates from diets was used as a remedy before the discovery of insulin in 1921 (Dyson, 2015; Lennerz *et al*., 2018). However, consumption of whole grain foods has been suggested in the prevention and management of diabetes (Saleh *et al*., 2013). Additionally, a diet that focuses on a good glycaemic control and the reduction of postprandial hyperglycaemia is considered crucial in the management of type 2 diabetes (Nambiar et al., 2011).

Epidemiological studies found diabetes related cases to be low in millet-consuming populations (Saleh *et al*., 2013; Sarita & Singh, 2016). This could be because of pearl millet's low glycaemic index (55) (Nambiar *et al*., 2011). Pearl millet's high proportion of insoluble dietary fibre enables the slow release of glucose, further making pearl millet ideal for people suffering from type 2 diabetes (Kumari et al., 2019).

Alyami *et al*., (2019) investigated the glycaemic, gastrointestinal and appetite responses to breakfast porridges from various cereal grains in healthy adults. These authors found that the consumption of pearl millet porridge led to a reduction in gastric emptying rate and subsequently blunted its glycaemic response. Nani *et al*., (2016) studied the impact of pearl millet on carbohydrate metabolism in diabetic rats. One group of rats was fed a corn-starch-based diet and the other group was fed a pearl millet based diet for a period of 28 days. At the end of the feeding trial, rats on the pearl millet diet had a glycaemic response of 1.39 g glucose/L compared to that of 4.35 g glucose/L at the beginning of the feeding trial. This study concluded that whole grain pearl millet may be useful in correcting hyperglycaemia. Shukla & Srivastava, (2014) evaluated the glycaemic index of 30% finger millet incorporated noodles and refined wheat flour noodles in female subjects between the ages of 24 and 26. They found that noodles containing 30% finger millet had a lower GI (45.13) compared to noodles made from refined wheat flour (62.59). Another study by Ugare, Chimmad, Naik, Bharati, & Itagi, (2014), found that barnyard millet reduced fasting plasma glucose levels by 6% in diabetic test subjects and by 7% in non-diabetic test subjects. These authors concluded that dehulled and heat-treated barnyard millet grains had a positive effect on blood glucose and serum lipid levels in both diabetic and non-diabetic humans.

2.2 Edible mushrooms

Edible mushrooms which can either be wild or cultivated are an important food source. According to FAOSTAT, Asia is the largest (72.6%) producer of mushrooms with Africa only responsible for less than 1% of the world's production (Figure 2).

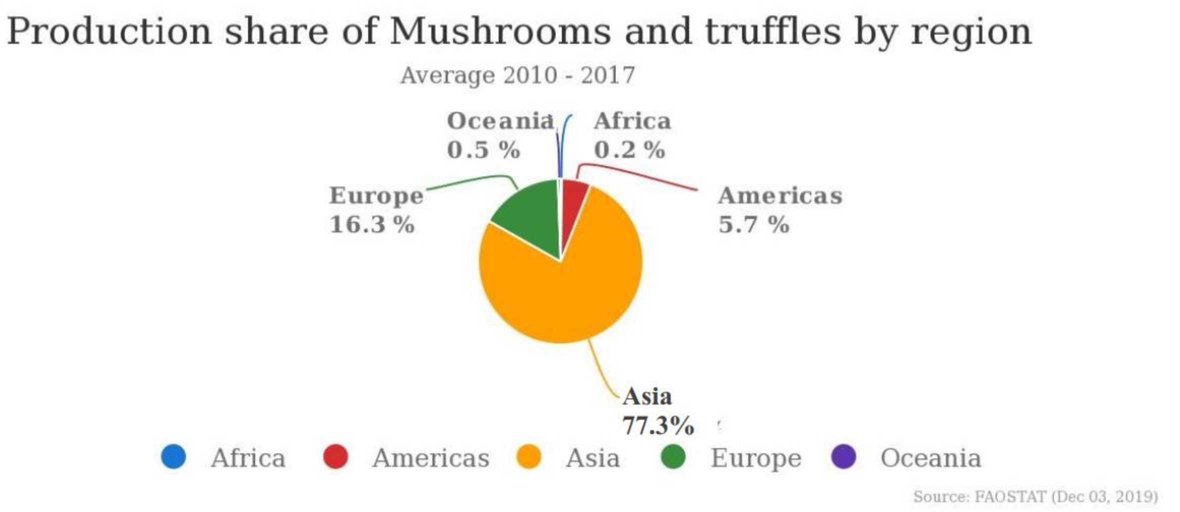

Figure 2 Production Share of mushrooms and truffles by region (FAOSTAT, 2019).

Mushrooms are low in fat, rich in proteins with an impressive amount of essential amino acids, vitamins (B1, B2, B12, C, D and E), unsaturated fatty acids, fibres and minerals (Islam et al., 2016; Kadnikova et al., 2015; Reis et al., 2012). Edible mushrooms are not only used as food, they are also used as a raw material for producing therapeutic and medicinal products (Kadnikova *et al*., 2015). However, compared to medicinal mushroom species, knowledge on the composition and nutritional value of culinary mushrooms is limited, mainly because these mushrooms are only viewed as a delicacy (Deepalakshmi & Mirunalini, 2014). Edible mushrooms contain several bioactive molecules such as phenolic compounds, terpenes and steroids known as effective antioxidants capable to scavenge free radicals (Islam *et al*., 2016). Mushrooms also contain β glucans, which are said to positively influence postprandial glycaemic response in humans (Ng, Robert, Ahmad, & Ishak, 2017).

2.2.1 Oyster mushrooms (*Pleurotus ostreatus)* and Kalahari truffle (*Kalaharituber pfeilli*)

For the purposes of this study, the focus will be on the Oyster and Kalahari truffle mushrooms.

2.2.2 Oyster mushrooms

The genus *Pleurotus* has about 40 species (Deepalakshmi & Mirunalini, 2014; Jeena et al., 2014). These include *P. ostreatus, P.sajorcaju, P. florida, P. flabellatus, P. highbing 5, P. eryngii, P. Pulmonarius* and many other species that are considered important, due to their superior nutritional values and medicinal importance (Deepalakshmi & Mirunalini, 2014). *Pleurotus* species are all commonly known as Oyster mushrooms (Adebayo & Martínez-Carrera, 2015; Deepalakshmi & Mirunalini, 2014).

2.2.2.1 Production and Distribution

In terms of worldwide edible mushrooms production, oyster mushrooms are ranked third after *Agaricus bisporus* and *Lentinula edodes,* respectively (Adebayo & Martínez-Carrera, 2015; Corrêa et al., 2016; Fernandes et al., 2015; Liu et al., 2016). Oyster mushrooms (*Pleurotus* spp) (Figure 3 and Figure 4) are edible mushrooms with apparently a good flavour and taste (Ovat et al., 2017). Naturally, these mushrooms are found in tropical or sub-tropical forests and they can also be cultivated (Bonatti et al., 2004; Fernandes et al., 2015; Kayode et al., 2015).

Pleurotus ostreatus is a saprotroph that acts as a primary decomposer on dead wood (Bonatti *et al*., 2004; Hearst *et al*., 2009). Due to their rich mineral content and medicinal properties, their commercial cultivation is on the rise (Deepalakshmi & Mirunalini, 2014; Jafri et al., 2013). Additionally, the cultivation of *Pleurotus* spp. is on the increase due to their short life cycle compared to other edible mushrooms, low demand on resources and technology (Bonatti *et al*., 2004; Deepalakshmi & Mirunalini, 2014)..

2.2.2.2 Structure

Pleurotus species have a characteristic white spore print attached to the gills and may or may not have a stipe (Adebayo & Martínez-Carrera, 2015). They have an oyster shaped cap, their colours range from white to grey or tan to dark-brown and they often appear lobbed or wavy with distinctive white, firm and fleshy fruiting bodies (Deepalakshmi & Mirunalini, 2014; Jonathan et al., 2012).

Figure 3 Fresh Oyster mushroom fruiting bodies (Coffey, 2019).

Figure 4 Dry Oyster mushroom fruiting bodies

2.2.2.3 Composition

Pleurotus ostreatus protein content (Table 3) is reported to vary from strain to strains due to physical and chemical differences in growing media (Deepalakshmi & Mirunalini, 2014). *P. ostreatus* was reported to have a high carbohydrate content (Reis *et al.*, 2012). This mushroom species contains more folacine, vitamin B_1 and Vitamin B_3 compared to other mushroom species (Deepalakshmi & Mirunalini, 2014). *P. ostreatus* contains more lysine (Table 4) content (22.9 g/100g db.) compared to other *Pleurotus* mushrooms such as *Pleurotus sajor-caju* (5.04 g/100g) (Deepalakshmi & Mirunalini, 2014; Kayode *et al.*, 2015).

Table 3 Oyster mushroom proximate composition.

Source	**(Reis *Et Al.*, 2012) (g/100 g fw)**	**(Deepalakshmi & Mirunalini, 2014)**	**(Bonatti *Et Al.*, 2004)**
Moisture	89.17	85 – 87 %	85.64 – 88.06 (g/100g w.b)
Ash	0.62	N/A	5.58 – 6.13 (g/100g d.b)
Protein	0.76	17 – 42 (g/100g d.b)	13.1 – 16.9 (g/100g d.b)
Fat	0.15	0.5 – 5 (g/100g d.b)	5.97 – 6.32 (g/100g d.b)
Carbohydrates	9.30	37 – 48 (g/100g d.b)	47.0 – 47.6 (g/100g d.b)
Fibre	N/A	24 – 31 (g/100g d.b)	9.41 – 9.86 (g/100g d.b)
Ash	N/A	4 – 10 (g/100g d.b)	N/A

N/A = Not Available

Table 4 Amino acids profile of P. ostreatus mushrooms' (Deepalakshmi & Mirunalini, 2014).

Amino Acid	Value (g/100g Dried Mushroom)
Histidine	12.4
Valine	21.0
Lysine	22.9
Tryptophan	4.8
Isoleucine	16.2
Leucine	25.7
Phenylalanine	15.2
Threonine	17.1
Methionine	3.8

2.2.2.4 Processing and uses

High moisture content, high respiration and the present microflora make fresh mushrooms highly perishable (Liu *et al*., 2016). Due to their highly perishable nature, much of the processing involving oyster mushrooms is aimed at extending their shelf life and quality. To extend their shelf life, mushrooms are usually salted and canned (Liu *et al*., 2016). Additionally, other preservation methods may be applied, these include fermentation (Liu et al., 2016; Radzki et al., 2016; Zheng et al., 2018), drying (Aishah & Rosli, 2013; Fan et al., 2012; Tolera & Abera, 2017; Tulek, 2011) and modified atmosphere packaging (Xiao et al., 2011).

It is not common practice for mushrooms to be eaten raw, they usually require thermal or hydro-thermal processing before consumption (Radzki *et al*., 2016). Coupled with its high perishability, the diversification of oyster mushroom consumption by incorporating it in popular foods was investigated. Eissa, Hussein, & Mostafa, (2007) investigated the rheological properties and quality of balady bread and biscuits supplemented with the oyster mushroom (*Pleurotus. sajor-caju*). These authors found that biscuits incorporated with 10% oyster mushroom were of acceptable sensory quality. These authors further reported that compared to the control bread, balady bread incorporated with 15% oyster mushroom scored high in terms of its general appearance, taste and crust colour.

Okafor, Okafor, Ozumba, & Elemo, (2012) looked at the quality characteristics of bread made from wheat and the oyster mushroom (*Pleurotus plumonarius*). These authors reported that compared to the control, 25% oyster mushroom incorporation resulted in a significant increase in the bread's crude protein (7.96-14.62%), ash (0.90-2.64) and crude fibre (0.51-2.48). It was further found that, 25% oyster mushroom incorporated bread had the highest water absorption percentage as compared to the 100% wheat bread. However, the addition of 25% oyster mushroom resulted in a low loaf volume (375 cc/g) when compared to the 100% wheat bread (675 cc/g). Finally, the sensory acceptability of the bread kept declining as the amount of oyster mushroom increased.

Wan Rosli, Nurhanan, & Aishah, (2012) investigate the effects of oyster mushroom (*Pleurotus sajor-caju)* incorporation on the nutritional composition and sensory properties of butter biscuits. The protein content proportionally increased with oyster mushroom incorporation. Butter biscuits containing 6% oyster mushroom had significantly higher protein content (6.94%) when compared to the control (6.50%). These authors also reported an increase in ash content, from 0.70% to 0.88 in the control and 6% oyster mushroom incorporated butter biscuits respectively. Butter biscuits incorporated with 4% and 6% oyster mushroom were found to have a significantly high amount of β-glucans (0.72 and 0.79 g/100g respectively). On sensory aspects, these authors found that 4% oyster mushroom incorporated butter biscuits had the most acceptable flavour (4.54). However, 2% oyster mushroom incorporated butter biscuits had the highest overall acceptance (5.16).

Likewise, Ng, Robert, Ahmad, & Ishak, (2017) studied the effect of oyster mushroom (*Pleurotus sajor-caju*) incorporation on starch digestibility of biscuits. Oyster mushroom incorporation significantly increased the biscuits protein content, from 6.50% in the control biscuits to 7.85% in biscuits containing 12% oyster mushroom powder. Equally so, ash content increased from 0.86% to 1%. The amount of β-glucans increased from 0.12% in the control biscuits to 1.78% in biscuits containing 12% oyster mushroom. In contrast, the carbohydrate content had an inverse relationship with oyster mushroom incorporation level. It was further found that 12% oyster mushroom incorporation reduced the amount of rapidly digestible starch by 60%.

The nutritional composition, physical qualities and sensory properties of wheat bread supplemented with oyster mushroom *(Pleurotus ostreatus)* were studied by Ndung`u, Otieno, Onyango, & Musieba, (2015). Oyster mushroom inclusion significantly increased both the protein and ash contents of composite breads. Oyster mushroom incorporation proportionally increased iron, potassium and zinc content of the composite breads. Mushroom incorporation further increased the amount of lysine, from 0.55mg/100g to 0.83mg/100g. The founders also observed an increase in the amount of riboflavin as the mushroom content in the bread increased.

2.2.2.5 Health benefits of oyster mushroom consumption

Oyster mushrooms are known to possess several medicinal properties, amongst many others, these include anti-diabetic properties, antimicrobial properties and anti-oxidant properties (Deepalakshmi & Mirunalini, 2014; Kumar, 2018; Y. Patel et al., 2012).

2.2.2.5.1 Anti-diabetic properties

The effect of oyster mushroom incorporation on biscuits' *in vitro* and *in vivo* starch digestibility was studied by Ng, Robert, Ahmad, & Ishak, (2017). *In vitro* starch digestibility investigations revealed that, increasing amounts of oyster mushroom powder led to a decline in the biscuits' rapidly digestibly content. At the same time a significant increase in slowly digestible and resistant starches was also observed. These results were an indication of oyster mushroom's potential in hyperglycaemia modulation. To further consolidate the potential shown during the *in vitro* starch digestibility studies, the authors conducted an *in vivo* investigation in which eleven (11) healthy participants took part. Blood glucose samples were taken at 15, 30, 45, 60, 90 and 120 minutes. At 30 minutes, the blood glucose levels of the test subjects after consuming the test foods were at their peak, with the reference food having the highest blood glucose concentration (8.3 mmol/L), followed by the control biscuit, 4%, 8% and 12% oyster mushroom biscuits with concentrations of 6.7 mmol/L, 6.6 mmol/L and 6.6 mmol/L respectively. These *in vivo* results provide affirmation that, oyster mushroom incorporation could positively impact postprandial glucose response or release in individuals, thus making these biscuits potentially safe for type 2 diabetes patients.

2.2.2.5.2 Antimicrobial and anti-oxidant properties

A comparative study by Iwalokun, Usen, Otunba, & Olukoya, (2007) documented the potential antimicrobial and antioxidant properties of the oyster mushroom (*Pleurotus ostreatus*). The authors tested the antibacterial and antifungal activity of petroleum ether and acetone extracts from oyster mushroom fruiting body on several multi-drug resistant bacterial pathogens. They concluded that *P. ostreatus* possesses "a broad spectrum antibacterial and antifungal activities". Okafor *et al*., (2017) investigated the antimicrobial and antioxidant activities of oyster mushroom. These authors found oyster mushroom to have inhibition zones of 14 and 12 mm against gram positive bacteria *Bacillus cereus* and *Streptococcus agalactiae*. Against the gram negative bacteria *Pseudomonas aeruginosa*, oyster mushroom had an inhibition zone of 11 mm. Moreover, oyster mushroom showed an antioxidant activity of up to 35.36µM. Oyster mushroom mycelial hot water extract was found to exhibit antimicrobial properties by Morris *et al*., (2017).

2.2.3 Desert Truffles (*Kalaharituber pfeilii*)

Desert truffles are found in arid and semi-arid regions (Akyüz et al., 2015; Slama et al., 2010). Truffles are mycorrhizal fungi that form either ecto- or endomycorrhizae symbiotic associations with the roots of the host plants (Chaturvedi et al., 2015; Minter, 2008).

There are three species of desert truffles found in the Kalahari Desert, namely, *Kalaharituber pfeilii* (Henn.) Trappe & Kagan– Zur, *Eremiomyces echinulatus* (Trappe & Marasas) Trappe & Kagan–Zur and *Mattirolomyces austroafricanus* (Trappe & Marasas) Trappe & Kovacs (Trappe et al., 2008). *K. pfeilii* (Figure 5) is found in Namibia, Botswana and adjacent areas of South Africa (Ferdman et al., 2005), from April through July (Trappe *et al*., 2008). *E. echinulatus* species is found in South Africa’s Northern Cape and Botswana (Ferdman *et al*., 2005), around June (Trappe *et al*., 2008). *M. austroafricanus* is found in South Africa’s Northern Cape Province around April (Trappe *et al*., 2008).

Figure 5 Washed Kalahari truffle tubers

2.2.3.1 Growth and distribution

Kalaharituber pfeilii are hypogeous, ascomycete fungi that grows in the Kalahari desert and northern parts of Namibia (Adeleke & Dames, 2014; Haileka, 2015; Trappe *et al*., 2008). For years, it has been misidentified as belonging to the Mediterranean genus Terfezia (Trappe *et al*., 2008). Locally, *Kalaharituber pfeilii* is known as Kalahari Truffle in English, Omatumbula in Oshiwambo and N’abba in Khoisan (Adeleke & Dames, 2014; Minter, 2008).

In the northern parts of Namibia, truffles manifest themselves towards the end of the pearl millet growing season (June) (Trappe *et al*., 2008). During this season, truffles push towards the surface forming cracks in the ground, which enable their discovery by truffle hunters (Ntshakaza, 2013). In the central and northern Kalahari, their fruiting season is from March to July (Trappe *et al*., 2008). In Saudi Arabia, truffles occur during the months of February to April (Hussain & Al-Ruqaie, 1999). While in Zambia, truffles are found from early April to early July (Siachoono et al., 2015).

Kalaharituber pfeilii was noted to grow on compact, pink or white sands with a pH of 5.5-6.5 and a $CaCO_3$ range of 0.3-3.1% (Trappe et al., 2014). On the Namibian side of the Kalahari desert, *Acacia melifera* and *Stipagrsfis uniplumis* were identified as host plants (Taylor, Thamage, Baker, Roth-Bejerano, & Kagan-Zur, 1995). However, attempts to cultivate *Kalaharituber pfeilii* by Kagan-Zur, Roth-Bejerano, & Taylor (1995) proved futile. These authors performed field experiments in Botswana and found it difficult to successfully inoculate the suspected *K. pfeilii* host plants and thus allow the successful cultivation of *K. pfeilli*.

