DEVELOPMENT OF PROTEIN ENRICHED MAIZE - CASSAVA LEAF COMPOSITE EXTRUDED INSTANT PORRIDGE FLOUR

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Doctor of Philosophy Degree in Food Science of Egerton University

EGERTON UNIVERSITY

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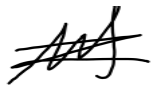
DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this thesis is my original work and has never been submitted elsewhere for the award of any degree.

Signature:  Date: 21/10/2022

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Recommendation

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DEDICATION

This Thesis is dedicated to my late brother-in-law, Tadele Gelaye and my late supervisor, Prof. Abdul K. Faraj.

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First, I thank the Almighty God for the courage. I also thank Egerton University through the department of Dairy and Food Science and Technology (DAFTECH) for giving me the opportunity in undertaking this study. I thank the Netherlands Universities Foundation for International Cooperation (NUFFIC) project through Q-point (NICHE/ ETH/179) for awarding me the scholarship that catered for the tuition and research expenses of this study. I would like to express my heartfelt gratitude to my supervisors, Prof. Mary Omwamba, Dr. John Nduko, and the late Prof. Abdul Faraj for their unlimited support, enthusiastic encouragement and useful critiques in the course of undertaking this research. Special thanks go to Prof. Symon Mahungu for his unreserved support and advice throughout my study. I sincerely acknowledge the staff members of the Ethiopian Biodiversity Institute for providing me with the starter culture. I would also like to extend my gratitude to the staff members of the School of Nutrition, Food Science and Technology at Hawassa University for their assistance and encouragement during my study. My heartfelt thanks to Mr. Andualem Tekle and his beautiful family members for their kindness and unlimited support during the write-up of the thesis. My deepest gratitude goes to my mother, Sewasew Chekol for her prayers and for being with me all the time. I am also grateful for having caring sisters and brothers who are always with me in many ways. Finally, I am very much happy to have handsome boys, Eyuel Zemenu and Eyob Zemenu who always understood me when I was away from them for this study.

ABSTRACT

Food insecurity and malnutrition especially protein deficiency is a major public health problems in developing countries. The aim of this study was, therefore to develop protein-enriched extruded instant porridge flour using composite flour of maize and cassava leaf through microbial fermentation and extrusion cooking technology. Microbial fermentation using starter cultures of *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and their co-cultures were used particularly to assure the safety of ingredients. Hence, these starter cultures showed a significant improvement in the nutritional qualities of maize flour and cassava leaves by reducing the anti-nutritional factors, particularly the hydrogen cyanide level in cassava leaves below the threshold limit (HCN < 10 mg/kg). Response Surface Methodology (RSM) in Box-Behnken design was used for formulation and optimization of the process variables for the production of protein-enriched instant flour. There was a significant ($p < 0.001$) increase in the protein and essential amino acid contents of the extruded flour due to the supplementation of maize with cassava leaf flour. The optimum extrusion variables that could give an optimum proximate composition and essential amino acid profiles were at extrusion temperature (118^{o}C), feed composition (8%) and feed moisture (14%) with composite desirability of 99.8 per cent. The water solubility index showed a significant ($p < 0.05$) positive correlation between *in vitro* protein digestibility and mineral contents. *In vitro* protein digestibility was significantly ($p < 0.05$) increased by extrusion temperature and feed composition. The descriptive panel developed a lexicon of 12 attributes to profile the product's appearance, aroma, flavour, taste and texture. In comparison to the control, the inclusion of cassava leaf flour increased the intensity of bitterness by up to 59% and the number of specks by up to 46%. The control sample had the highest consumer acceptability score for all the attributes followed by instant porridge developed at a feed composition of 5%, feed moisture of 14% and extrusion temperature of 100^{o}C. Paper packaging material had higher scores for desirable attributes such as overall aroma and sweetness while low-density polyethylene had a higher score for undesirable attributes such as bitterness and rancid off-flavours. Production of protein-enriched foods from locally available ingredients can be an ideal solution for the ever-increasing food and nutrition insecurity particularly protein-energy malnutrition in developing countries. In this study, the use of cassava leaf and maize composite flour for the production of protein-enriched flour through the application of extrusion technology has been found ideal for protein and other micro-nutrient improvements.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Unit
CHO	Carbohydrate
CL	Confidence Limit
CLEF	Cassava Leaf Enriched Flour
CSA	Central Statistical Agency
DM	Dry Matter
EDHS	Ethiopian Demographic Health Survey
EAA	Essential Amino Acids
FAO	Food and Agriculture Organization of the United Nations
HCN	Hydrogen Cyanide
HPLC	High Performance Liquid Chromatography
LP	Lactobacillus Plantarum
MRS	Man Rogasa Sharpe
PCA	Principal Component Analysis
PDA	Potato Dextrose Agar
PEM	Protein Energy Malnutrition
RSM	Response Surface Methodology
SC	Saccharomyces Cerevisiae
SAS	Statistical Analysis System
TTA	Total Titratable Acidity
TVC	Total Viable Count
UV-VIS	Ultraviolet-Visible Spectroscopy
WAI	Water Absorption Index
WHO	World Health Organization
WSI	Water Solubility Index
YM	Yeast and Moulds