2.2.3.2 Structure

Generally, truffles have no stalk or gills and its mycelium grows underground (Wang & Marcone, 2011). Kalahari truffle's size and shape may be compared to that of a potato, with an average diameter of 2 – 5 cm (Adeleke, 2007). Sliced Kalahari truffle (Figure 8) has a cream white colour that eventually becomes yellow due to exposure to air (Adeleke, 2007). In general, mature truffles are usually firm, dense and woody (Wang & Marcone, 2011). Mature Kalahari truffles have been found to grow to about 12 cm in diameter, may weigh up to 200 g and their weight may go up to 400 – 500 g on years with relatively good rainfall (Ntshakaza, 2013). Truffles' unique physical appearance makes it very easy to differentiate them from common mushrooms (Wang & Marcone, 2011).

2.2.3.3 Composition

Truffles are highly nutritious with a good taste, they are rich in fibre, proteins, vitamins and minerals (Haileka, 2015; Slama *et al.*, 2010). Although they vary depending on the environmental conditions where they grow, truffles' nutritional value is almost identical across the different species (Saritha *et al.*, 2016). On a dry weight basis, desert truffles contain 3-7% fat, 7-13% crude fibre, 60%carbohydrates and 20-27% crude protein, of which 85% is digestible by humans (Kagan-zur & Roth-bejerano, 2008). Gadallah & Ashoush, (2016) found that powdered desert truffle (*Terfezia claveryi*) contains 5.30% ash, 18.74% crude protein, 2.78% lipids, 8.5% crude fibre and 64.64% carbohydrates. However, when Dundar *et al.*, (2012) evaluated the chemical composition of the desert truffle (*Terfezia boudieri* (Chatin)) from different locations in Turkey, they found them to contain 9.4-28.8% protein, 4.4-17.7% crude fat, 5.3-20.5% ash, 24.9-127.2% carbohydrate and 11.6-39.1% crude fibre. Bouatia *et al.*, (2018) analysed the nutrient content of the Moroccan truffle (*Tirmania pinoyi*) and found it to contain 26.96 g/100g protein, 3.01 g/100g fat, 5.28 g/100g ash and 64.74 g/100g carbohydrate. Kalahari truffle fruiting bodies contain fructose, glucose, sucrose and sorbitol (Adeleke, 2007).

2.2.3.4 Uses

Truffles are a delicacy and they are considered to be the world's most expensive mushroom. Using the exchange rate of 2011, a kilogram of truffles sold for N$ 6 846 - N$ 68 460 in the European markets whereas the same kilogram sold for N$18 977 - N$56 933 in the USA, depending on the species (Slama *et al.*, 2010; Wang & Marcone, 2011). Truffles' expensiveness rises from their scarcity and medicinal value (Saritha *et al.*, 2016). Truffles may be eaten raw or cooked when fresh and they can also be dried as a way of preserving them (Chaturvedi *et al*., 2015; Haileka, 2015).

2.2.3.5 Health benefits of truffles consumption

Truffles are mainly known for their aromatic appeal. Apart from their use in the culinary world, truffles have some beneficial health attributes, these include their roles as antioxidants, antimicrobial, immunomodulators and anti-tumours (El Enshasy et al., 2013). Akyüz, Kırbağ, & Bircan, (2015) investigated the medical characteristics of several Turkish truffles including *Terfezia boudieri* Chatin and *Terfezia claveryi* Chatin. The truffles were assessed for their antimicrobial properties and free radical scavenging activity. This study demonstrated that truffles *T. boudieri, T. claveryi, T. olbiensis, P. lefebvrei* and *P. juniper* exhibit antimicrobial activities towards certain bacteria, yeast and dermatophyte. Dahham, Al-Rawi, Ibrahim, Abdul Majid, & Abdul Majid, (2018) studied the effect of different *Terfezia claveryi* extractions on 4 cancer cell lines (U-87 MG, HT 29, MCF-7 and PC3). The different extraction methods exhibited varying cytotoxicity levels with hexane extraction being most potent against U-87 MG and PC3 cell lines. Whereas ethanol and methanol were most potent against the MCF7 and HT29 cell lines respectively. Chaturvedi, Khare, Kwape, & Makholwa, (2015) investigated the antioxidant and free radical scavenging properties of *Kalaharituber pfeilii* and found that both methanol and water extraction exhibited significant 2, 2-diphenyl-1-picryl hydrazyl (DPPH), 2-Azobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS), Hydroxyl and nitric oxide (NO) radicals scavenging activity. The health benefits derived from truffles consumption are further discussed in reviews by Patel (2012) and Patel, Rauf, Khan, Khalid, & Mubarak (2017).

2.3 Crackers Manufacturing

Crackers (Figure 6) are usually distinguished by their crispness, open texture and savoury flavours (Davidson, 2016). The crackers' family is composed of saltine crackers (soda crackers) cream crackers and snack crackers (Davidson, 2016; Miller, 2016). Saltine and cream crackers undergo fermentation, using the sponge and dough method, whereas snack crackers are chemically leavened (Miller, 2016). Chemically leavened crackers are quick to produce and leavening agents such as sodium bicarbonate are employed. Savoury crackers undergo fermentation during which herbs and spices are used as additional ingredients (Millar *et al*., 2017). Post fermentation, the dough is sheeted to about 0.3 mm thick and laminated into six or eight layers (Miller, 2016). Lamination increases dough thickness to about 2.5 cm, the laminated dough is thus passed through a number of sheeting rollers to bring the thickness back to 0.3mm before cutting it into 5 cm squares and three rowed perforations are made in each square (Miller, 2016). If desired, the now sheeted and perforated dough is sprinkled with salt and baked at 250 - 300°C for 2.5 – 3 minutes (Miller, 2016).

Figure 6 Typical crackers (Anderson, 2019)

Although both biscuits and crackers are low moisture baked products and are similar in many ways, the main distinguishing factor is that crackers undergo fermentation and biscuits do not. Cookies or biscuits are typically characterised by high sugar and fat presence, whereas crackers usually contain little amounts of the said ingredients (Kweon et al., 2014).

2.3.1 Why crackers?

In recent years, the global demand for convenient functional foods has risen and crackers were identified as a fast growing segment as far as baked products are concerned (Qadri et al., 2018). Their long shelf life and attractive appearance make them a suitable product for scalability (Qadri *et al*., 2018). Crackers possess an admirable market share in the snacks market and has been a source of new product development opportunities, especially in the functional foods arena (Millar *et al*., 2017). Several studies involving crackers as functional foods have been conducted. Crackers for use as functional foods are usually produced using composite flours and less sugar, which may not disturb glucose homeostasis. Schmidt, Geweke, Struck, Zahn, & Rohm, (2018) investigated the dough characteristics and product properties of savoury crackers from wheat flour partially substituted with blackcurrant pomace. Similarly, Gangopadhyay *et al*., (2019) looked at the fate of beta-glucans, polyphenols and lipophilic compounds in baked crackers fortified with different barley-milled fractions. Whereas Venkatachalam & Nagarajan, (2017) looked at the physicochemical and sensory properties of green gram flour incorporated savoury crackers. The effects of pulse flours on the physiochemical characteristics and sensory acceptance of backed crackers were also studied by Millar *et al*., (2017). Further studies included the investigation of nutritional and storage stability of brown rice flour and carboxymethyl cellulose (CMC) incorporated wheat based crackers by Qadri *et al.*, (2018).

From literature, crackers are an important tool in the functional foods segment. Their convenience and long shelf being some of the attractive features placing crackers at the centre of new cereal products development. In this light, it is therefore ideal to constantly identify new, health promoting and locally available foods to incorporate into these new products.

2.3.2 Crackers and nutrition

Baking usually results in the destruction of some nutrients, especially those found in the crust portions of baked goods (Ranhotra & Bock, 1988). During baking proteins and fats undergo an alteration of their physical state whereas starch gets gelatinized and hydrolysed to dextrins and eventually to reducing sugars (Fellows, 2017). Compared to other baked goods, the destruction of nutrients is much greater in breakfast cereals, biscuits and other baked snack foods, this is mainly due to the thin profile of these food items(Fellows, 2017). Apart from the destruction of nutrients, baking may also result in the production of carcinogenic acrylamide (Fellows, 2017). On a positive note, baking may improve the nutritional profile of food products through the inactivation of undesirable microorganisms, some anti-nutrients and by breaking up complexes that would normally hinder the absorbance of certain nutrients (Ranhotra & Bock, 1988).

To offset the effects of baking on the nutritional content of crackers and other similar baked goods, there is a need to optimise time and temperature combinations. Another option would be to utilise nutrient rich foods to produce crackers that will still maintain an improved nutritional content post baking. This shift in cracker technology is becoming popular and several studies have looked at improving the nutritional content of crackers by incorporating different cereal grain flours, as well as other sources. Benjakul & Karnjanapratum, (2018) developed whole wheat crackers fortified with tuna bone bio-calcium powder. The incorporation of tuna bone bio-calcium powder increased the crackers' protein (11.53% to 13.59%), ash (3.97% to 16.21%), calcium (0.14% to 4.85), and phosphorus (0.44% to 2.02%) contents. Tuna bone bio-calcium powder incorporation also led to a decline in the amount of total carbohydrates (67.03% to 56.88%). Whole buckwheat crackers developed by Sedej *et al*., (2011) had a relatively higher protein content (11.4%) than whole grain wheat crackers (10.5%). Whole buckwheat crackers were also found to contain less starch (46.7%) when compared to whole wheat crackers (48.9%). From literature, it is evident that cracker technology is evolving towards the production of composite cracker that are of an improved nutritional content. Despite this shift in cracker technology, studies on the starch digestibility of crackers is very rare.

2.4 Starch digestibility

The increase in lifestyle related diseases such as diabetes, consumers' awareness of the link between food, nutrition and health has sparked an interest in starch digestion kinetics (Naidoo et al., 2015). In humans, starch is broken down into glucose, which is the main source of energy for body cells.

Starch digestibility is used to classify starchy foods (Singh, Dartois, & Kaur, 2010). Starch digestibility is characterised by the rate and duration of glycaemic response (Singh *et al*., 2010). Starch digestion begins in the mouth and is completed in the small intestines where glucose is absorbed into the bloodstream and undigested starch passes through to the large intestine (Parada & Santos, 2016). The resultant change in the blood glucose levels after ingestion of food is referred to as glycaemic response (Jones, 2007).

Glycaemic response is measured using the concept of glycaemic index (GI) (Sheard *et al*., 2004). Glycaemic index is defined as "a measure of the change in glucose following ingestion of carbohydrate-containing foods" (Sheard *et al*, 2004), and it is expressed as a percentage of the area under the blood glucose response curve after taking the same amount of carbohydrates as glucose (Jenkins *et al*., 1981).With regards to glycaemic response, carbohydrates are of interest because they have the greatest influence on blood glucose levels (Sheard *et al*, 2004).

Starch can be grouped into three categories (Englyst, Kingman, & Cummings, 1992). Starch that is hydrolysed within 20 minutes is referred to as Rapidly Digestible Starch (RDS), beyond that it is then classified as slowly digestible starch (SDS) (Englyst *et al*, 1992). These authors also concluded that, once starch digestion goes beyond 120 minutes, the remaining undigested starch can be referred to as Resistant Starch (RS). According to these authors, things such as properties of food (intrinsic factors) and chewing (extrinsic factors) influence starch digestion. For instance, if the structure of the food is such that it cannot be readily accessed by starch digesting enzymes, then starch digestion will be slow. Furthermore, actions such as chewing and to the extent which they are carried out influences the physical accessibility of starch that is within rigid structures.

2.5 Sensory evaluation

Sensory evaluation involves a set of techniques that are employed to accurately measure human responses to foods (Lawless & Heimann, 2010). Furthermore, sensory analysis deals with the isolation of food's sensory characteristics (Lawless & Heymann, 2010).

Measuring foods sensory properties and determining their importance to consumers is integral to sensory analysis (Stone & Sidel, 1985). Sensory scientists have an interest in knowing how consumers would respond to food products, both pre and post-purchase (Stone et al., 2012), thus making sensory analysis a crucial tool to product developers and food scientists alike (Lawless & Heymann, 2010).

Sensory evaluation started to gain relevance to food businesses in the 1950s and 1960s, this was after the development of the flavour profile to quantify flavour characteristics of food by Arthur D. Little Inc. (Moskowitz, Beckley & Resurreccion, 2012). Another notable milestone in sensory analysis was the development of the 9-point hedonic scale in 1957 (Moskowitz, Beckley & Resurreccion, 2012). Prior to the above developments, sensory evaluation faced challenges such as management failure to recognise its importance (Stone & Sidel, 1985). Nowadays, food companies have sensory evaluation branches under their quality departments.

Although a food item may be nutritious, it is ideal that the consumers are willing to accept it, hence consumer acceptance tests form an important part of any product development initiative.

CHAPTER 3

RESEARCH

This chapter is divided into two sections. The first section looks at the formulation of pearl millet, wheat, Kalahari truffle and Oyster mushroom composite flours. In this section, selected nutritional and functional properties of the different composite flours are reported and discussed.

Section two of chapter three looks at the development of Kalahari truffle and Oyster mushroom incorporated crackers. Similar to section one, selected nutritional and functional properties of the different mushroom incorporated crackers are discussed.

To highlight the different aspects of starch digestibility, results are presented as rapidly available and slowly available glucose in section one, whereas in section two, starch digestibility results are presented as the amount of glucose released after 20, 60 and 120 minutes (G20, G60 and G120) of hydrolysis.

Pearl millet-wheat, Kalahari truffle and Oyster mushroom composite flours: Some functional and nutritional aspects

Abstract

Cereal grains for a huge part of many people's diets, with wheat being the most consumed of the three major cereal grains. Most developing countries are unable to sufficiently grow their own wheat, thus relying on imports. There has been an increased interest in the development of functional composite flours using locally available cereal grains, tubers or legumes. The aim of this work was to develop pearl millet-wheat, Kalahari truffle and Oyster mushroom composite flours. Fermented whole grain pearl millet flour was mixed with bread wheat flour to have a 1:1 (w/w) pearl millet-wheat composite (control) flour. Parts of this control flour were replaced with 5, 10 and 15% of dry Kalahari truffle powder to have Kalahari truffle composite flours. The same procedure was repeated using oyster mushroom powder. The composite flour flours were analysed for moisture, protein, ash, fat, starch and crude fibre contents, starch digestibility as well as water absorption and solubility indices. Mushroom incorporation significantly ($p<0.05$) increased the composite flours' protein and ash contents. Mushroom incorporation also led to a decline in rapidly available glucose following *in vitro* starch digestibility. Compared to the control, oyster mushroom composite flours had high slowly available glucose, whereas Kalahari truffle composite flours had significantly low slowly available glucose. This study has demonstrated that the two studied mushrooms have the potential to increase the protein content of pearl millet-wheat composite flours. Moreover, the low rapidly available glucose content suggests that the resultant composite flours can be used in formulating food products with low glycaemic index, which could be beneficial to people suffering from type 2 diabetes.

3.1 Introduction

Cereal grains serve as the backbone of agriculture in many civilisations and are an important source of dietary energy for humans (Serna-Saldivar, 2010). Major cereal grains are wheat, rice and maize. While oats, rye, barley, buckwheat, sorghum and millets are considered to be minor cereals (Linquist et al., 2012; Singhal & Kaushik, 2016). In Namibia, pearl millet is an important cereal crop, with more than 60% of the population farming with it at a subsistence level. Other important cereal grains are wheat and white maize.

Compared to other cereal grains, wheat is widely used in the baking industry (Kadam et al., 2012), wheat flour has gluten that when hydrated and mechanically worked forms a cohesive and elastic dough that is ideal for the development of baked goods (Serna-Saldivar, 2010). Several developing countries have shown interest in utilising alternative and local available flours as substitutes for wheat (Raihan & Saini, 2017). This is achieved by formulating composite flours where flour from a locally available cereal crop supplants a portion of wheat flour (Raihan & Saini, 2017). In terms of wheat, composite flour formulation involves mixing wheat flour with other cereals or legumes (Kadam *et al*., 2012), and the resultant composite flour may be used for the production of pasta, snacks, porridges and leavened or unleavened baked products (Chandra *et al*., 2015).

Pearl millet (*Pennisetum glaucum*) is a staple food in many dry regions of Africa and Asia, where it is cultivated via subsistence farming (Dias-Martins *et al*., 2018; Singhal & Kaushik, 2016). It is one of the gluten-free cereal which is also an important source of energy in many developing countries (Akinola *et al*., 2017; Serna-Saldivar, 2010). Pearl mill*et al*so exhibits, anti-inflammatory, antihypertensive, anti-carcinogenic and hypoglycaemic properties (Dias-Martins *et al*., 2018).

Mushrooms are macro-fungus that are composed of a distinctive fruiting body which can either be above or underground (Wani et al., 2010). Mushrooms like truffles grow underground. They are considered to be delicacies that are popularly consumed because of their aromatic and textural appeal (Kalac, 2013). In general, mushrooms contain 19-35% protein, 57% carbohydrate and 2-8% lipids (Beluhan & Ranogajec, 2011; Ekunseitan *et al*., 2017; Wani *et al*., 2010). Mushrooms are highly perishable, hence the need for preservative processing (Ekunseitan *et al*., 2017). There are several studies that investigated the incorporation of mushroom powder in wheat based food products (Gadallah & Ashoush, 2016; Ng *et al*., 2017; Okafor *et al*., 2012). However, there are no studies that investigated compositing pearl millet-wheat and mushrooms. The aim of this study was to determine some physical and chemical characteristics of whole pearl millet-wheat and mushroom (Kalahari truffle and oyster mushroom) composite flours.

3.1.2 Materials and methods

3.1.2.1 Pearl millet and wheat flours

Pearl millet grains were purchased from subsistence farmers around Omuthiya gwIipundi in Oshikoto region. Grains were cleaned to remove foreign objects and fermented at a ratio of 1:2 (w/v) for 24 hours 37°C as outlined by (Onweluzo & Nwabugwu, 2009). Fermented grain was dried at 40°C in an oven (Scientific, Model 222, South Africa) for 48 hours before milling using a hammer mill (Trapp TRF 4000, Metalúrgica Trapp Ltda. Brazil) and passed through a 250 µm screen. Bread wheat flour was purchased from a local retail shop in Windhoek, Namibia.

3.1.2.2 Mushrooms

Kalahari truffles were purchased from informal vendors at Omuthiya gwIipundi Informal (open) Market in Oshikoto region. Truffles were sliced (Figure 8) and dried at 40˚C for 48 hours. Dried Oyster mushrooms were obtained from the University of Namibia's Zero Emissions Research Initiatives Division. Both dry mushrooms were milled using a blender (Waring Commercial 7011HS 2-Speed Food Blender) before passing through a 250 µm screen.

Figure 7 Sliced fresh Kalahari truffle.

3.1.2.3 Composite flours formulation

Composite flours were prepared by mixing equal amounts of wheat and pearl millet flours to make a pearl millet-wheat composite flour (control), 5, 10 and 15% of this composite flour were replaced with either Kalahari truffle powder or Oyster mushroom powder.

3.1.2.4 Proximate composition of composite flours

Moisture, ash, crude fat, crude protein and crude fibre contents were determined following AACC methods 44-15.02 A, 08-01, 30-10.01, 46-30.01 and 32-10.01 respectively. Total starch was determined using the Megazyme Total Starch Assay Kit. Minerals were determined according to the method described by Giron (1973), using Inductively Coupled Plasma (ICP) Spectrometry (ICAP 6000 Series, Thermo Scientific).

3.1.2 .5 Water absorption and Solubility indices

Water absorption (WAI) and solubility (WSI) were determined following the method by Anderson (1982) with some modifications. One gram (1 g) of sample was suspended in 10 mL of distilled water in a 15 mL centrifuge tube. The sample was left at room temperature for 30 minutes with shaking every 5 minutes before centrifuging at 3689 × g for 20 minutes and working out WAI as the wet pellet weight per dry sample weight. Water solubility index was determined by continuing the process used in WAI determination. The supernatant from WAI determination was poured into tarred evaporation dishes. The supernatant was evaporated at 100°C for 3 hours in a convection oven (Scientific, Model 222, South Africa). WSI was worked out as the weight of soluble solids per dry sample weight, multiplied by 100.

3.1.2.6 *In vitro* starch digestibility

In vitro starch digestibility was determined using a modified Englyst method (Englyst *et al.*, 1992). 100 mg of dry sample was suspended in 2 mL 0.1M sodium acetate buffer (pH 5.2) and incubated at 37°C for 5 minutes before the addition of 100 µL of diluted α-amylase and 100 µL of diluted amyloglucosidase. Samples were incubated in a shaking water bath at 37°C for 120 minutes. 50 µL aliquots were pipetted into 2 mL micro-centrifuge tubes containing 400 µL of cold ethanol after 20, 60 and 120 minutes of digestion. Glucose released at the specified time intervals was determined using the Megazyme D-Glucose Assay Procedure (GOPOD).

3.1.2.7 Statistical analysis

All analyses were carried out in duplicates (unless stated otherwise). Results represent the average of duplicates and are presented as means ±standard deviation. Data obtained was subjected to an analysis of variance (ANOVA) and Duncan's least significant differences (LSD) test using SPSS Statistics Software, Version 25 (IBM, USA).

3.1.3 Results and Discussion

3.1.3.1 Moisture content

The moisture contents of all the composite flours ranged from 6.81%-10.22% with Kalahari truffle powder having the least moisture content (Table 6). These results are within range of what Ekunseitan *et al*. (2017) found. These authors reported a moisture content of 10.6-13.22% in wheat, mushroom and high quality cassava composite flours. Moisture content is important in flour storage, with low moisture contents being preferred (Butt et al., 2004). For safe storage, the moisture content of flour and/or grains need to be less than 14% (Delcour & Hoseney, 2010). Low moisture content also retards the deterioration of the flour's baking quality (Butt *et* *al*., 2004). The moisture contents of the oyster mushroom and Kalahari truffle composite flours in this study were all below 14%, which makes the less susceptible to microbial spoilage and can possibly be store safely for relatively much longer.

Table 5 Proximate and physicochemical properties of composite flours.

Composite Flours	**Moisture (%)**	**Ash (%)**	**Crude protein (%)**	**Crude Fat (%)**	**Crude fibre (%)**	**Starch (%)**	**WAI (g/g)**	**WSI (%)**
Kalahari truffle	6.81 ±0.10[a]	5.62 ±0.02[h]	23.65 ±0.07[e]	16.73 ±0.05[h]	7.46 ±0.71[b]	22.91 ±9.32[b]	2.71 ±0.01[d]	24.56 ±1.04[f]
Oyster mushroom	7.33 ±0.03[b]	6.83 ±0.01[i]	25.60 ±0.28[f]	1.44 ±0.06[a]	9.45 ±1.10[c]	12.50 ±0.85[a]	5.26 ±0.06[e]	28.38 ±0.47[g]
Control flour	10.19 ±0.03[d]	0.87 ±0.04[a]	11.65 ±0.07[a]	2.30 ±0.01[d]	0.72 ±0.16[a]	72.90 ±2.45[d]	2.05 ±0.03[ab]	4.75 ±0.08[a]
5% Oyster flour	10.11 ±0.05[cd]	1.12 ±0.01[c]	12.35 ±0.07[b]	2.13 ±0.02[b]	1.40 ±0.45[a]	76.57 ±4.13[d]	1.93 ±0.05[a]	6.81 ±0.06[b]
10% Oyster flour	10.03 ±0.04[c]	1.43 ±0.00[e]	12.9 ±0.00[c]	2.21 ±0.04[c]	1.88 ±0.47[a]	76.57 ±2.78[d]	2.01 ±0.06[ab]	8.17 ±0.16[cd]
15% Oyster flour	10.02 ±0.07[c]	1.72 ±0.02[g]	13.6 ±0.00[d]	2.24 ±0.01[cd]	1.85 ±0.05[a]	65.06 ±4.59[c]	2.20 ±0.21[b]	10.72 ±0.52[e]
5% Truffle flour	10.22 ±0.04[d]	1.05 ±0.04[b]	12.2 ±0.14[b]	3.08 ±0.00[e]	1.21 ±0.13[a]	71.06 ±1.39[d]	1.89 ±0.08[a]	5.54 ±0.01[a]
10% Truffle flour	10.08 ±0.01[e]	1.31 ±0.00[d]	12.8 ±0.00[c]	3.89 ±0.00[f]	1.33 ±0.07[a]	73.02 ±2.80[d]	1.89 ±0.07[a]	7.87 ±0.18[bc]
15% Truffle flour	10.01 ±0.01[c]	1.53 ±0.00[f]	13.45 ±0.07[d]	4.66 ±0.02[g]	1.75 ±0.02[a]	60.65 ±3.95[c]	2.44 ±0.04[c]	8.85 ±0.25[d]

*Values are means (n = 2, except for starch where n = 4) ± standard deviation, values with the same letter in a column are not significantly different (p >0.05). WAI = Water absorption index, WSI = Water solubility index.

3.1.3.3 Ash content

Ash content of the composite flours ranged from 0.87-1.72% (Table 6). Oyster mushroom incorporation significantly ($p<0.05$) increased the composite flour's ash content. There was no significant difference between 5% and 10% oyster mushroom composite flours. Incorporation with 15% oyster mushroom significantly increased the composite flour's ash content. This could have been due to the high (6.83%) ash content observed in the oyster mushroom flour. All Kalahari truffle composite flours had ash contents that were significantly ($p<0.05$) higher than the control flour. Similar to oyster mushroom incorporation, ash content was directly proportional to Kalahari truffle powder incorporation in the composite flour with 15% Kalahari truffle composite flour having the highest ash content amongst the Kalahari truffle composite flours. The incorporation of Kalahari truffle which has a high ash content (5.62%) resulted in an increased amount of ash in the composite flour. These results are consistent with the findings of Ekunseitan *et al*., (2017) who observed a significant increase on ash content as a result of mushroom incorporation in wheat, mushroom and high quality cassava composite flours. Mushrooms have relatively high amounts of minerals (Manzi *et al*., 1999). The relatively high ash contents suggest that these two mushrooms probably have more minerals and they may significantly increase the minerals content of the resultant composite flours.

3.1.3.4 Crude protein

Protein content of the composite flours was between 11.65% and 13.60% (Table 6). Compared to the control flour, 5, 10 and 15% oyster mushrooms composite flours had significantly ($p<0.05$) high protein contents. Among the oyster mushroom composite flours, 15% oyster mushroom composite flour had the highest protein content amongst all the composite flours. This could have been due to the considerably high (25.60%) protein content observed in oyster mushroom powder.

Kalahari truffle incorporation also led to a significant ($p<0.05$) increase in the composite flour's protein content. Following the trend observed in oyster mushroom composite flours, increasing the amount of Kalahari truffle in the composite flour led to significant increases in the composite flours' protein content. Between mushrooms, there was no significant difference ($p<0.05$) between 5% oyster mushroom and 5% Kalahari truffle composite flours. The same trend was true for 10% oyster mushroom and 10% Kalahari truffle composite flours. Mushroom incorporation significantly increased the protein content in both Kalahari truffle and Oyster mushroom composite flours at 15% incorporation level. Protein is an essential biomolecule that is central to proper body function (Bello et al., 2017) and it is the primal element of animal and human tissues (Wu, 2016). The results are in line with the findings of Tumwine, Atukwase, Tumuhimbise, Tucungwirwe, & Linnemann, (2019) who reported that protein content of pearl millet, skimmed milk and vegetables composite flours increased with a percentage increase in skimmed milk. Fenn, Lukow, Humphreys, Fields, & Boye, (2010) also reported a similar trend in wheat-legumes composite flours. Oyster mushroom and Kalahari truffle powders had relatively high protein contents, hence the increase in protein content of the composite flours may be a direct result of mushroom incorporation. These results suggest that these two mushrooms can be employed in the improvement of cereal flours' protein contents.

3.1.3.5 Crude Fat

Crude fat content ranged between 2.13% and 3.89% (Table 6). Among the oyster mushroom composite flours, 15% oyster mushroom composite flour had the highest fat content. There was a significant difference ($p<0.05$) between all oyster mushroom composite flours and the control. Composite flours containing 10 and 15% oyster mushroom did not differ significantly. Fat content was directly proportional to oyster mushroom incorporation in the composite flours. Kalahari truffle incorporation significantly ($p<0.05$) increased the crude fat content of the composite flours. 10% Kalahari truffle composite flour had the highest fat content among all the composite flours. These results show a similar trend like the findings of Udomkun *et al*., (2019) who reported significant increases in the crude fat content of wheat-rice composite flour. Fat play a significant role in the stabilisation of gas cells in cake batters and bread dough, allowing expansion without rapture (Chevallier et al., 2000). Hydrolytic and oxidative rancidity may occur due to high fat content especially when it comprises high levels of unsaturated fatty acids and this has a negative impact on the sensory quality and functional properties of whole grain flour (Doblado-Maldonado et al., 2012). Therefore, flours with low fat content could be less susceptible to fat oxidation.

3.1.3.6 Crude fibre

The composite flours' crude fibre content range was 1.21-1.88% (Table 6). There was a significant difference ($p<0.05$) between the control and all the oyster mushroom composite flours. However, there were no significant differences between the different oyster mushroom composite flours. Among the oyster mushroom composite flours, the highest crude fibre content was observed in 15% oyster flour. The increase in oyster mushroom composite flours could be due to oyster mushroom's high crude fibre, which is in the range of 5.4-30% (Michael et al., 2011). The crude fibre contents of all Kalahari truffle composite flours were not significantly different from the control flour, except for the 15% Kalahari truffle composite flour. Like oyster mushroom composite flours, Kalahari truffle composite flours' crude fibre contents were not significantly different from each other. Kalahari truffle powder's crude fibre content was 7.46%, this was relatively lower than what was found by Gadallah & Ashoush, (2016) in dry desert truffle flour to (8.54%). The difference in the truffles' crude fibre contents could be due to a difference in species and geographical origins. Kalahari truffle's high crude fibre (compared to pearl millet-wheat composite flour) might have contributed to the increased crude fibre content of the Kalahari truffle composite flours.

Overall, the crude fibre contents of oyster mushroom and Kalahari truffle composite flours are within range with what was found by Udomkun *et al.*, (2019), who reported a crude fibre content of 2.1-2.9%. Similarly, when Ekunseitan *et al.*, (2017) investigated the crude fibre content of wheat, high quality cassava and mushroom composite flours, they reported that the crude fibre content ranged between 0.74 and 2.76%. Dietary fibre is an important aspect in the human diet as it can impart a range of health benefits, dietary fibre intake reduces the risk of being vulnerable to diseases such as coronary heart disease, stroke, hypertension, diabetes, obesity and other several gastrointestinal disorders (Anderson *et al.*, 2009; Rathore, Prasad, & Sharma, 2017). Oats, barley and mushrooms are regarded as good sources of dietary fibre including β-glucans (Ng *et al.*, 2017). These results suggest that, these two mushrooms may be successfully used to improve the fibre content of pearl millet-wheat composite flours.

3.1.3.5 Starch

Starch content of the composite flours was between 60.65 and 76.57%. The starch contents for 5 and 10% oyster mushroom composite flours were similar and were the highest among all the composite flours. Oyster mushroom incorporation of up to

15% significantly ($p<0.05$) reduced the composite flour's starch content due the low starch contribution of oyster mushroom. Kalahari truffle composite flours had a starch content of 60.65-73.02%, with the 15% composite flour having the lowest and 10% composite flour having the highest starch content. Incorporation of 15% Kalahari truffle significantly ($p<0.05$) reduced the composite flour's starch content. Of all the carbohydrates found in cereals, starch is the most abundant (Taylor, Emmambux, & Kruger, 2015a). The starch content observed in this study is in correspondence with the findings of Ekunseitan *et al.*, (2017), whereby wheat, mushroom and cassava composites had a starch content range of 68.5-73.91%. Jisha *et al.*, (2010) reported a starch range of 62.06-72.1% in extruded cassava, cereal and legume composite flours, whereas Udomkun *et al.*, (2019) found carbohydrates content (by difference) range of 58.3-66.5% in wheat-cassava, wheat-rice and wheat-banana composite flours.

3.1.3.8 Water absorption (WAI) and solubility (WSI) indices

The water absorption index of the composite flours was in the range of 1.89-2.44 g water/g flour (Table 6). There was no significant difference ($p<0.05$) between the control and all the oyster mushroom composite flours. Composite flours containing 10 and 15% oyster mushroom did not differ significantly. These findings are comparable with those (1.88-2.01 g/g) reported by Bolarinwa & Muhammad, (2019) for germinated brown rice and rice starch composite flours. Kalahari truffle had a low water absorption index (2.71 g/g) compared to oyster mushroom (5.26 g/g). There were no significant differences ($p<0.05$) between the control composite flour and the 5%, and 10% Kalahari truffle composite flours. No significant differences were observed between the control composite flour, 5% and 10% Kalahari truffle composite flours. The increase in the composite flours' WAI could be due to increased amylose leaching and solubility as well as loss of starch crystalline structure (Chandra *et al.*, 2015). High water absorption is associated with the presence of hydrophilic components like polysaccharides (Aathira & Siddhuraju, 2017). Water absorption index is a crucial parameter in baking and it also has importance when the viscosity, bulking and consistency of products are of interest (Julianti et al., 2017).

WSI ranged between 4.75 and 10.72% for the composite flours (Table 6). The composite flour with 15% Oyster mushroom had the highest (10.72%) WSI. This could have been due to oyster mushroom powder's high WSI. This paper's findings were within the range of 5.12-7.76% as found by Obadina *et al.*, (2016) when they investigated the physical properties of native and roasted pearl millet flour. WSI measures the volume occupied by the starch granule after percolated from starch granules in the presence of excess water (Savlak et al., 2016). It is essentially an indicator of the flour particles ability to dissolve in water (Aathira & Siddhuraju, 2017).

3.1.3.9 Minerals content

The composite flours' mineral content is given in Table 7. Three minerals were analysed for and iron was the most abundant mineral in both oyster mushroom and Kalahari truffle. The amount of iron found in oyster mushroom is consistent with the findings of Sakellari *et al.*, (2019) who reported an iron content of 64 – 185 mg/kg in oyster mushrooms grown on different substrates. On the other hand, the amount of iron in Kalahari truffle powder was much lower than 2170.05 mg/kg found by Hamza, Jdir, & Zouari, (2016) in *Tirmania nivea*, a desert truffle from Tunisia. Copper was the lowest of the three minerals in both the oyster mushroom and Kalahari truffle.

Table 6 Composite flours minerals (Cu, Fe and Zn) content (mg/kg).

	Copper	**Iron**	**Zinc**
Composite flours			
Kalahari truffle	19.03 ±0.68^{e}	66.21 ±0.13^{d}	42.27 ±0.83^{f}
Oyster mushroom	10.37 ±0.20^{d}	77.21 ±0.88^{e}	62.19 ±0.91^{g}
Control	3.94 ±0.24^{ab}	40.13 ±2.41^{a}	23.22 ±0.56^{a}
5% OMF	3.31 ±0.37^{a}	41.11 ±1.85^{ab}	25.19 ±0.12^{b}
10% OMF	5.47 ±0.74^{c}	46.36 ±0.94^{c}	28.74 ±1.20^{de}
15% OMF	3.84 ±0.78^{ab}	44.83 ±2.07^{c}	30.05 ±0.37^{e}
5%KTF	3.11 ±0.19^{a}	45.49 ±0.35^{c}	26.19 ±0.88^{bc}
10%KTF	5.30 ±0.67^{c}	45.73 ±0.19^{c}	27.45 ±0.59^{cd}
15%KTF	4.93 ±0.28^{bc}	44.00 ±0.26^{bc}	28.81 ±0.12^{de}

Values are means ±standard deviation; Values with the same letter in a column are not significantly different (p >0.05); results presented as is and n = 2.OMF = Oyster mushroom flour, KTF = Kalahari truffle flour.

Iron was highest in 10% oyster mushroom flour, whereas zinc was highest in 15% oyster mushroom flour. The increase in the composite flours' zinc and iron contents can be directly linked to their high presence in oyster mushroom. There was no significant difference (p<0.05) between the control and 5% oyster mushroom composite flour's copper content. At 10% oyster mushroom incorporation, the composite flour's copper content significantly increased.

All Kalahari truffle composite flours had significantly higher amounts of iron as compared to the control. The same was observed for the composite flours' zinc content. Kalahari truffle had a significantly (p<0.05) higher copper content as compared to oyster mushroom (10.37). Consequently, Kalahari truffle composite flours had copper contents. There was no significant difference between the control and 5% Kalahari truffle composite flours' copper content. Similarly, for copper, no significant differences were observed between 15% Kalahari truffle composite flour and the control. However, 10% Kalahari truffle composite flours' copper contents differed significantly.

In summary, the minerals content of oyster mushroom and Kalahari truffle composite flours is comparable to the findings of Tumwine *et al*., (2019) and Udomkun *et al*., (2019). For example, Udomkun *et al*., (2019) investigated the minerals content of cereal and legume based composite flours and found an iron content that was in the range of 41 – 145 mg/kg and zinc was in the range of 34 mg/kg – 83 mg/kg.

3.1.3.10 *In vitro* Starch Digestibility

In vitro starch digestibility results are given in Table 8**.** The composite flours' rapidly available glucose (RAG) was in the range of 9.22 - 11.91%. Incorporation with 15% oyster mushroom significantly ($p<0.05$) reduced the amount of rapidly digestible starch.

Table 7 Composite flours starch digestibility.

Composite flours	**RAG**	**SAG**
Kalahari truffle	14.73 ±0.42^{e}	5.62 ±0.02^{h}
Oyster mushroom	8.03 ±0.77^{a}	6.83 ±0.01^{i}
Control	11.50 ±0.72^{cd}	0.87 ±0.04^{a}
5% Oyster mushroom flour	11.33 ±1.20^{cd}	1.12 ±0.01^{c}
10% Oyster mushroom flour	11.91 ±0.55^{d}	1.43 ±0.00^{e}
15% Oyster mushroom flour	9.22 ±0.55^{b}	1.72 ±0.02^{g}
5% Kalahari truffle flour	10.74 ±0.72^{c}	1.05 ±0.04^{b}

10% Kalahari truffle flour	11.15 ±0.28[cd]	1.31 ±0.00[d]
15% Kalahari truffle flour	10.88 ±0.43[cd]	1.53 ±0.00[f]

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different ($p > 0.05$). RAG = rapidly available glucose, SAG = slowly available glucose.