CHAPTER ONE

INTRODUCTION

1.1 Background information

The variety of factors that cause food and nutrition insecurity in developing nations makes it a persistent problem (FAO, 2017). In most African nations, including Ethiopia, food instability and malnutrition, especially protein shortage, are serious public health issues (Tefera *et al*., 2009). Protein energy malnutrition, also known as PEM, is a broad category of malnutrition that mostly affects young infants in underdeveloped nations. Marasmus, kwashiorkor, and a combined characteristic known as marasmic-kwashiorkor are its severe clinical variants (Tefera *et al*., 2009). In Africa and Southeast Asia, it affects 20 to 40 percent of the population (Tefera *et al*., 2009). The prevalence of malnutrition varies greatly among Ethiopia's regions, according to the Ethiopian Demographic and Health Survey. The Southern, Nation and Nationalities Peoples Regional State has an estimated 44% prevalence of chronic malnutrition, which is higher than the national average of 38% (EDHS, 2016). The high level of malnutrition in the Southern region is majorly attributed to low dietary diversity (EDHS, 2016). The overuse of supplemental foods made from maize flour, which are deficient in nutrients, also contributes to protein-energy malnutrition. Development of nutrient-dense food formulations employing readily available native ingredients, such as cassava (*Manihot esculenta* Crantz) leaves flour, has been encouraged as a solution to this issue (Latif & Müller, 2015). However, the use of cassava leaves is restricted by the high concentration of cyanogenic glycosides in those leaves. Due to this, even though Ethiopia produces a lot of cassava in its southern regions, cassava leaf consumption as food is still uncommon there at the moment (Kebede *et al*., 2012). This can be caused by a lack of effective cyanogenic reduction technologies as well as a lack of knowledge about the nutritional profiles and advantages of cassava leaves.

Depending on the cultivar and climatic conditions, cassava leaves have a high crude protein content (17.7–38.1%) (Latif & Müller, 2015). Additionally, it contains significant amounts of the vitamins B1, B2, and C, carotenoids, and minerals such as phosphorus, magnesium, potassium, and calcium, but low manganese, zinc, iron, copper, or sodium (Latif & Müller, 2015). Cassava leaves vary in moisture content (6.27-9.55%), crude protein (17.7-38.1%), crude fat (5.59-13.27%), total ash (3.40-5.56%), and total carbohydrate (27.31-38.71%) depending on cultivars and growth conditions on dry weight basis, according to Oresegun *et al*. (2016). In Brazil, instant flour is made by combining cassava flour and cassava leaves flour (Trombini *et al*., 2016). Since maize is lacking in important amino acids like lysine and

tryptophan but has reasonable levels of sulfur-containing amino acids like methionine and cysteine, this is one of the potential methods to cover the gap in nutrient shortage (Scott *et al*., 2006). However, the significant amount of necessary amino acids found in cassava leaves makes them appropriate for value addition (Bokanga, 1994). The proximate composition of maize is as follows: moisture (11.6–20%), protein (4.50–9.87%), fat (2.17–4.43%), fibre (2.10–26.70%), ash (1.10–2.95%), and carbohydrate (44.60–69.60%) (Enyisi *et al*., 2014). 1.5% of the kernel dry weight (DW) and 60 to 90% of the kernel phosphate in maize, respectively, are made up of phytic acid and myo-inositol hexa-phosphate, two naturally occurring compounds (Singh *et al*., 2018).