According to its digestibility, starch can be classified as either rapidly digestible, slowly digestible or resistant starch (Englyst, Liu, & Englyst, 2007). The amount of glucose released after 20 minutes (G20) of digestion is referred to as Rapidly Available Glucose (RAG), whereas Slowly Available Glucose (SAG) is the difference between G120 and G20 (Englyst, Englyst, Hudson, Cole, & Cummings, 1998). RAG is considered to be a good indicator of starch hydrolysis as it includes both free glucose and rapidly digestible starch. Foods with relatively low rapidly digestible starch are preferred because high amounts of rapidly digestible starch can be detrimental to people suffering from postprandial hyperglycaemia (Vujic et al., 2015).

The slowly available glucose (SAG) content for the composite flour was in the range of 0.87 – 1.72%. Oyster mushroom significantly ($p<0.05$) increased the composite flours' slowly available glucose. Similarly, there was a significant ($p<0.05$) increase in the composite flour's slowly available glucose as a result of Kalahari truffle incorporation. Kalahari truffle composite flours had significantly higher SAG compared to the control. This could be due to the presence of insoluble dietary fibre which acts as a physical barrier and entraps starch molecules, making them less susceptible to enzymatic hydrolysis (Ng *et al*., 2017). These findings point out that oyster mushroom and Kalahari truffle have the potential to increase SAG in pearl millet-wheat composite flours. Increasing SAG content is crucial in changing starch hydrolysis rates and consequently the glycaemic index of the resultant products (Vujic et al., 2015).

3.1.4 Conclusions

Mushrooms incorporation resulted in composite flours that had improved protein, ash and crude fibre content. Oyster mushroom incorporation of up to 15% resulted in an increased crude protein, ash and zinc contents. Whereas 10% oyster mushroom led to relatively high crude fibre, copper and iron contents. Composite flours containing 15% Kalahari truffle powder had relatively high amounts of ash, crude protein, crude fibre and zinc. Relatively high amounts of copper and iron were present in 10% Kalahari truffle incorporated flour. Furthermore, oyster mushroom incorporation of up to 15% resulted in a composite flour with low rapidly available glucose levels, which can be beneficial in manufacturing food with a low GI.

In vitro starch digestibility of Kalahari truffle and Oyster mushroom incorporated pearl millet-wheat crackers.

Abstract

Type 2 diabetes is a global problem that can be mitigated by amongst others, a diet that consists of carbohydrates with substantial amounts of slowly digestible starch. The aim of this study was to investigate the effects of Kalahari truffle and Oyster mushroom incorporation on pearl millet-wheat crackers. Equal amounts of pearl millet and wheat bread flour were mixed. Of this mixture, 5%, 10% and 15% were substituted with Oyster mushroom and Truffle powders to have 0% (control), 5, 10 and 15% Oyster mushroom as well as 5, 10 and 15% Truffle flour formulations. The moisture ash, protein, starch, crude fibre and crude fat contents, water absorption (WAI) and solubility (WSI) indices, physical characteristics, minerals' content, *in vitro* starch digestibility, and sensory evaluation crackers were determined. The ash content, WSI and WAI were higher in the mushrooms than in the cereals composite flour. However, mushrooms had the lowest starch content compared to the cereal composite flour. For the crackers, the ash content, water absorption and water solubility indices were directly proportional to the levels of mushroom incorporation. The starch digestibilities of mushroom incorporated crackers were lower than that of the control. The rate of glucose released during in vitro starch digestion decreased as the mushroom incorporation levels increased. The increase in ash content indicates that mushroom incorporation may potentially improve wheat-pearl millet-mushroom crackers' minerals content. The slow release of glucose during starch digestion suggests that these mushroom incorporated wheat-pearl millet crackers may be beneficial to people with Type 2 diabetes.

3.2.1 Introduction

Generally, cereal grains are responsible for the huge part of human carbohydrates intake, thus the alteration of carbohydrate especially starch quality in cereal products has a potential positive effect on nutrition (Englyst, Vinoy, Englyst, & Lang, 2003). Starch is composed of glucose molecules joined together by α-1,4, and α-1,6 glycosidic bonds that can be hydrolysed by amylases and alpha-glucosidases (Ng *et al*., 2017). *In-vitro* starch digestibility assist in the description of the carbohydrate component of a meal (Englyst & Englyst, 2005). Starch can be classified as rapidly digestible, slowly digestible or resistant starch (Englyst *et al*., 1992). Starch that is hydrolysed within 20 minutes is regarded as rapidly digestible starch, whereas starch that is digested between 20 and 120 minutes is called slowly digestible starch, beyond 120 minutes, it is regarded as resistant starch (Englyst, Kingman, & Cummings, 1992). With the exception of lactose, Englyst *et al*., (2003) further categorised glycaemic fractions of carbohydrates into Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG), with RAG explained as the glucose released after 20 minutes of hydrolysis and SAG as the difference between the glucose released after 120 and 20 minutes ($SAG = G_{120} – G_{20}$).

Cereals grains are an important food source for mankind all over the world. Rice, barley, maize, wheat, sorghum, oat, rye and millets are classified as major cereals in terms of their contribution to people’s diets (Adebiyi, Obadina, Adebo, & Kayitesi, 2018). The word millet is reserved for cereal species or grasses with small round seeds (Graybosch, 2016; Taylor, 2017). Due to their short growing cycle, millets are adapted to semi-desert, tropical and subtropical areas where they are mainly grown at a subsistence level (Serna-Saldivar, 2010). However, millets’ perceived health benefits could see them being grown more on a commercial scale. Millets are classified as either major or minor millets (Taylor, 2017). Fonio, teff, pearl, finger, proso and foxtail millets are regarded as major millets classification, whereas barnyard, kodo and minor millets are identified as minor millets (Taylor, 2017). Based on its annual production and superior nutritional value, pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the most important millet (Graybosch, 2016; Serna-Saldivar, 2010). Pearl millet is cultivated in Southern Africa, Brazil and the United States of America, however, the major pearl millet cultivation areas are India, West and Central Africa (Taylor, 2016). In Namibia, pearl millet is commonly known as *Mahangu* and it is one of three controlled cereal crops, together with wheat and white maize. It is consumed by over half of the Namibian population, with the largest estimated planting area of 268 359 hectares (Namibian Agronomic Board, 2017) in Namibia.

Pearl millet is gluten free, making it one of the low-cost alternative cereal for people suffering celiac disease (Dias-Martins *et al*., 2018). Additional health benefits include the management of blood glucose levels (Kaur *et al*., 2018), as well as anti-cancer and prebiotic properties (Dias-Martins *et al*., 2018). On the other hand, millets contain less than the recommended lysine content (only 17% of the 1.93 g) lysine required by adults (Taylor & Kruger, 2016). This impediment may be overcome by incorporating mushrooms in pearl millet products. Edible mushrooms are known to be an excellent source of protein, with exceptional amounts of essential amino acids (Islam *et al*., 2016; Kadnikova *et al*., 2015; Reis *et al*., 2012). Additionally, mushrooms contain β-glucans which are known to positively influence postprandial glycaemic response in humans (Ng *et al*., 2017). Namibia is home to indigenous and or endemic species of edible mushrooms such as Kalahari truffles (*Kalaharituber* *pfeilii*), *Termitomyces schimperi*, *Termitomyces sagittiformis,* and *Ganoderma* mushroom species, which are barely utilised commercially.

With an increased consumer interest in functional foods and biscuits and crackers can be excellent vessels for functional ingredients due to their popular consumption (Pasqualone *et al*., 2015; Sedej *et al*., 2011). Crackers are of significance in the baking industry due to their low moisture and sugar contents which ensures a better shelf life and they also offer variety in taste, texture and aroma (Florence-Suma et al., 2014; Reddy et al., 2019). Crackers belong to a group of crisp, chemically leavened or fermented bakery products that may contain up to 30% fat (Reddy *et al*., 2019; Sedej *et al*., 2011). Crackers can either be fermented (soda, saltine and cream crackers) or chemically leavened (snack crackers) (Reddy *et al*., 2019). The aim of this project was to develop mushroom incorporated pearl millet-wheat crackers and evaluate the mushroom's influence on the resultant crackers' nutrient content and starch digestibility.

3.2.2 Materials and Methods

3.2.2.1 Ingredients

The pearl millet grain, Kalahari truffle and oyster mushroom samples were treated as described in sections 3.1.2.1 and 3.1.2.2 in the previous paper on composite flours.

3.2.2.2 Crackers formulation

Whole grain pearl millet flour, Kalahari truffle and Oyster mushroom powders were kept frozen until required for crackers formulation. The composite crackers were formulated as presented in Table 9. The dough was rested for 20 minutes followed by sheeting and cutting (Figure 9**)**. The crackers were baked at 180 °C in a conventional oven (Macadams Convecta 7, Cape Town, South Africa) for 8 minutes.

Table 8 Crackers formulation

Ingredients (%)	**Crackers**						
	Control	**5%OMC**	**10%OMC**	**15%OMC**	**5%KTC**	**10%KTC**	**15%KTC**
Wheat flour	25.70	24.40	23.13	21.85	24.40	23.13	21.85
Pearl millet flour	25.70	24.40	23.13	21.85	24.40	23.13	21.85
Oyster M. Flour	0	2.60	5.14	7.70	0	0	0
Kalahari T. Flour	0	0	0	0	2.60	5.14	7.70
Baking powder	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Sugar	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Salt	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Vegetable oil	17.8	17.8	17.8	17.8	17.8	17.8	17.8
Water	26.8	26.8	26.8	26.8	26.8	26.8	26.8
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**

OMC = Oyster mushroom cracker, KTC = Kalahari truffle crackers.

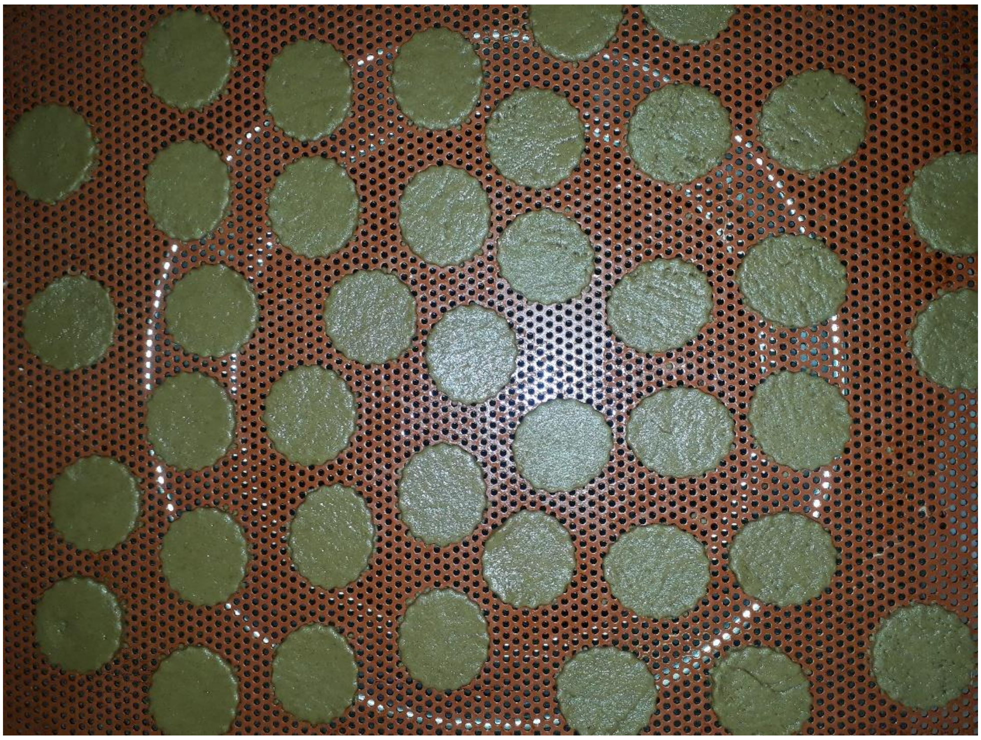

Figure 8 Kalahari truffle crackers before baking

3.2.2.3 Proximate composition of crackers

Crackers were grounded using a blender (Waring Commercial 7011HS 2-Speed Food Blender) and passed through a 500 µm screen. Moisture, ash, crude fat, crude protein and crude fibre contents were determined following AACC methods 44-15.02 A, 08-01, 30-10.01, 46-30.01 and 32-10.01 respectively. Total starch, WAI, WSI, minerals content and starch digestibility were determined as described in respective sections 3.1.2.4, 3.1.2.5 and 3.1.2.6 in the previous paper on composite flours.

3.2.2.6 Crackers' physical characteristics

The pearl millet-wheat and mushroom composite crackers' physical parameters were determined as described by Bello, Oluwamukomi, & Enujiugha, (2017). The diameter (cm) was measured using a ruler. The crackers' thickness was determined by stacking 6 well shaped crackers together, measuring their height and dividing it by 6 to get the average thickness of the crackers. A weighing balance was used to measure the crackers' weight.

3.2.2.9 Preliminary sensory evaluation (Consumer acceptance test)

A voluntary consumer acceptance sensory evaluation was conducted in two sessions on different days. The evaluation of Kalahari truffle incorporated crackers was conducted in the first session with 30 participants (14 females and 16 males, age group 18-30) took part. Oyster mushroom incorporated crackers were evaluated in the second session involving 27 participants (13 females and 14 males, age group 18-50) took part. Four crackers, each identifiable by a three digit random number were presented to each panellist. The crackers were evaluated on a 9 point hedonic scale as described by Lawless & Heimann, (2010), with 1 = dislike extremely and 9 = like extremely. Parameters evaluated were appearance, flavour, texture and overall acceptance.

3.2.2.10 Statistical analyses

The experiment (baking) was done twice. All analyses were done in duplicates and results represent the average of duplicates, presented as means ± standard deviation. Data obtained was subjected to an analysis of variance (ANOVA) and Duncan's least significant differences (LSD) test using SPSS Statistics Software, Version 25 (IBM, USA).

3.2.3 Results and discussion

3.2.3.2 Chemical characteristics of mushroom supplemented pearl millet-wheat crackers.

Results for the chemical characteristics of mushroom supplemented pearl millet-wheat crackers are presented in Table 10.

Table 9 Chemical characteristics of mushroom supplemented pearl millet-wheat crackers.

Crackers	Moisture (%)	Ash (%)	Protein	Total Starch (%)	Crude Fibre	Crude Fat	WAI (g/g)	WSI (%)
Control	3.44 ±0.01^{d}	3.77 ±0.02^{a}	8.72 ±0.26^{a}	58.99 ±3.03^{b}	0.93 ±0.19^{a}	25.50 ±0.47^{a}	3.61 ±0.05^{e}	10.70 ±0.11^{a}
5% OMC	2.23 ±0.02^{a}	3.91 ±0.01^{b}	9.22 ±0.30^{b}	49.50 ±3.56^{a}	2.80 ±0.78^{d}	25.02 ±1.78^{a}	3.03 ±0.04^{b}	16.89 ±0.99^{bcd}
10% OMC	2.32 ±0.07^{b}	3.98 ±0.13^{b}	9.74 ±0.21^{c}	48.41±3.16^{a}	1.43 ±0.29^{abc}	25.47 ±0.52^{a}	3.17 ±0.03^{c}	17.12 ±0.38^{cd}
15% OMC	3.70 ±0.03^{e}	4.27 ±01^{c}	10.35 ±0.06^{d}	46.44 ±2.60^{a}	1.60 ±0.07^{c}	26.11 ±0.23^{a}	3.38 ±0.03^{d}	17.86 ±0.10^{d}
5% KTC	4.27 ±0.00^{f}	3.83 ±0.00^{a}	9.13 ±0.09^{b}	50.04 ±3.13^{a}	1.06 ±0.18^{ab}	25.79 ±1.23^{a}	2.92 ±0.02^{a}	16.03 ±0.33^{b}
10% KTC	3.18 ±0.04^{c}	3.96 ±0.05^{b}	9.67 ±0.14^{c}	48.97 ±4.77^{a}	1.51 ±0.12^{abc}	26.11 ±0.97^{a}	3.07 ±0.03^{b}	16.10 ±0.24^{bc}
15% KTC	2.22 ±0.01^{a}	4.19 ±0.00^{c}	10.55 ±0.47^{d}	48.62 ±2.56^{a}	1.68 ±0.29^{c}	26.21 ±0.29^{a}	3.18 ±0.09^{c}	16.23 ±0.23^{bc}

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different (p >0.05). Values were adjusted to dry weight conditions. OMC = Oyster mushroom cracker, KTC = Kalahari truffle cracker

3.2.3.3 Moisture content

Oyster mushroom incorporation led to a significant ($p<0.05$) decrease in the moisture content of 5 and 10% oyster mushroom crackers. It was also observed that, there was an increase in the crackers' moisture content as oyster mushroom percentage in the formulation increased. These results are in contradiction with Ng *et al.*, (2017)'s findings who observed a decrease in the moisture content of oyster mushroom fortified biscuits. The difference in results could be due to the differences in baking times as these authors baked for 14 minutes compared to the 8 minutes used in this study. The longer baking time could have resulted in more moisture loss hence the observed decrease in the moisture content of oyster mushroom fortified biscuits. These results are however similar to those of Srivastava, Genitha, & Yadav (2012), who noted an increase in the moisture content of biscuits fortified with sweet potato flour. With every increase in the percentage of sweet potato flour, an increase in the moisture content was noted, and this could be attributed to the sweet potatoes' high water binding capacity (Srivastava *et al.*, 2012). Furthermore, oyster mushroom powder recorded the highest water absorption index, this could potentially be an indication that it somehow has a high water binding capacity and possibly be the reason for the crackers observed moisture content increase.

All Kalahari truffle crackers were significantly ($p<0.05$) different from the control cracker. Their moisture content was in the range of 2.22-4.27%. Kalahari truffle crackers' moisture content decreased as the percentage of Kalahari truffle powder increased. These results are also consistent with the findings of Ng *et al.*, (2017). In contrast, Gadallah & Ashoush, (2016) reported that the moisture content of biscuits fortified with desert truffle powder increased as the truffle powder percentage was increased.

The dietary fibre in the mushrooms' powders absorbs water and that could be the reason for the inverse relationship between moisture content and mushroom incorporation (Ng *et al.*, 2017). All the crackers had a moisture content of less than 5% which is the desired moisture content for cookies, crackers and biscuits. A moisture content of less than 5% can be used as a quality parameter and may be used to make inferences on biscuits' microbial and textural properties and eventually their shelf life (Ng *et al.*, 2017).

3.2.3.4 Ash content

Ash content serves as an estimate of the overall mineral content of food samples (Harris & Marshall, 2017). The control cracker had the least ash content (3.77%). Although there was no significant difference ($p<0.05$) between the control cracker's ash content and those of the oyster mushroom and truffle crackers at 5%, and there was a gradual increase in the cracker's ash content with every increase in mushroom percentage. This is consistent with the findings of Farzana & Mohajan, (2015) who found that the ash content of mushroom fortified biscuits increased (from 1.50% to 1.76%) with every increase in the percentage of soy flour. Similarly, Ng, Robert, Ahmad, & Ishak, (2017) also observed a gradual increase in the ash content (0.86% to 1%) of oyster mushroom fortified biscuits. Overall, the 15% oyster mushroom cracker had the highest ash content (4.11%), with the 15% Kalahari truffle cracker in second place. These two crackers were not significantly different ($p<0.05$). The high ash content in the 15% oyster mushroom and 15% Kalahari truffle crackers could be a result of these two mushrooms high ash content (6.80 and 5.61 respectively). These results show that the addition of mushroom powder increased the ash content of Kalahari truffle and oyster mushroom enriched crackers. These findings imply that these two mushrooms can potentially be used in improving the mineral content of cereal based baked products.

3.2.3.5 Protein content

The control cracker's protein content was 8.75%, this was significantly ($p<0.05$) lower than all the oyster mushroom crackers' protein content. The results show a trend of an increasing protein content with every percentage increase in the formulation's oyster mushroom content. Protein content significantly differed among the oyster mushroom crackers. The highest oyster mushroom incorporation (15%) significantly increased the crackers' protein from 8.75 to 10.35%.

Kalahari truffle incorporation resulted in a significant increase in the crackers' protein content. Similar to oyster mushroom crackers, the relationship between Kalahari truffle incorporation and the crackers' protein content was directly proportional. The highest protein content amongst the Kalahari truffle crackers was found in 15% Kalahari truffle crackers, whereas 5% Kalahari truffle crackers had the least protein content.

Overall, both oyster mushroom and Kalahari truffle incorporation significantly increased the crackers' protein content. This could have been due to the two mushrooms' superior protein content as compared to that of wheat or pearl millet. The highest protein content amongst both oyster mushroom and Kalahari truffle crackers was found in the 15% Kalahari truffle cracker. Compared to the findings of Adebowale, Adegoke, Sanni, Adegunwa, & Fetuga (2012) who reported a decrease in the amount of protein in sorghum incorporated wheat biscuits, the study's findings show a gradual increase in protein content as the mushrooms' content in the formulation increased. The results are however in agreement with the trend and protein content found in composite biscuits by Kolawole, Akinwande, & Ade-Omowaye (2018).

3.2.3.6 Total starch

Cereal grain carbohydrate include starch, non-carbohydrate polysaccharides, pentosans, beta glucans and trace amounts of simple sugars and oligosaccharides (Taylor, Emmambux, & Kruger, 2015). Compared to the other components, starch is found in large amounts (Taylor *et al*., 2015). The control cracker had the highest (58.99%) starch content and this was significantly different from 15% oyster mushroom cracker had the least (46.44%) starch content. Overall, Oyster mushroom incorporation resulted in a significant decrease ($p<0.05$) in the crackers' starch content. Within the crackers, no significant differences were observed between 5, 10 and 15% oyster mushroom crackers.

Kalahari truffle incorporation significantly reduced the cracker's starch content (58.99 to 48.62%). Amongst the different Kalahari truffle crackers, 5% Kalahari truffle crackers had the highest starch content, and this was not significantly different from the 10 and 15 % Kalahari truffle cracker's starch contents.

All in all, the decrease in the mushroom incorporated cracker's starch content could be attributed to the two mushrooms' low starch content as presented in Table 9. The decrease in starch content is consistent with the findings of Farzana and Mohajan, (2015) who recorded a reduction (from 65.62% to 56.38%) in the starch content of mushroom fortified biscuits. Similarly, Ng *et al*., (2017) also reported a decrease in the starch content of mushroom fortified biscuits as the mushroom percentage increased. Gadallah and Ashoush, (2016) also reported similar findings on biscuits fortified with desert truffle powder.

3.2.3.7 Crude fibre

The crackers' crude fibre content was in the range of 0.93 – 2.80%. 5 and 15% oyster mushroom incorporation significantly ($p<0.05$) increased the crackers' crude fibre content. Crackers with 5% oyster mushroom had the highest crude fibre content, thereafter, crude fibre content decreased to 1.43 and 1.60% for 10 and 15% oyster mushroom crackers respectively. Crackers with 10% oyster mushroom incorporation did not significantly increase the crackers' crude fibre content.

Kalahari truffle crackers had a crude fibre content of 1.06 – 1.68%. Although 5 and 10% Kalahari truffle incorporation resulted in an increase in the crackers' crude fibre content, there was no significant difference between them and the control cracker. Kalahari truffle incorporation of up to 15% significantly increased the crackers' crude fibre content from 0.93 to 1.68%.

Apart from the sudden rise in the crackers' crude fibre content after 5% oyster mushroom incorporation, the general trend was that of a gradual increase in fibre as mushroom incorporation increased. The gradual increase is comparable with Bello *et al*., (2017)'s findings. A similar trend was reported by (Adebowale et al., 2012; Kolawole et al., 2018; Ogunlakin et al., 2018).

3.2.3.8 Crude fat

The crackers' fat content was between 25.02 and 26.21%. There was no significant difference between the control and all oyster mushroom crackers. Similarly, no significant differences were observed between the control and all Kalahari truffle crackers. In comparison to the composite flours where the crackers were derived, the fat content sharply increased. The sunflower oil added to the crackers' dough could be the cause of the sharp increase. Although it was lower than this study's findings, Bello *et al*., (2017) reported a crude fat content of 19.05 – 21.71% in mushroom composited wheat biscuits.

3.2.3.9 Water absorption and Solubility indices

The results for water absorption and water solubility indices are presented in table 10. The control cracker had the highest WAI and the lowest WSI, 3.61 g/g and 10.70 % respectively. Compared to the control, the addition of Kalahari truffle and oyster mushroom powders led to a significant ($p<0.05$) reduction in WAI. These results are consistent with the findings of (Brennan, Derbyshire, Tiwari, & Brennan, 2012) who reported that the WAI of extruded snacks enriched with Chestnut mushroom co-products reduced from 5.03 g/g (control) to 4.23 (15% MCM). The 15% oyster mushroom cracker was found to have the highest WSI 17.12%. This could have resulted from the high water solubility property of oyster mushroom powder as shown in table 9.

WAI and WSI are mostly used as indicators of starch gelatinisation (Tongdang et al., 2008). WAI measures the extent to which granules can absorb a certain volume of water when suspended in excess water in the absence of heat and I high WAI is an indication that starch was fully gelatinised (Tongdang *et al*., 2008). WSI on the other hand is used to determine the solubility of free polysaccharides (Tongdang *et al*., 2008). These authors further state that WSI is directly proportional to the molecular breakdown of starch during processing.

3.2.3.10 Crackers' physical characteristics

Physical properties of the composite crackers are presented in Table 11. The composite crackers' weights ranged between 3.42 – 5.25g. Crackers containing 5% oyster mushroom had a significantly lower weight than the control.

Table 11 Oyster mushroom and Kalahari truffle crackers' physical characteristics

Crackers	Weight (g)	Diameter (cm)	Thickness (cm)
Control	5.25 ±0.84^{b}	3.58 ±0.22^{a}	0.53 ±0.09^{a}
5% OMC	3.42 ±0.35^{a}	4.02 ±0.04^{c}	0.44 ±0.01^{a}
10% OMC	4.25 ±0.12^{ab}	3.93 ±0.05^{bc}	0.52 ±0.02^{a}
15% OMC	4.25 ±0.12^{ab}	4.00 ±0.00^{c}	0.45 ±0.02^{a}
5% KTC	3.83 ±0.24^{a}	3.83 ±0.10^{b}	0.42 ±0.07^{a}
10% KTC	4.42 ±0.59^{ab}	3.82 ±0.15^{b}	0.43 ±0.04^{a}
15% KTC	3.92 ±0.12^{a}	3.80 ±0.11^{b}	0.40 ±0.00^{a}

*Values are means (n = 2) ± standard deviation, values with the same letter in a column are not significantly different (p >0.05). OMC = Oyster mushroom cracker, KTC = Kalahari truffle cracker.

There were no significant differences between the control, 10 and 15% oyster mushroom crackers. In terms of diameter, all oyster mushroom crackers significantly differed from the control. However, there no significant differences between all the oyster mushroom crackers and the control cracker's thickness.

Kalahari truffle crackers' stack weights were in the range of 3.83 – 4.42, these were significantly lower than the control's weight, except for the 10% oyster mushroom cracker which was not significantly (p<0.05) different from the control. The control cracker's diameter was significantly less compared to all the Kalahari truffle crackers.

The general observation was that, mushroom incorporation significantly increased the composite crackers' diameter. This observation is consistent with the findings of Ogunlakin, Oni, & Olaniyan, (2018) who noticed an increase in the diameter of edible termite fortified biscuits. A similar trend was reported by Akubor, (2003) when investigating the physical properties of cowpea, plantain and wheat composite biscuits. The crackers' wet thickness are consistent with the findings of Ogunlakin *et al*., (2018) and Bello *et al*., (2017).

3.2.3.11 Minerals content

Minerals content is shown in Table 12. Among the three minerals analysed in the crackers, iron was the most abundant. For all the mushroom incorporated crackers, only OMC (10% and 15%) had a significantly lower copper content than the control. The zinc and iron contents of all the crackers were statistically the same.

Table 12 Composite crackers minerals (Cu, Fe and Zn) content (mg/kg).

	Copper	**Iron**	**Zinc**
Crackers			
Control	3.18 ±1.43^{c}	39.10 ±5.12^{a}	22.28 ±2.19^{a}
5% OMC	2.80 ±0.78^{bc}	44.02 ±6.06^{a}	23.84 ±3.24^{a}
10% OMC	1.32 ±0.95^{ab}	43.00 ±7.88^{a}	24.66 ±3.98^{a}
15% OMC	0.68 ±0.13^{a}	38.57 ±6.53^{a}	23.86 ±2.00^{a}
5%KTC	2.19 ±0.62^{abc}	41.23 ±4.92^{a}	23.09 ±2.43^{a}
10%KTC	2.44 ±1.34^{bc}	41.03 ±5.73^{a}	22.36 ±2.82^{a}
15%KTC	1.92 ±1.00^{abc}	41.43 ±2.77^{a}	21.50 ±2.71^{a}

Values are means ±standard deviation; Values with the same letter in a column are not significantly different ($p > 0.05$); results presented as is and n = 4. OMC = Oyster mushroom cracker, KTC = Kalahari truffle cracker

All in all, these results are different from what was reported by Bello, Oluwamukomi, & Enujiugha, (2017). These authors reported significant increases in the amount of iron, copper and zinc present in mushroom-wheat composite biscuits

(5 – 30% oyster mushroom incorporation). Furthermore, these authors found these elements in much lower amounts as compared with this study's findings.

3.2.3.12 *In vitro* Starch digestibility

In-vitro starch digestibility results are presented in Table 13. Compared to the mushroom powder enriched crackers, the control cracker had the highest digestible starch at both G20 (16.32), G60 (26.63) and G120 (37.71) respectively.

Table 10 In vitro starch digestibility of Kalahari truffle and Oyster mushroom powder enriched crackers (%).

Crackers	G20	G60	G120
Control	16.32 ±0.55[c]	26.63 ±1.08[b]	37.71 ±1.05[d]
5% OMC	16.09 ±0.41[b]	26.33 ±1.52[b]	34.45 ±1.82[c]
10% OMC	13.97 ±0.78[a]	23.99 ±1.24[ab]	32.53 ±1.06[bc]
15% OMC	13.69 ±0.60[a]	23.31 ±1.66[a]	31.16 ±1.93[ab]
5% KTC	15.89 ±0.39[bc]	25.84 ±1.40[ab]	33.75 ±0.34[c]
10% KTC	15.09 ±0.04[b]	23.54 ±2.92[a]	32.78 ±1.88[bc]
15% KTC	14.16 ±0.42[a]	23.67 ±1.82[a]	30.11 ±0.86[a]

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different (p >0.05). OMC = Oyster mushroom cracker, KTC = Kalahari truffle cracker.

It was observed that starch digestibility significantly ($p<0.05$) decreased as mushroom powder incorporation increased. This could be the a result of the low starch contents of both Kalahari truffle and oyster mushroom powders and/or the effect of mushroom powder on the digestibility of starch. These results are consistent with the findings of Ng *et al*., (2017), who reported a similar trend in mushroom fortified biscuits. Similarly Brennan, Derbyshire, Tiwari, and Brennan, (2013) recorded a decline in the amount of glucose released from mushroom enriched extruded snacks.

The decline in the release of glucose could be due to the increase in mushroom percentage, which could have in turn resulted in an increase in the crackers' dietary fibre content. Dietary fibre is especially important as it improves glucose metabolism and insulin response in patient suffering from Type 2 diabetes, by facilitating the gradual hydrolysis of carbohydrates and glucose release (Brennan, 2005). Wan Rosli, Nurhanan, and Aishah, (2012) reported an increase in the amount of dietary fibre as the mushroom percentage increased in butter biscuits. These authors reported that butter biscuits formulated with 4% and 6% mushroom powder had high amounts of β-glucans, 0.72% and 0.79% respectively. Mushroom dietary fibre has been of interest lately, as it has been found to contain β-glucans which have positive effects on blood glucose modulation (Cheung, 2013). The soluble fraction of β-glucans is apparently responsible for their ability to lower blood glucose levels (De Paula et al., 2017).

These *in vitro* starch digestibility results show that the addition of oyster mushroom powder to cereal based baked products can significantly reduce the amount of glucose released at 20, 60 and 120 minutes time intervals. The addition of Kalahari truffle powder also resulted in a similar outcome. Therefore, the addition of these two mushrooms to pearl millet-wheat crackers can potentially be beneficial to subjects with the Type 2 diabetes.

3.2.3.13 Sensory evaluation (Consumer acceptance test)

All the oyster mushroom incorporated crackers scored higher than 5 on the 9-point hedonic scale (Figure 11**)**. The 5% oyster mushroom incorporated cracker scored highest points for flavour (6.6), texture (6.7) and overall appearance (7.1). It can be argued that oyster mushroom incorporation of up to 5% imparts desirable flavour and texture, thus contributing to its overall acceptance. These findings are almost similar to those of Ng, Robert, Ahmad, & Ishak, (2017) who reported that 4% oyster mushroom incorporated biscuits scored the highest in terms of overall acceptance.

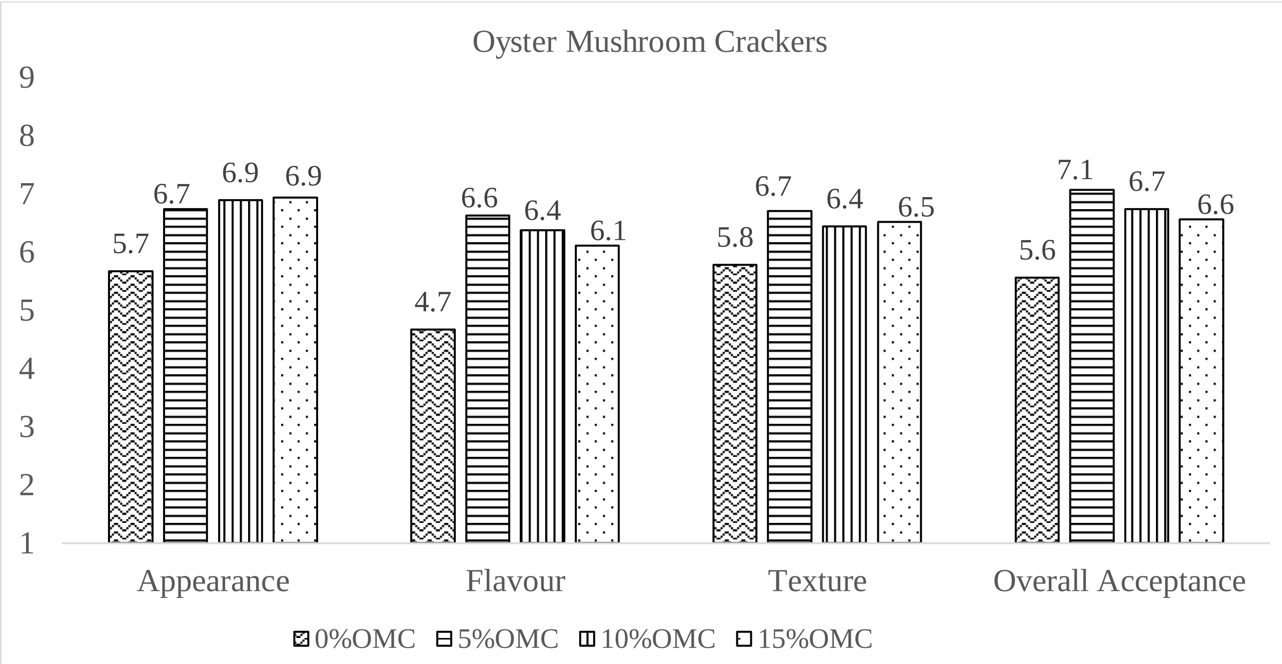

Figure 9 Oyster mushroom crackers' consumer sensory evaluation results. (n = 27

The 5% Kalahari truffle incorporated cracker scored the highest across all the evaluated parameters (Figure 10**)**. The Kalahari truffle incorporated crackers' appearance, flavour, texture and overall acceptance both declined with increasing amounts of Kalahari truffle powder in the formulation. The declining score in the crackers' appearance could be due to the dark colour of the Kalahari truffle incorporated crackers. The decline in flavour scoring could be due to the presence of polyphenols in the truffle powder, which is associated with slight bitterness (Gadallah & Ashoush, 2016). Gadallah & Ashoush, (2016) reported similar trends in desert truffle incorporated biscuits.

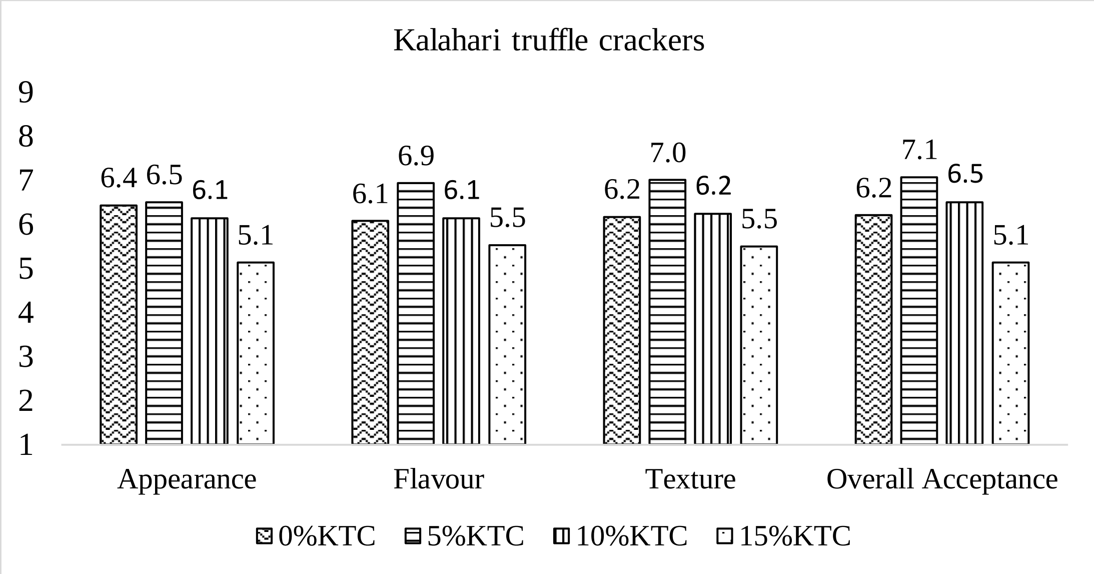

Figure 10 Kalahari truffle crackers' consumer sensory evaluation results. (n = 30).