To address the issue of malnutrition and food insecurity, numerous food products with increased nutrition have been created by various researchers. One of them is the widespread usage of food products manufactured from cassava flour in various West African and South East Asian nations, since cassava is regarded as a crop with 21st-century potential as it adapts to difficulties posed by both the global economy and climate change (Latif & Müller, 2015). However, the utilization of cassava leaves flour in food product development is scanty even though it is consumed as a vegetable in many African countries (Latif & Müller, 2015).

Therefore, this study was aimed at developing protein-enriched instant porridge flour using composite flour of maize and cassava leaf through microbial fermentation and extrusion cooking technology. It was hypothesized that supplementation of maize with cassava leaf flour will improve both the macro and micro-nutrients of instant flour.

1.2 Statement of the problem

Maize (*Zea mays* L.) is the most-produced cereal crop in the world and a major source of calories for most of the world's population. Despite its increased consumption, maize is known to be poor nutritional quality, especially protein. As a result, protein-energy malnutrition is high in areas where maize is the only option as a staple food. Due to its capacity to adapt to challenging environmental conditions and its status as one of the potential crops for food security, Ethiopia has placed increasing attention on the cultivation of cassava. In order to combat food instability and malnutrition, this crop, which is mostly grown in Southern Ethiopia, is now being disseminated across the entire nation. However, the dispersion of the leaves, which are nutrient-dense and eaten as vegetables yet have significant concentrations of cyanogenic glycosides, is not supported by tested utilization and processing methods. Despite the widespread usage of extrusion technology in the production of maize-based instant porridge

flour, it is rarely combined with cassava leaves to create instant flour. Therefore, the goal of this study was to produce protein-enriched extruded instant porridge flour using composite flour made of maize and cassava leaf through microbial fermentation and extrusion cooking technology.

1.3 Objectives

1.3.1 General objective

To contribute to food and nutrition security through the development of protein-enriched extruded instant porridge flour using the composite of maize and cassava leaf flour

1.3.2 Specific objectives

i. To determine the effect of microbial fermentation on nutritional and anti-nutritional contents of maize and cassava leaf flour
ii. To optimize extrusion cooking variables for the production of protein-enriched maize-cassava leaf composite instant porridge flour
iii. To evaluate the sensory characteristics of maize-cassava leaf composite extruded instant porridge
iv. To determine the effect of packaging materials and storage durations on physicochemical properties, microbial quality and sensory characteristics of maize-cassava leaf composite extruded porridge flour

1.4 Hypotheses

i. Microbial fermentations have no significant effect on the nutritional and anti-nutritional contents of a cassava leaf and maize flour
ii. Extrusion cooking variables have no significant effect on the protein contents of maize-cassava leaf composite instant porridge flour
iii. There is no significant difference in sensory characteristics of maize-cassava leaf composite instant porridge compared to the control
iv. Packaging materials and storage durations have no significant effect on the physicochemical properties, microbial quality and sensory characteristics of maize-cassava leaf composite extruded instant porridge flour

1.5 Justification of the study

Malnutrition, particularly a lack of protein and calories, is a serious issue for public health in underdeveloped nations. By employing locally accessible crops to create nutrient-rich foods,

efforts have been made to lessen these issues. Although cassava leaves have superior nutritional profiles to the roots, they have not been effectively used to produce food products. This is because there are more cyanogenic glycosides present in the leaves than in the roots (Latif & Müller, 2015). Boiling, drying, and pounding have all been used as cyanogenic glycoside reduction procedures, but none of these processes are successful since they alter other dietary constituents. The use of this technology is restricted to cyanide reduction in cassava leaves, while microbial fermentations have been employed for a variety of food products as a way to increase nutrition by reducing anti-nutritional elements. There is some evidence that using *Lactobacillus plantarum* and *Saccharomyces cerevisiae* as starter cultures can improve the nutritional content of maize and cassava leaf flour by lowering anti-nutritional elements. Due to its improved versatility, lower cost, high product quality, and lack of process effluents, extrusion technology has recently gained popularity as a processing unit operation. Extruded food includes a very wide range of items, including snacks made from cereal that also contain dietary fibre, infant foods, breakfast cereals, and products made with modified starches from cereals, crackers, and nuts. However, composite extruded instant porridge flour made from maize and cassava leaf flour is not readily available.