3.3.4 Conclusions

Incorporating Kalahari truffle and Oyster mushrooms in pearl millet-wheat crackers increased their ash content. Mushroom incorporation of up to 15% can significantly increase pearl millet and wheat-based crackers' protein content. Furthermore, Kalahari truffle and Oyster mushroom may be used in the development of low *in vitro* starch digestibility foods. Both 5% Oyster and Kalahari truffle incorporated crackers were moderately accepted by consumers.

CHAPTER 4

GENERAL DISCUSSION

This chapter first discusses availability and cultivation of oyster mushroom and Kalahari truffles. It then covers the necessity of the use of composite flours and briefly discusses the validity of *in vitro* starch digestibility methods as an approximation of *in vivo* results. Lastly, a brief discussion on the effect of compositing pearl millet-wheat-mushroom on nutrient content, starch digestibility and consumer sensory responses.

4.1 Oyster mushrooms and Kalahari truffles: Availability and cultivation

Mushroom cultivation has been on the rise with commercial production reaching 7 959 979 tonnes in 2012 (Corrêa *et al*., 2016). China is currently the world's largest oyster mushroom producer with more 46% of global production attributed to Chinese producers (Jongman et al., 2018). Africa is the least mushroom producing continent accounting for less than 1% of the world's mushroom production (Chioza & Ohga, 2014; Jongman *et al*., 2018). In Africa, most of the mushrooms are grown in South Africa, with oyster and button mushrooms being the most cultivated (Chioza & Ohga, 2014).

Kalahari truffles are seasonal and they usually grow well in years where there is adequate and well distributed rainfall (Trappe *et al*., 2008). They are associated with several host species such as *Acacia haematoxylon* (grey camelthorn), *Acacia erioloba*, *Stipagrostis* species, *Cynodon datylon* (R. A. Adeleke, 2007) and pearl millet (*Pennisetum glaucum*) (Ntshakaza, 2013). Although species found in the vicinity of Kalahari truffles may be considered as hosts, this is apparently not usually the case (Ntshakaza, 2013). The inability to successfully identify Kalahari truffle's host plants has hampered efforts for its cultivation (Ntshakaza, 2013).

The seasonality of Kalahari truffles may present a challenge in their availability throughout the year. However, finding alternative processing and preservation streams could ensure that in the years when they are abundantly available, they do not go to waste, given mushroom's perishable nature. Although drying as a preservation method may lead to a loss of the desirable flavours associated with truffles, dried Kalahari truffles can be repurposed to develop alternative products such as composite flours, extruded snacks, crackers or biscuits and many other food items.

4.2 Composite flours and their relevance in the agro-business and nutritional landscape

The development of a composite flour is influenced by economic or nutritional reasons (Chandra et al., 2015). The use of composite flours can reduce wheat importation and may act as a boost to local agro-businesses as farmers can be encouraged to grow more of the crops found to be suitable wheat substitutes (Ekunseitan *et al*., 2017). Nutritionally, people are becoming more health conscious and their diets are centred around the prevention of many diseases (Erukainure *et al*., 2016; Raihan & Saini, 2017). Thus, consumers are shifting towards the consumption of functional foods, some of which can be produced using composite flours.

4.3 Validity of *in vitro* starch digestibility methods as an approximation of *in vivo* results

Measuring Glycaemic Index (GI) involves human test subject to evaluate postprandial blood glucose response up to 2 hours after ingestion (Germaine *et al*., 2008). To cost effectively assess GI, *in vitro* starch digestibility methods have been developed, these methods are faster and allow product developers in the selection of which products may be used for *in vivo* GI tests (Germaine *et al*., 2008). Several studies on the *in vitro* prediction of postprandial GI have been conducted (Bornet *et al*., 1989; Englyst, Kingman, & Cummings, 1992; Englyst, Veenstra, & Hudson, 1996; Goñi, Garcia-Alonso, & Saura-Calixto, 1997; Granfeldt, Bjorck, Drews, & Tovar, n.d.; Jenkins *et al*., 1980; Snow & O'Dea, 1981). Each of these methods employ different sample preparations, some use only amylases and others use a combination of amylases and proteolytic enzymes (Goñi *et al*., 1997). However, the most widely used method for starch digestibility and classification of starch fractions in food substances is that of Englyst, Kingman, & Cummings, (1992). The fact that this method was developed hand in hand with validated *in vivo* studies has contributed to its wide use (Englyst *et al*., 2018). The method is reproducible and this was confirmed through inter-laboratory studies as reported by Englyst *et al*., (2018).

Most of the starch digestibility methods used around the world have been validated in conjunction with *in vivo* studies. For instance, Goñi, Garcia-Alonso, and Saura-Calixto (1997) found good correlation (r = 0.894) between values obtained *in vivo* and those obtained *in vitro*. Similarly, Englyst, Kingman, & Cummings, (1992) developed an *in vitro* starch digestibility method whose validation involved a series of *in vivo* studies. Therefore, several *in vitro* starch digestibility methods that exist have been validated and are good predictors of what happens in the human body. However, for better accuracy, it is important to closely mimic the mechanisms that take place *in vivo*. The mechanisms in question include replicating the mastication of a food. The accuracy of these chemical methods might also be improved by using enzymes of human origin.

4.4 Effect of compositing crackers with mushrooms on the nutrient content, starch digestibility and consumer acceptance

Baked foods are primary made with wheat flours. In recent times, the incorporation of locally available cereal grains in the development of different baked foods has become popular. This investigation developed composite flours and crackers from whole grain pearl millet, bread wheat flour, Kalahari truffle and oyster mushroom.

One of the objectives of this investigation was to develop composite flours and crackers with an improved nutritional content. The results presented in this work suggest that Kalahari truffle and oyster mushroom incorporation can improve the nutritional content of crackers. Thus, it can be concluded that this objective has been achieved.

Another objective was to determine how mushroom incorporation affects the *in vitro* starch digestibility of the composite flours and crackers. This objective has also been achieved as it was determined that mushroom incorporation resulted in flours and crackers that had low rapidly digestible starch.

Edible mushrooms are mostly prized for their culinary appeal. To diversify the use of mushrooms, there is a need to normalise the use of mushrooms for reasons other than those that a culinary related. Although oyster mushroom has been used in the development of a similar product (biscuits), the use of Kalahari truffle in the development of crackers has never been reported before. Additionally, the use of pearl millet in the production of composite flours and crackers is also new. The use of pearl millet in the production of these composite flours and crackers may contribute to diversifying the use of this locally available cereal grain.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Mushroom incorporation positively affects pearl millet-wheat composite flour's protein, crude fibre ash and rapidly available glucose contents. The use of these composite flours in the production of crackers yielded products with an increased protein, ash and minerals (Cu, Fe and Zn) contents. The water absorption and water solubility indices results improved as a result of mushrooms incorporation, suggesting that the composite flours possess the required functional properties for use in the production of baked products. Mushrooms incorporation also positively impacted starch digestibility by significantly reducing the amount of RAG present in pearl millet, wheat, Kalahari truffle and Oyster mushroom crackers. This positively asserts the importance pearl millet-wheat and mushroom crackers as a potential healthy snack especially for those living with diabetes.

Although the composite flours and crackers show potential nutritional and functional capabilities, further studies on aspects such as protein digestibility, *in* vivo starch digestibility, minerals availability, amino acids profile, pasting and rheological properties and shelf life of the composite flours and crackers need to be done.

CHAPTER 6

REFERENCES

AACC. (2009). *AACC International Approved Methods, 44-15.02 A, 08-01, 30-10.01, 46-30.01 and 32-10.01* (9th ed.,). https://doi.org/10.1094/aaccintmethods

Aathira, M., & Siddhuraju, P. (2017). Evaluation on suitability of differentially processed D . bulbifera tubers (Aerial and Underground) as alternative in composite flours for future food innovations. *International Journal of Food Science and Nutrition*, *2*(6), 142–148.

Adebayo, E. A., & Martínez-Carrera, D. (2015). Oyster mushrooms (Pleurotus) are useful for utilizing lignocellulosic biomass. *African Journal of Biotechnology*, *14*(1), 52–67. https://doi.org/10.5897/ajb2014.14249

Adebiyi, J. A., Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2018). Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. *Critical Reviews in Food Science and Nutrition*, *58*(3), 463–474. https://doi.org/10.1080/10408398.2016.1188056

Adebiyi, Janet Adeyinka, Obadina, A. O., Adebo, O. A., & Kayitesi, E. (2017). Comparison of nutritional quality and sensory acceptability of biscuits obtained from native , fermented , and malted pearl millet (Pennisetum glaucum) flour. *Food Chemistry*, *232*, 210–217. https://doi.org/10.1016/j.foodchem.2017.04.020

Adebowale, A. A., Adegoke, M. T., Sanni, S. A., Adegunwa, M. O., & Fetuga, G. O. (2012). Functional properties and biscuit making potentials of sorghum-wheat flour composite. *American Journal of Food Technology*, Vol. 7, pp. 372–379. https://doi.org/10.3923/ajft.2012.372.379

Adeleke, R. A. (2007). *Isolation, Propagation and Rapid Molecular Detection of the Kalahari Truffle, A Mycorrhizal Fungus Occurring In South Africa.* Rhodes University.

Adeleke, R., & Dames, J. F. (2014). Kalaharituber pfeilii and associated bacterial interactions. *South African Journal of Botany*, *90*, 68–73. https://doi.org/10.1016/j.sajb.2013.10.003

Aishah, M. S., & Rosli, W. I. W. (2013). Effect of Different Drying Techniques on the Nutritional Values of Oyster Mushroom (Pleurotus sajor-caju) (Kesan Teknik Pengeringan Berbeza terhadap Nilai Pemakanan Cendawan Tiram (Pleurotus sajor-caju). *Sains Malaysiana*, *42*(7), 937–941.

Ajitaa, T., & Jha, S. K. (2017). Extrusion Cooking Technology: Principal mechanism and effect on direct expanded snacks: An overview. *International Journal of Food Studies*, *6*, 113–128. https://doi.org/10.7455/ijfs/6.1.2017.a10

Akinola, S. A., Badejo, A. A., Osundahunsi, O. F., & Edema, M. O. (2017). Original article Effect of preprocessing techniques on pearl millet flour and changes in technological properties. *International Journal of Food Science and Technology*, *54*(4), 992–999. https://doi.org/10.1111/ijfs.13363

Akubor, P. I. (2003). Functional properties and performance of cowpea / plantain / wheat flour blends in biscuits. *Plant Foods for Human Nutrition*, *58*, 1–8. https://doi.org/10.1023/B

Akyüz, M., Kırbağ, S., & Bircan, B. (2015). Medical Characteristics of Arid-Semi Arid Truffle (Terfezia and Picoa) in the Elazığ-Malatya region of Turkey. *Hacettepe Journal of Biology and Chemistry*, *4*(43), 301–301. https://doi.org/10.15671/hjbc.20154315989

Alyami, J., Ladd, N., Pritchard, S. E., Hoad, C. L., Sultan, A. A., Spiller, R. C., … Taylor, M. A. (2019). Glycaemic, gastrointestinal and appetite responses to breakfast porridges from ancient cereal grains: A MRI pilot study in healthy humans. *Food Research International*, *118*(December 2017), 49–57. https://doi.org/10.1016/j.foodres.2017.11.071

Amadou, I., Gounga, M. E., & Le, G. W. (2013). Millets: Nutritional composition, some health benefits and processing - A review. *Emirates Journal of Food and Agriculture*, *25*(7), 501–508. https://doi.org/10.9755/ejfa.v25i7.12045

Anderson, J. (2019). Gluten-Free Cracker Brand List. Retrieved October 19, 2019, from VeryWell Fit Website website: https://www.verywellfit.com/gluten-free-crackers-562449

Anderson, J. W., Baird, P., Davis Jr, R. H., Ferreri, S., Knudtson, M., Koraym, A., … Williams, C. L. (2009). Health benefits of dietary fiber. *Nutrition Reviews*, *67*(4), 188–205. https://doi.org/10.1111/j.1753-4887.2009.00189.x

Anderson, R. A. (1982). Water Absorption and Solubility and Amylograph Characteristics of Roll-Cooked Small Grain Products. *Cereal Chemistry*, *59*(4), 265–269.

Annor, G. A., Tyl, C., Marcone, M., Ragaee, S., & Marti, A. (2017). Why do millets have slower starch and protein digestibility than other cereals? *Trends in Food Science and Technology*, *66*, 73–83. https://doi.org/10.1016/j.tifs.2017.05.012

Barrion, S. C. (2008). *Pearl millet milling: Comparison between traditional Namibian fermentation- semi-wet milling and dry milling* (University of Pretoria). https://doi.org/10.1109/PGC.2010.5706077

Bello, M., Oluwamukomi, M. O., & Enujiugha, V. N. (2017). Nutrient Composition and Sensory Properties of Biscuit from Mushroom-Wheat Composite Flours. *Archives If Current Research International*, *9*(3), 1–11. https://doi.org/10.9734/ACRI/2017/35686

Beluhan, S., & Ranogajec, A. (2011). Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry*, *124*(3), 1076–1082. https://doi.org/10.1016/j.foodchem.2010.07.081

Benjakul, S., & Karnjanapratum, S. (2018). Characteristics and nutritional value of whole wheat cracker fortified with tuna bone bio-calcium powder. *Food Chemistry*, *259*(November 2017), 181–187. https://doi.org/10.1016/j.foodchem.2018.03.124

Bolarinwa, I. F., & Muhammad, K. (2019). *Functional Properties , Antioxidant Activities and Storage Stability of Cookies from Germinated Brown Rice and Rice- Potato Starch Composite Flour*. *42*(2), 503–518.

Bonatti, M., Karnopp, P., Soares, H. M., & Furlan, S. A. (2004). Evaluation of Pleurotus ostreatus and Pleurotus sajor-caju nutritional characteristics when cultivated in different lignocellulosic wastes. *Food Chemistry*, *88*(3), 425–428. https://doi.org/10.1016/j.foodchem.2004.01.050

Bornet, F. R., Fontvieille, A.-M., Rizkalla, S., Colonna, P., Blayo, A., Mercier, C., & Slama, G. (1989). Insulin and glycemic responses starches processed in different in vitro a-amylase in healthy humans to native ways : correlation with in vitro a-amylase hydrolysis13. *American Journal of Clinical Nutrition*, *50*, 315–323.

Bouatia, M., Touré, H. A., Cheikh, A., Eljaoudi, R., Rahali, Y., Oulad Bouyahya

Idrissi, M., … Draoui, M. (2018). Analysis of nutrient and antinutrient content of the truffle (Tirmania pinoyi) from Morocco. *International Food Research Journal*, *25*(1), 174–178. Retrieved from http://www.ifrj.upm.edu.my

Brennan, C. S. (2005). Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition and Food Research,* *49*(6), 560–570. https://doi.org/10.1002/mnfr.200500025

Brennan, M. A., Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2012). Enrichment of extruded snack products with coproducts from chestnut mushroom (Agrocybe aegerita) production: Interactions between dietary fiber, physicochemical characteristics, and glycemic load. *Journal of Agricultural and Food Chemistry*, *60*(17), 4396–4401. https://doi.org/10.1021/jf3008635

Brennan, M. A., Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2013). Integration of β-Glucan Fibre Rich Fractions from Barley and Mushrooms to Form Healthy Extruded Snacks. *Plant Foods for Human Nutrition*, *68*(1), 78–82. https://doi.org/10.1007/s11130-012-0330-0

Butt, M. S., Nasir, M., Akhtar, S., & Sharif, K. (2004). Effect Of Moisture and Packaging on the Shelf Life of Wheat Flour. *Internet Journal of Food Safety*, *4*(1), 1–6.

Chandra, S., Singh, S., & Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology*, *52*(6), 3681–3688. https://doi.org/10.1007/s13197-014-1427-2

Chaturvedi, P., Khare, K., Kwape, T., & Makholwa, K. (2015). Free radical scavenging and antioxidant properties of a truffle, Kalaharituber pfeilii found in

Kalahari deserts of Botswana. *International Jornal of Bioassays*, *4*(11), 4533–4537.

Cheung, P. C. K. (2013). Mini-review on edible mushrooms as source of dietary fiber : Preparation and health benefits. *Food Science and Human Wellness*, *2*(3–4), 162–166. https://doi.org/10.1016/j.fshw.2013.08.001

Chevallier, S., Colonna, P., Della Valle, G., & Lourdin, D. (2000). Contribution of major ingredients during baking of biscuit dough systems. *Journal of Cereal Science*, *31*(3), 241–252. https://doi.org/10.1006/jcrs.2000.0308

Chioza, A., & Ohga, S. (2014). Cultivated Mushrooms in Malawi: A Look at the Present Situation. *Advances in Microbiology*, *04*(01), 6–11. https://doi.org/10.4236/aim.2014.41002

Coffey, J. (2019). MycoTerraFarm. Retrieved December 3, 2019, from Oyster Mushroom – Pleurotus Ostreatus website: http://mycoterrafarm.com/oyster-mushroom-pleurotus-ostreatus/

Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2016). Biotechnological, nutritional and therapeutic uses of Pleurotus spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends in Food Science and Technology*, *50*, 103–117. https://doi.org/10.1016/j.tifs.2016.01.012

Dahham, S. S., Al-Rawi, S. S., Ibrahim, A. H., Abdul Majid, A. S., & Abdul Majid, A. M. S. (2018). Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle Terfezia claveryi. *Saudi Journal of Biological Sciences*, *25*(8), 1524–1534. https://doi.org/10.1016/j.sjbs.2016.01.031

Davidson, I. (2016). *Biscuit Baking Technology* (2nd ed.). Oxford: Academic Press.

De Paula, R., Abdel-Aal, E. S. M., Messia, M. C., Rabalski, I., & Marconi, E. (2017). Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. *Journal of Cereal Science*, *75*, 124–131. https://doi.org/10.1016/j.jcs.2017.03.030

Deepalakshmi, K., & Mirunalini, S. (2014). Pleurotus ostreatus : an oyster mushroom with nutritional and medicinal properties. *J Biochem Tech*, *5*(2), 718–726.

Delcour, J. A., & Hoseney, R. C. (2010). *Principles of Cereal Science and Technology* (3rd ed.). St Paul, Minnesota: AACC International Inc.

Dias-Martins, A. M., Letícia, K., Pessanha, F., Pacheco, S., Avelino, J., Rodrigues, S., … Carvalho, P. (2018). Potential use of pearl millet (Pennisetum glaucum (L .) R . Br .) in Brazil : Food security , processing , health bene fi ts and nutritional products. *Food Research International*, *109*(April), 175–186. https://doi.org/10.1016/j.foodres.2018.04.023

Doblado-Maldonado, A. F., Pike, O. A., Sweley, J. C., & Rose, D. J. (2012). Key issues and challenges in whole wheat flour milling and storage. *Journal of Cereal Science*, *56*(2), 119–126. https://doi.org/10.1016/j.jcs.2012.02.015

Dundar, A., Yesil, O. F., Acay, H., Okumus, V., Ozdemir, S., & Yildiz, A. (2012). Antioxidant properties, chemical composition and nutritional value of Terfezia boudieri (Chatin) from Turkey. *Food Science and Technology International ·*, *18*(4), 317–328. https://doi.org/10.1177/1082013211427954

Dyson, P. (2015). Low Carbohydrate Diets and Type 2 Diabetes: What is the Latest Evidence? *Diabetes Therapy*, *6*(4), 411–424. https://doi.org/10.1007/s13300-

015-0136-9

Eissa, H. A., Hussein, A. S., & Mostafa, B. E. (2007). Rheological Properties and Quality Evaluation of Egyptian Balady Bread and Biscuits ... *Polish Journal of Food and Nutrition Sciences*, *57*(4), 487–496.