By incorporating cassava leaves in the production of food items to create food products with superior nutritional profiles, the results of this study can help to attain food security in underdeveloped nations where cassava and maize are grown in large quantities. Additionally, it will provide a complete grasp of how to produce low-cyanide cassava leaf flour in regions with significant cassava output. It also makes it possible for someone to understand how to combine maize and cassava leaf flour to create food products that are protein-rich.

CHAPTER TWO
LITERATURE REVIEW

2.1 Food and nutrition security

Food security is defined as having enough of the right foods at all times and is reliant on both local and global food availability, as well as household and individual access to and proper use of that food. While appropriate access to the right foods is necessary for nutritional security, it also calls for access to proper feeding, caring for, and hygiene practices, as well as to health, water, and sanitation services (FAO, 2017). Thus, access to a nutritious diet that contains all the nutrients needed for a healthy existence and being healthy so that the body can use these nutrients as effectively as possible for its various activities are the two requirements for nutrition security (FAO, 2015).

Africa's development goal is centred on ensuring food and nutrition security, with a stronger commitment to eradicating hunger, attaining food security, and increasing optimal nutrition for all citizens. These exist when all people, at all times, have physical, social, and economic access to food, which is consumed in an environment with enough sanitization and health services and care, enabling a healthy and active existence (FAO, 2017).

In sub-Saharan Africa, food and nutrition insecurity are very common. Southern Africa and Western Africa were predicted to have the lowest frequency of food and nutrition insecurity in sub-Saharan Africa at 20% and 23%, respectively. On the other hand, projections for middle Africa and eastern Africa are 31% and 28%, respectively (Mohamed, 2017). Middle and eastern Africa have the highest rates of acute food insecurity, which correspond to 26 million and 62 million people, respectively, who are 15 years of age or older (FAO, 2016). Numerous factors, as shown in Figure 2.1, can have an impact on a person's nutritional health.