Eissa, H., Hussein, A., & Mostafa, B. (2007). Rheological properties and quality evaluation of Egyptian balady bread and biscuits supplemented with flours of ungerminated and germinated legume seeds or mushroom. *Polish Journal of Food and Nutrition Sciences*, *57*(4), 487–496.

Ekunseitan, O. F., Obadina, A. O., Sobukola, O. P., Omemu, A. M., Adegunwa, M. O., Kajihausa, O. E., … Keith, T. (2017). Nutritional composition, functional and pasting properties of wheat, mushroom, and high quality cassava composite flour. *Journal of Food Processing and Preservation*, *41*(5), 1–8. https://doi.org/10.1111/jfpp.13150

El Enshasy, H., Elsayed, E. A., Aziz, R., & Wadaan, M. A. (2013). Mushrooms and truffles: Historical biofactories for complementary medicine in Africa and in the middle East. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 10. https://doi.org/10.1155/2013/620451

El Hag, M. E., El Tinay, A. H., & Yousif, N. E. (2002). *Effect of fermentation and dehulling on starch , total polyphenols , phytic acid content and in vitro protein digestibility of pearl millet*. *77*, 193–196.

Elyas, S. H. ., El Tinay, A. H., Yousif, N. E., & Elsheikh, E. A. E. (2002). Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet vitro protein digestibility of pearl millet. *Food Chemistry*, *78*, 75–79. https://doi.org/10.1016/S0308-8146(01)00386-7

Englyst, H.N., Kingman, S. M., & Cummings, J. . (1992). Classification and measurement of nutritionally important starch fractions.pdf. *European Journal of Clinical Nutrition*, *46*(2), S33–S50.

Englyst, Hans N., Veenstra, J., & Hudson, G. J. (1996). Measurement of rapidly available glucose (RAG) in plant foods : a potential in vitro predictor of the glycaemic response. *British Journal of Nutrition*, *75*(1996), 327–337.

Englyst, K., Goux, A., Meynier, A., Quigley, M., Englyst, H., Brack, O., & Vinoy, S. (2018). Inter-laboratory validation of the starch digestibility method for determination of rapidly digestible and slowly digestible starch. *Food Chemistry*, *245*(November 2017), 1183–1189. https://doi.org/10.1016/j.foodchem.2017.11.037

Englyst, K N, Liu, S., & Englyst, H. N. (2007). *Nutritional characterization and measurement of dietary carbohydrates*. *61*, 19–39. https://doi.org/10.1038/sj.ejcn.1602937

Englyst, Klaus N., Vinoy, S., Englyst, H. N., & Lang, V. (2003). Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition*, *89*(3), 329–339. https://doi.org/10.1079/bjn2002786

Englyst, Klaus N, & Englyst, H. N. (2005). Carbohydrate bioavailability. *British Journal of Nutrition*, *94*(1), 1–11. https://doi.org/10.1079/bjn20051457

Englyst, Klaus N, Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1998). Rapidly available glucose in foods : an in vitro measurement that reflects the glycemic response 1 , 2. *American Journal of Clinical Nutrition*, *69*, 448–454.

Erukainure, O. L., Okafor, J. N. C., Ogunji, A., Ukazu, H., Okafor, E. N., & Eboagwu, I. L. (2016). Bambara–wheat composite flour: rheological behavior of dough and functionality in bread. *Food Science and Nutrition*, *4*(6), 852–857. https://doi.org/10.1002/fsn3.356

Fan, L., Li, J., Deng, K., & Ai, L. (2012). Effects of drying methods on the antioxidant activities of polysaccharides extracted from Ganoderma lucidum. *Carbohydrate Polymers*, *87*(2), 1849–1854. https://doi.org/10.1016/j.carbpol.2011.10.018

Farzana, T., & Mohajan, S. (2015). Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuits fortified with mushroom. *Food Science and Nutrition*, *3*(5), 363–369. https://doi.org/10.1002/fsn3.228

Fenn, D., Lukow, O. M., Humphreys, G., Fields, P. G., & Boye, J. I. (2010). Wheat-Legume Composite Flour Quality. *International Journal of Food Properties*, *13*(2), 381–393. https://doi.org/10.1080/10942910802571729

Ferdman, Y., Aviram, S., Roth-Bejerano, N., Trappe, J. M., & Kagan-Zur, V. (2005). Phylogenetic Studies of Terfezia pfeilii and Choiromyces echinulatus (Pezizales) support new genera for southern African truffles: Kalaharituber and Eremiomyces. *Mycological Research*, *109*(2), 237–245. https://doi.org/10.1017/S0953756204001789

Fernandes, Â., Barros, L., Martins, A., Herbert, P., & Ferreira, I. C. F. R. (2015). Nutritional characterisation of Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm. produced using paper scraps as substrate. *Food Chemistry*, *169*, 396–400. https://doi.org/10.1016/j.foodchem.2014.08.027

Florence-Suma, P., Urooj, A., Asha, M. ., & Rajiv, J. (2014). Sensory, Physical and

Nutritional Qualities of Cookies Prepared from Pearl Millet (Pennisetum Typhoideum). *Journal of Food Processing & Technology*, *5*(10). https://doi.org/10.4172/2157-7110.1000377

Gadallah, G. E. M., & Ashoush, S. I. (2016). Value Addition on Nutritional and Sensory Properties of Biscuit Using Desert Truffle (Terfezia claveryi) Powder. *Food and Nutrition Sciences*, *07*(12), 1171–1181. https://doi.org/10.4236/fns.2016.712109

Gangopadhyay, N., O'Shea, N., Brunton, N. P., Gallagher, E., Harrison, S. M., & Rai, D. K. (2019). Fate of beta-glucan, polyphenols and lipophilic compounds in baked crackers fortified with different barley-milled fractions. *LWT - Food Science and Technology*, *114*(June), 108413. https://doi.org/10.1016/j.lwt.2019.108413

Gençcelep, H., Uzun, Y., Tunçtürk, Y., & Demirel, K. (2009). Determination of mineral contents of wild-grown edible mushrooms. *Food Chemistry*, *113*(4), 1033–1036. https://doi.org/10.1016/j.foodchem.2008.08.058

Germaine, K. A., Samman, S., Fryirs, C. G., Griffiths, P. J., Johnson, S. K., & Quail, K. J. (2008). Comparison of in vitro starch digestibility methods for predicting the glycaemic index of grain foods. *Journal of the Science of Food and Agriculture*, *658*(December 2006), 652–658. https://doi.org/10.1002/jsfa

Giron, H. C. (1973). Comparison between dry ashing and wet digestion in the preparation of plant material for atomic absorption analysis. *Atomic Absorption Newsletters*, *12*(28).

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, *17*(3), 427437.1.

Granfeldt, Y., Bjorck, I., Drews, A., & Tovar, J. (1992). An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products..pdf. *European Journal of Clinical Nutrition*, *46,* 649–660.

Graybosch, R. A. (2016). The Grain Crops: An Overview. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains, Volume 1* (Second Edi, pp. 16–22). Oxford: Academic Press, Elsevier.

Guyot, J. P. (2012). Cereal-based fermented foods in developing countries: Ancient foods for modern research. *International Journal of Food Science and Technology*, *47*(6), 1109–1114. https://doi.org/10.1111/j.1365-2621.2012.02969.x

Haileka, V. L. (2015). Screening, isolation and characterization of laccase enzumes from Namibian Termitomyces Schimperi and Kalaharituber Pfeilii (Namibia; Vol. 53). https://doi.org/10.1017/CBO9781107415324.004

Hamza, A., Jdir, H., & Zouari, N. (2016). Antioxidant and Antibacterial Properties of Tirmania nivea , A Wild Edible Desert Truffle from Tunisia Arid Zone. *Medicinal & Aromatic Plants*, *5*(4). https://doi.org/10.4172/2167-0412.1000258

Harris, G. K., & Marshall, M. R. (2017). Ash Analysis. In S. S. Nielsen (Ed.), *Food Analysis* (5th ed., pp. 287–297). Cham, Switzerland: Springer International Publishing.

Hearst, R., Nelson, D., McCollum, G., Millar, B. C., Maeda, Y., Goldsmith, C. E., … Moore, J. E. (2009). An examination of antibacterial and antifungal properties of constituents of Shiitake (Lentinula edodes) and Oyster (Pleurotus ostreatus) mushrooms. *Complementary Therapies in Clinical Practice*, *15*(1), 5–7. https://doi.org/10.1016/j.ctcp.2008.10.002

Hussain, G., & Al-Ruqaie, I. M. (1999). Occurance, Chemical composition and nutritional value of truffles: An Overview. *Pakistan Journal of Biological Sciences*, *2*(2), 510–514.

International Food Information Service. (2009). IFIS Dictionary of Food Science and Technology. In *International Journal of Dairy Technology* (2nd ed.). https://doi.org/10.1111/j.1471-0307.2007.00242.x

Islam, T., Yu, X., & Xu, B. (2016). Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. *LWT - Food Science and Technology*, *72*, 423–431. https://doi.org/10.1016/j.lwt.2016.05.005

Iwalokun, B. A., Usen, U. A., Otunba, A. A., & Olukoya, D. K. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of Pleurotus ostreatus. *African Journal of Biotechnology*, *6*(15), 1732–1739. https://doi.org/10.5897/AJB2007.000-2254

Jafri, M., Jha, A., Bunkar, D. S., & Ram, R. C. (2013). Quality retention of oyster mushrooms (Pleurotus florida) by a combination of chemical treatments and modified atmosphere packaging. *Postharvest Biology and Technology*, *76*, 112–118. https://doi.org/10.1016/j.postharvbio.2012.10.002

Jeena, G. S., Punetha, H., Prakash, O., Chandra, M., & Kushwaha, K. P. S. (2014). Study on in vitro antioxidant potential of some cultivated pleurotus species (oyster mushroom). *Indian Journal of Natural Products and Resources*, *5*(1), 56–61.

Jenkins, D. J. A., Wolever, T. M. S., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., … Goff, D. V. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, *34*(3), 362–366.

Jenkins, D. J. A., Wolever, T. M. S., Taylor, R. H., Ghafari, H., Jenkins, A. L., Barker, H., & Jenkins, M. J. A. (1980). Rate of digestion of foods and postprandial glycaemia in normal and diabetic subjects. *British Medical Journal*, (July), 14–17.

Jisha, S., Sheriff, J. T., Padmaja, G., Jisha, S., Sheriff, J. T., & Padmaja, G. (2010). Nutritional , Functional and Physical Properties of Extrudates from Blends of Cassava Flour with Cereal and Legume Flours. *International Journal of Food Properties*, *2912*(13), 5. https://doi.org/10.1080/10942910902934090

Jonathan, S. G., Okon, C. B., Oyelakin, A. O., & Oluranti, O. O. (2012). Nutritional values of oyster mushroom (Pleurotus ostreatus) (Jacq. Fr.) Kumm. cultivated on different agricultural wastes. *Nature and Science*, *10*(9), 186–191.

Jones, J. M. (2007). Glycemic response definitions. *Cereal Foods World*, *52*(2), 54–55. https://doi.org/10.1094/CFW-52-1-0054

Jongman, M., Khare, K. B., & Loeto, D. (2018). Oyster mushrrom cultivation at different production systems: a review. *European Journal of Biomedical and Pharmaceutical Sciences*, *5*(5), 72–79.

Julianti, E., Rusmarilin, H., & Yusraini, E. (2017). Functional and rheological properties of composite flour from sweet potato, maize, soybean and xanthan gum. *Journal of the Saudi Society of Agricultural Sciences*, *16*(2), 171–177. https://doi.org/10.1016/j.jssas.2015.05.005

Kadam, M., Salve, R., Mehrajfatema, Z., & More, S. (2012). Development and

Evaluation of Composite Flour for Missi roti /chapatti. *Journal of Food Processing & Technology*, *03*(1), 1–7. https://doi.org/10.4172/2157-7110.1000134

Kadnikova, I. A., Costa, R., Kalenik, T. K., Guruleva, O. N., & Yanguo, S. (2015). *Chemical Composition and Nutritional Value of the Mushroom Auricularia auricula-judae*. *3*(8), 478–482. https://doi.org/10.12691/jfnr-3-8-1

Kagan-zur, V., & Roth-bejerano, N. (2008). Desert Truffles. *Fungi Magazine*, *1*, 32–37. https://doi.org/10.1007/978-3-642-40096-4

Kagan-Zur, V., Roth-Bejerano, N., & Taylor, F. W. (1995). *Cultivation of Terfezia Pfeilii - The Kalahari Desert Truffle*. Beer-Sheva, Israel.

Kalac, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, *93*, 209–218. https://doi.org/10.1002/jsfa.5960

Kaur, P., Purewal, S. S., Sandhu, K. S., Kaur, M., & Salar, R. K. (2018). Millets: a cereal grain with potent antioxidants and health benefits. *Journal of Food Measurement and Characterization*, *13*(1), 793–806. https://doi.org/10.1007/s11694-018-9992-0

Kayode, R. M. O., Olakulehin, T. F., Adedeji, B. S., Ahmed, O., Aliyu, T. H., & Badmos, A. H. A. (2015). Evaluation of amino acid and fatty acid profiles of commercially cultivated oyster mushroom (Pleurotus sajor-caju) grown on gmelina wood waste. *Nigerian Food Journal*, *33*(1), 18–21. https://doi.org/10.1016/j.nifoj.2015.04.001

Kolawole, F. L., Akinwande, B. A., & Ade-Omowaye, B. I. O. (2018).

Physicochemical properties of novel cookies produced from orange-fleshed sweet potato cookies enriched with sclerotium of edible mushroom (Pleurotus tuberregium). *Journal of the Saudi Society of Agricultural Sciences*, 1–5. https://doi.org/10.1016/j.jssas.2018.09.001

Kumar, K. (2018). Nutraceutical potential and processing aspects of oyster mushrooms (Pleurotus species). *Current Nutrition & Food Science, 14,* 1–12. https://doi.org/10.2174/1573401314666181015111724

Kumari, R., Singh, K., Singh, R., Bhatia, N., & Nain, M. S. (2019). Development of healthy ready-to-eat (RTE) breakfast cereal from popped Development of healthy ready-to-eat (RTE) breakfast cereal from popped pearl millet. *Indian Journal of Agricultural Sciences*, *89*(5), 877–881.

Kweon, M., Slade, L., Levine, H., & Gannon, D. (2014). Cookie- Versus Cracker-Baking — What ' s the Difference ? Flour Functionality Requirements Explored by SRC and Alveography. *Critical Reviews in Food Science and Nutrition*, *54*, 115–138. https://doi.org/10.1080/10408398.2011.578469

Lawless, H. T., & Heimann, H. (2010). Introduction, Descriptive Analysis, Acceptance Training. In *Sensory Evaluation of Food: Principles and Practices* (Second, pp. 1–17, 227–253, 325–344). https://doi.org/10.1007/978-1-4419-6488-5

Lennerz, B. S., Barton, A., Bernstein, R. K., Dikeman, R. D., Diulus, C., Hallberg, S., … Ludwig, D. S. (2018). Management of type 1 diabetes with a very low–carbohydrate diet. *Pediatrics*, *141*(6). https://doi.org/10.1542/peds.2017-3349

Linquist, B., van Groenigen, K. J., Adviento-Borbe, M. A., Pittelkow, C., & van Kessel, C. (2012). An agronomic assessment of greenhouse gas emissions from major cereal crops. *Global Change Biology*, *18*(1), 194–209. https://doi.org/10.1111/j.1365-2486.2011.02502.x

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

Lu, X., Brennan, M. A., Serventi, L., Liu, J., Guan, W., & Brennan, C. S. (2018). Addition of mushroom powder to pasta enhances the antioxidant content and modulates the predictive glycaemic response of pasta. *Food Chemistry*, *264*(April), 199–209. https://doi.org/10.1016/j.foodchem.2018.04.130

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., Pizzoferrato, L., & Smr, P. (1999). Nutrients in edible mushrooms : an inter-species comparative study. *Food Chemistry*, *65*, 1–6.

McDonough, C. M., & Rooney, L. W. (1989). Structural Characteristics of Pennisetum Americanum (Pearl Millet) Using Scanning Electron and Fluorescence Microscopy. *Food Microstructure*, *8*(1), 137–149.

Michael, H. W., Bultosa, G., & Pant, L. M. (2011). Nutritional contents of three edible oyster mushrooms grown on two substrates at Haramaya, Ethiopia, and sensory properties of boiled mushroom and mushroom sauce. *International Journal of Food Science and Technology*, *46*(4), 732–738. https://doi.org/10.1111/j.1365-2621.2010.02543.x

Millar, K. A., Barry-Ryan, C., Burke, R., Hussey, K., McCarthy, S., & Gallagher, E. (2017). Effect of pulse flours on the physiochemical characteristics and sensory acceptance of baked crackers. *International Journal of Food Science and Technology*, *52*, 1155–1163. https://doi.org/10.1111/ijfs.13388

Miller, R. (2015). Biscuits, Cookies and Crackers: Nature of the Products. In *Encyclopedia of Food and Health* (1st ed.). https://doi.org/10.1016/B978-0-12-384947-2.00075-1

Minter, D. W. (2008). *Kalaharituber pfeilii* (Vol. 75).

Moreno, M. de L., Comino, I., & Sousa, C. (2014). Alternative grains as potential raw material for gluten– free food development in the diet of celiac and gluten–sensitive patients. *Austin Journal of Nutrition and Food Sciences*, *2*(3).

Morris, H., Beltrán, Y., Llauradó, G., Batista, P., Perraud, I., Garcia, N., … Diez, J. (2017). Mycelia from Pleurotus sp. (oyster mushroom): a new wave of antimicrobials, anticancer and antioxidant bio-ingredients. *International Journal of Phytocosmetics and Natural Ingredients*, *4*(1), 3. https://doi.org/10.15171/ijpni.2017.03

Mweu, B. M., Akuja, T. E., & Mburu, M. W. (2016). Yield components of six pearl millet (Pennisetum Glaucum (L .) R . Br) varieties in the arid South Eastern part of Kenya. *International Journal of Agricultural Research and Review: ISSN-2360-7971*, *4*(3), 514–521.

Naidoo, K., Amonsou, E. O., & Oyeyinka, S. A. (2015). In vitro digestibility and some physicochemical properties of starch from wild and cultivated amadumbe corms. *Carbohydrate Polymers*, *125*, 9–15. https://doi.org/10.1016/j.carbpol.2015.02.066

Nambiar, V. S., Dhaduk, J. J., Neha, S., Tosha, S., & Rujuta, D. (2011). Potential functional implications of pearl millet (Pennisetum glaucum) in health and disease. *Journal of Applied Pharmaceutical Science, 1*(10), 62–67.

Namibian Agronomic Board. (2017). Mahangu: Production, Imports And Local Procurement Statistics. Retrieved September 9, 2019, from https://www.nab.com.na/controlled-crops/grain/mahangu/production-imports.php

Nani, A., Belarbi, M., Soualem, Z., Ghanemi, F. ., Borsali, N., & Amamou, F. (2016). Study of the impact of millet (Pennisetum glaucum) on the glucidic metabolism in diabetic rats. *Annals of Biological Research*, *2*(4), 34–37.

Ndung`u, S. W., Otieno, C. A., Onyango, C., & Musieba, F. (2015). Nutritional Composition, Physical Qualities and Sensory Evaluation of Wheat Bread Supplemented with Oyster Mushroom. *American Journal of Food Technology*, *10*(6), 279–288. https://doi.org/10.3923/ajft.2015.279.288

Ng, S. H., Robert, S. D., Ahmad, W. A. N. W., & Ishak, W. R. W. (2017). Incorporation of dietary fibre-rich oyster mushroom (Pleurotus sajor-caju) powder improves postprandial glycaemic response by interfering with starch granule structure and starch digestibility of biscuit. *Food Chemistry*, *227*, 358–368. https://doi.org/10.1016/j.foodchem.2017.01.108

Ntshakaza, P. (2013). *Host relations of Kalaharituber pfeilii (Henn .) Trappe & Kagan-Zur*. Rhodes University.

Obadina, A., Oluwadamilare, I., Omobolanle, I., Gomes, A., Wanderlei, C., Carvalho, P. De, & Barboza, H. T. (2016). Nutritional and physico-chemical properties of flour from native and roasted whole grain pearl millet (Pennisetum glaucum [L .] R . Br .). *Journal of Cereal Science*, *70*, 247–252.

https://doi.org/10.1016/j.jcs.2016.06.005

Obilana, A. B. (2003). Importance of millets in Africa. In P. S. Belton & J. R. N. Taylor (Eds.), *Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa. 2–4 April 2003.* Pretoria, South Africa.

Ogunlakin, G. O., Oni, V. T., & Olaniyan, S. A. (2018). Quality Evaluation of Biscuit Fortified with Edible Termite (Macrotermes nigeriensis). *Asian Journal of Biotechnology and Bioresource Technology*, *4*(2), 1–7. https://doi.org/10.9734/ajb2t/2018/43659

Okafor, D. ., Onuegbu, N. ., Odimegwe, N. ., Ibeabuchi, J. ., Njoku, N. ., Agunwa, I. ., … Njoku, C. . (2017). Antioxidant and Antimicrobial Activities of Oyster Mushroom. *American Journal of Food Science and Technology*, *5*(2), 64–69. https://doi.org/10.12691/ajfst-5-2-6

Okafor, J. N. C., Okafor, G. I., Ozumba, A. U., & Elemo, G. N. (2012). Quality characteristics of bread made from wheat and nigerian oyster mushroom (Pleurotus plumonarius) powder. *Pakistan Journal of Nutrition*, *11*(1), 5–10. https://doi.org/10.3923/pjn.2012.5.10

Onweluzo, J. C., & Nwabugwu, C. C. (2009). Fermentation of millet (Pennisetum americanum) and Pigeon Pea (Cajanus cajan) seeds for flour production: Effects on composition and selected functional properties. *Pakistan Journal of Nutrition*, *8*(6), 737–744.

Ovat, O. ., Ijomah, J. ., Bukie, J. ., & Ugobo, W. . (2017). Effect of organic substrates on the propagation of *Pleurotus ostreatus* (Oyster Mushroom) In Obubra, Cross River State, Nigeria. *Journal of Research in Forestry, Wildlife & Environment*, *9*(2), 15–22.

Parada, J., & Santos, J. L. (2016). Interactions between Starch, Lipids, and Proteins in Foods: Microstructure Control for Glycemic Response Modulation. *Critical Reviews in Food Science and Nutrition*, *56*(14), 2362–2369. https://doi.org/10.1080/10408398.2013.840260

Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., Caponio, F., & Blanco, A. (2015). Production and characterization of functional biscuits obtained from purple wheat. *Food Chemistry*, *180*, 64–70. https://doi.org/10.1016/j.foodchem.2015.02.025

Patel, S. (2012). Food, Health and Agricultural Importance of Truffles: A Review of Current Scientific Literature. *Current Trends in Biotechnology*, *6*(1), 15–27. https://doi.org/10.1017/CBO9781107415324.004

Patel, S., Rauf, A., Khan, H., Khalid, S., & Mubarak, M. S. (2017). Potential health benefits of natural products derived from truffles: A review. *Trends in Food Science and Technology*, *70*, 1–8. https://doi.org/10.1016/j.tifs.2017.09.009

Patel, Y., Naraian, R., & Singh, V. K. (2012). Medicinal Properties of Pleurotus Species (Oyster Mushroom): A Review medicinal mushroom View project Diversity of Pleurotus species having many nutritional and medicinal properties View project. *World Journal of Fungal and Plant Biology*, *3*(1), 1–12. https://doi.org/10.5829/idosi.wjfpb.2012.3.1.303

Pelembe, L. A. M., Dewar, J., & Taylor, J. R. N. (2003). Food products from malted pearl millet. *Food Products from Malted Pearl Millet. In Proceeding of the Workshop on the Proteins of Sorghum and Millets:Enhancing Nutritional and Functional Properties for Africa, Pretoria, South Africa.*, *Paper 21*.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, *26*(7), 693–699. https://doi.org/10.1016/j.fm.2009.07.011

Qadri, T., Hussain, S. Z., Rather, A. H., Amin, T., & Naseer, B. (2018). Nutritional and storage stability of wheat-based crackers incorporated with brown rice flour and carboxymethyl cellulose (CMC). *International Journal of Food Properties*, *21*(1), 1117–1128. https://doi.org/10.1080/10942912.2018.1485033

Radzki, W., Ziaja-Sołtys, M., Nowak, J., Rzymowska, J., Topolska, J., Sławińska, A., … Kuczumow, A. (2016). Effect of processing on the content and biological activity of polysaccharides from Pleurotus ostreatus mushroom. *LWT - Food Science and Technology*, *66*, 27–33. https://doi.org/10.1016/j.lwt.2015.10.016

Raihan, M., & Saini, C. S. (2017). Evaluation of various properties of composite flour from oats, sorghum, amaranth and wheat flour and production of cookies thereof. *International Food Research Journal*, *24*(6), 2278–2284.

Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition*, *5*(2), 35–46. https://doi.org/10.1016/j.phanu.2017.02.001

Reddy, P. A., Pranusha, G., Rao, G. L., & Reddy, A. N. (2019). Development and Analysis of Sorghum Crackers. *International Journal of Scientific Research in Science and Technology*, *6*(2), 26–31. https://doi.org/10.32628/ijsrst196178

Regula, J., & Gramza-Michalowska, A. (2010). New cereal food products with dried shiitake mushroom (lentinula edodes) added as a source of selected nutrients. *Italian Journal of Food Science*, *22*(3), 292–297.

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology*, *50*(2), 191–197. https://doi.org/10.1016/j.fct.2011.10.056

Rooney, L. W. (2010). *Overview: sorghum and millet food research failures and successes*.

Sakellari, A., Karavoltsos, S., Tagkouli, D., Rizou, C., Sinanoglou, V. J., Zoumpoulakis, P., … Kalogeropoulos, N. (2019). Trace Elements in Pleurotus Ostreatus, P. Eryngii, and P. Nebrodensis Mushrooms Cultivated on Various Agricultural By-Products. *Analytical Letters*, *52*(17), 2692–2709. https://doi.org/10.1080/00032719.2019.1594865

Salar, K. R., Purewal, S. S., & Sandhu, K. S. (2017). Fermented pearl millet (Pennisetum glaucum) with in vitro DNA damage protection activity , bioactive compounds and antioxidant potential. *Food Research International*, *100*(June), 204–210. https://doi.org/10.1016/j.foodres.2017.08.045

Saleh, A. S. M., Zhang, Q., Chen, J., & Shen, Q. (2013). Millet grains: Nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 281–295. https://doi.org/10.1111/1541-4337.12012

Sarita, & Singh, E. (2016). Potential of Millets: Nutrients Composition and Health Benefits. *Journal of Scientific and Innovative Research JSIR*, *5*(52), 46–50.

Saritha, K. V., Prakash, B., Khilare, V. C., Khedkar, G. D., Reddy, Y. M., & Khedkar, C. D. (2016). Mushrooms and Truffles: Role in the Diet. In *Encyclopedia of Food and Health* (1st ed., pp. 1–8).

https://doi.org/10.1016/B978-0-12-384947-2.00473-6

Savlak, N., Türker, B., & Yesilkanat, N. (2016). Effects of particle size distribution on some physical, chemical and functional properties of unripe banana flour. *Food Chemistry*, *213*, 180–186. https://doi.org/10.1016/j.foodchem.2016.06.064

Schmidt, C., Geweke, I., Struck, S., Zahn, S., & Rohm, H. (2018). Blackcurrant pomace from juice processing as partial flour substitute in savoury crackers: dough characteristics and product properties. *International Journal of Food Science and Technology*, *53*, 237–245. https://doi.org/10.1111/ijfs.13639

Sedej, I., Sakač, M., Mandić, A., Mišan, A., Pestorić, M., Šimurina, O., & Čanadanović-Brunet, J. (2011). Quality assessment of gluten-free crackers based on buckwheat flour. *LWT - Food Science and Technology*, *44*(3), 694–699. https://doi.org/10.1016/j.lwt.2010.11.010

Seguchi, M., Morimoto, N., Abe, M., & Yoshino, Y. (2001). Effect of maitake (Grifola frondosa) mushroom powder on bread properties. *Journal of Food Science*, *66*(2), 261–264. https://doi.org/10.1111/j.1365-2621.2001.tb11328.x

Serna-Saldivar, S. O. (2010). *Cereal Grains: Properties, Processing and Nutritional Attributes*. https://doi.org/10.1017/CBO9781107415324.004

Sheard, N. F., Clark, N. G., Brand-Miller, J. C., Franz, M. J., Pi-Sunyer, F. X., Mayer-Davis, E., … Geil, P. (2004). Dietary carbohydrate (amount and type) in thr prevention and management of diabetes. *Diabetes Care*, *27*(9), 2266–2271. https://doi.org/10.1007/BF01032464

Shukla, K., & Srivastava, S. (2014). Evaluation of finger millet incorporated noodles for nutritive value and glycemic index. *Journal of Food Science and*

Technology, *51*(3), 527–534. https://doi.org/10.1007/s13197-011-0530-x

Siachoono, M. S., Shakachite, O., Muyenga, M. A., & Bwalya, J. (2015). Under Ground Treasure: A Preliminary Inquiry into the Ecology and Distribution of Zambian Truffles. *International Journal of Biology*, *8*(2), 1. https://doi.org/10.5539/ijb.v8n2p1

Singh, A. K., Rehal, J., Kaur, A., & Jyot, G. (2015). Enhancement of Attributes of Cereals by Germination and Fermentation: A Review. *Critical Reviews in Food Science and Nutrition*, *55*(11), 1575–1589. https://doi.org/10.1080/10408398.2012.706661

Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science and Technology*, *21*(4), 168–180. https://doi.org/10.1016/j.tifs.2009.12.001

Singhal, P., & Kaushik, G. (2016). Therapeutic Effect of Cereal Grains: A Review. *Critical Reviews in Food Science and Nutrition*, *56*(5), 748–759. https://doi.org/10.1080/10408398.2012.714417

Slama, a, Fortas, Z., Boudabous, a, & Neffati, M. (2010). Cultivation of an edible desert truffle (Terfezia boudieriChatin). *African Journal of Microbiology Research*, *4*(22), 2350–2356.

Snow, P., & O'Dea, K. (1981). Factors affecting in food1 ' 2 of hydrolysis of starch. *American Journal of Clinical Nutrition*, *34*, 2721–2727.

Srivastava, S., Genitha, T. R., & Yadav, V. (2012). Preparation and Quality Evaluation of Flour and Biscuit from Sweet Potato. *Journal of Food Processing & Technology*, *03*(12), 113–118. https://doi.org/10.4172/2157-7110.1000192

Stone, H., Bleibaum, R. N., & Thomas, H. A. (2012). Sensory Evaluation Practices Fourth Edition. In *Sensory Evaluation Practices* (4th ed.). https://doi.org/10.1016/B978-0-12-382086-0.00009-1

Stone, H., & Sidel, J. L. (1985). *Sensory Evaluation Practices* (1st ed.). Orlando, Florida: Academic Press Inc.

Taylor, F. W., Thamage, D. M., Baker, N., Roth-Bejerano, N., & Kagan-Zur, V. (1995). Notes on the Kalahari desert truffle, Terfezia pfeilii. *Mycological Research*, *99*(7), 874–878. https://doi.org/10.1016/S0953-7562(09)80744-2

Taylor, J.R.N., & Kruger, J. (2016). Millets. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (Volume 4, pp. 748–757). https://doi.org/10.1016/B978-0-12-384947-2.00466-9

Taylor, John R.N. (2016). Millet Pearl: Overview. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains, Volume 1* (2nd ed., pp. 190–198). Oxford: Academic Press, Elsevier.

Taylor, John R.N. (2017). Millets: Their Unique Nutritional and Health-Promoting Attributes. In *Gluten-Free Ancient Grains: Cereals, Pseudocereals, and Legumes: Sustainable, Nutritious, and Health-Promoting Foods for the 21st Century* (pp. 55–103). https://doi.org/10.1016/B978-0-08-100866-9.00004-2

Taylor, John R.N., Emmambux, M. N., & Kruger, J. (2015). Developments in modulating glycaemic response in starchy cereal foods. *Starch/Staerke Journal*, *67*(1–2), 79–89. https://doi.org/10.1002/star.201400192

Taylor, John R.N., & Kruger, J. (2019). Sorghum and Millets: Food and Beverage Nutritional Attributes. In John R.N Taylor & K. G. Duodu (Eds.), *Sorghum and*

Millets (2nd ed., pp. 171–224). https://doi.org/10.1016/b978-0-12-811527-5.00007-1

Tolera, K. D., & Abera, S. (2017). Nutritional quality of Oyster Mushroom (Pleurotus Ostreatus) as affected by osmotic pretreatments and drying methods. *Food Science and Nutrition*, *5*(5), 989–996. https://doi.org/10.1002/fsn3.484

Tongdang, T., Meenun, M., & Chainui, J. (2008). Effect of sago starch addition and steaming time on making cassava cracker (keropok). *Starch/Staerke*, *60*(10), 568–576. https://doi.org/10.1002/star.200800213

Trappe, J. M., Claridge, A. W., Arora, D., & Smit, W. A. (2008). Desert truffles of the African Kalahari: Ecology, ethnomycology, and taxonomy. *Economic Botany*, *62*(3), 497–506. https://doi.org/10.1007/s12231-008-9041-8

Trappe, J. M., Claridge, A. W., & Kagan-zur, V. (2014). Ecology and Distribution of Desert Truffles in the Kalahari of Southern Africa. In Varda Kagan-Zur, N. Roth-Bejerano, Y. Sitrit, & A. Morte (Eds.), *Desert Truffles. Phylogeny, Physiology, Distribution and Domestication* (Vol. 38, pp. 193–202). https://doi.org/10.1007/978-3-642-40096-4

Tulek, Y. (2011). Drying kinetics of oyster mushroom (Pleurotus ostreatus) in a convective hot air dryer. *Journal of Agricultural Science and Technology*, *13*(5), 655–664.

Tumuluru, J. S. (2015). Snack Foods: Role in Diet. In B. Caballero, M. P. Finglas, & F. Toldra (Eds.), *Encyclopedia of Food and Health* (1st ed., pp. 6–12). https://doi.org/10.1016/B978-0-12-384947-2.00632-2

Tumwine, G., Atukwase, A., Tumuhimbise, G. A., Tucungwirwe, F., & Linnemann,

A. (2019). Production of nutrient- - enhanced millet- - based composite flour using skimmed milk powder and vegetables. *Food Science and Nutrition*, *7*, 22–34. https://doi.org/10.1002/fsn3.777

Udomkun, P., Tirawattanawanich, C., Ilukor, J., Sridonpai, P., Njukwe, E., Nimbona, P., & Vanlauwe, B. (2019). Promoting the use of locally produced crops in making cereal-legume-based composite flours : An assessment of nutrient , antinutrient , mineral molar ratios , and aflatoxin content. *Food Chemistry*, *286*, 651–658. https://doi.org/10.1016/j.foodchem.2019.02.055

Ugare, R., Chimmad, B., Naik, R., Bharati, P., & Itagi, S. (2014). Glycemic index and significance of barnyard millet (Echinochloa frumentacae) in type II diabetics. *Journal of Food Science and Technology*, *51*(2), 392–395. https://doi.org/10.1007/s13197-011-0516-8

Venkatachalam, K., & Nagarajan, M. (2017). Physicochemical and sensory properties of savory crackers incorporating green gram flour to partially or wholly replace wheat flour. *Italian Journal of Food Science*, *29*, 599–612.

Vinoth, A., & Ravindhran, R. (2017). Biofortification in Millets : A Sustainable Approach for Nutritional Security. *Frontiers in Plant Science*, *8*(January), 1–13. https://doi.org/10.3389/fpls.2017.00029

Vujic, L., Cepo, D. V., & Dragojevic, I. V. (2015). Impact of dietetic tea biscuit formulation on starch digestibility and selected nutritional and sensory characteristics. *LWT - Food Science and Technology*, *62*(1), 647–653. https://doi.org/https://doi.org/10.1016/j.lwt.2014.06.003

Wan Rosli, W. I., Nurhanan, A. R., & Aishah, M. S. (2012). Effect of partial replacement of wheat flour with oyster mushroom {pleurotus sajor-caju) powder on nutritional composition and sensory properties of butter biscuit. *Sains Malaysiana, 41*(12), 1565–1570.

Wang, S., & Marcone, M. F. (2011). The biochemistry and biological properties of the world's most expensive underground edible mushroom: Truffles. *Food Research International, 44*(9), 2567–2581. https://doi.org/10.1016/j.foodres.2011.06.008

Wani, B. A., Bodha, R. H., & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research, 4*(24), 2598–2604. https://doi.org/10.5897/JMPR09.565

WHO. (2019). *Classification of diabetes mellitus*. Paris.

WHO. (2007). Preventing and controlling micronutrient deficiencies in populations affected by an emergency. *Joint statement by the WHO/FAO/UNICEF. Geneva: WHO*

Wu, G. (2016). Dietary protein intake and human health. *Food and Function, 7*(3), 1251–1265. https://doi.org/10.1039/c5fo01530h

Xiao, G., Zhang, M., Shan, L., You, Y., & Salokhe, V. M. (2011). Extension of the shelf-life of fresh oyster mushrooms (Pleurotus ostreatus) by modified atmosphere packaging with chemical treatments. *African Journal of Biotechnology*, *10*(46), 9509–9517. https://doi.org/10.5897/ajb08.974

Zheng, H. G., Chen, J. C., & Ahmad, I. (2018). Preservation of King Oyster Mushroom by the use of different fermentation processes. *Journal of Food Processing and Preservation*, *42*(1), 1–7. https://doi.org/10.1111/jfpp.13396