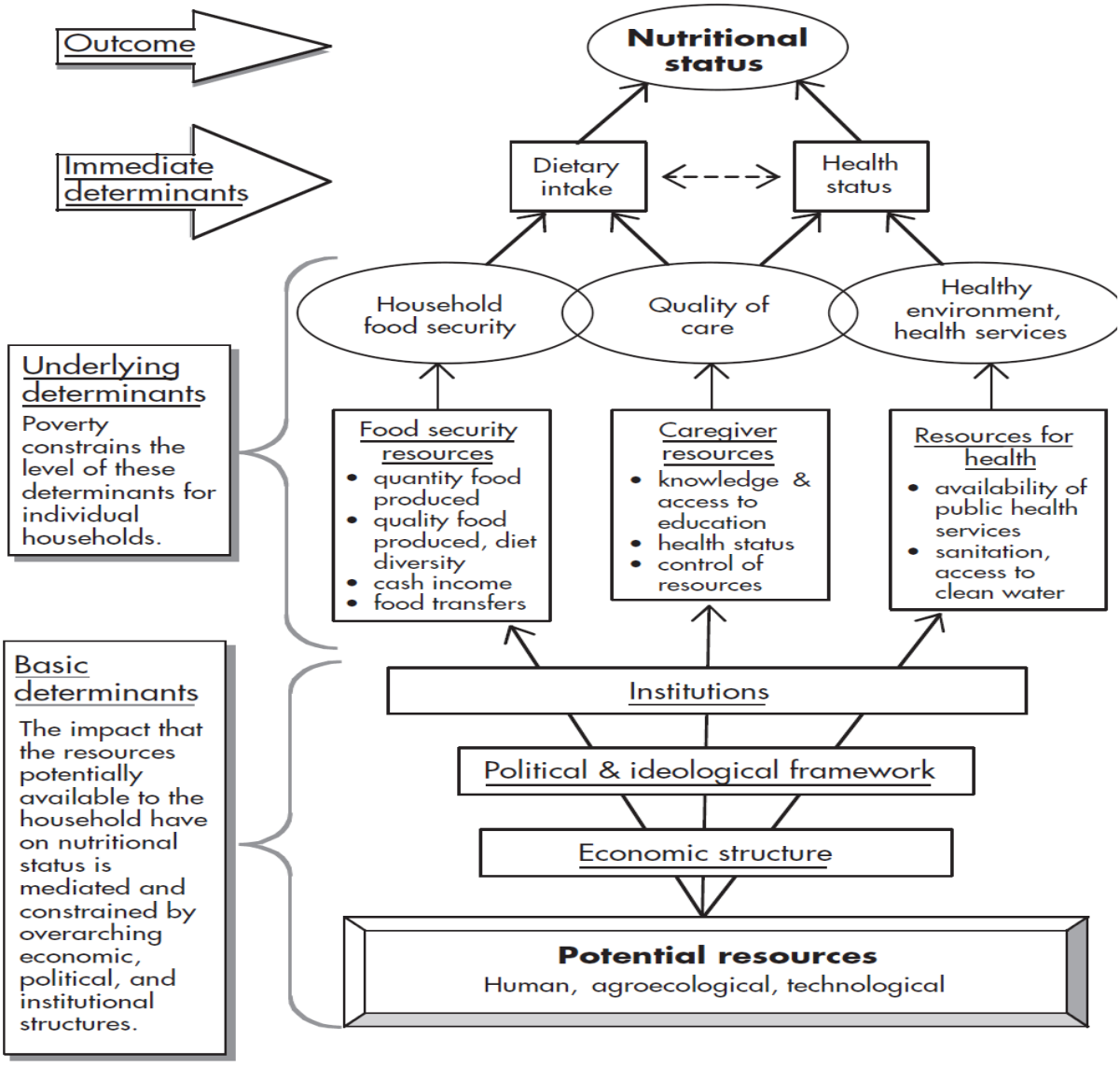


Figure 2.1. Conceptual framework of the determinants of nutritional status

Source: Benson (2004)

2.2 Protein-energy malnutrition

A wide definition of malnutrition is "any departures from adequate and optimal nutritional status" brought on by nutrient deficits or diets consisting of unsuitable food combinations or proportions (UNICEF, 2015). It is a group of ailments that develop when the intake of one or more nutrients falls short of the needs. The idea of the "protein gap" was previously used to explain protein-energy malnutrition (PEM) (deficiency of proteins in diet). The main causes of protein energy malnutrition among preschool-aged children include improper diet that is poor in energy density, protein, and micro-nutrients such vitamins and minerals like iron and zinc (FAO, 2013). The most prevalent health issue impacting both children and adults in underdeveloped nations is protein-energy malnutrition. With some of the highest rates of

stunting and underweight in the world, it is the primary factor in 57% of child fatalities in Ethiopia (Belachew *et al.*, 2009). Over half of all deaths among children under five are caused by hunger and poor sanitation, according to global statistics (UNICEF, 2015). In order for cells to best carry out their physiological functions of growth, reproduction, defence, and repairs, they need to get enough energy and nutrients (Young & Jaspars, 2009). The diseases Marasmus, kwashiorkor, anaemia, goitre, hypoglycemia, and vitamin insufficiency are all brought on by malnutrition (Belachew *et al.*, 2009).

One of the leading causes of disease and mortality among children worldwide is still malnutrition. One third of the 7.6 million children under five who die each year as a result of malnutrition (FAO, 2017). Meeting this problem is extremely important because chronic starvation has terrible and irreversible effects on children who survive. According to FAO (2014), there are six elements that combine to make people more susceptible to food: Food production and preservation in agriculture, including food processing, population density and child spacing, the prevalence of poverty, political ideologies, and illness and infection epidemiology (Figure 2.2).

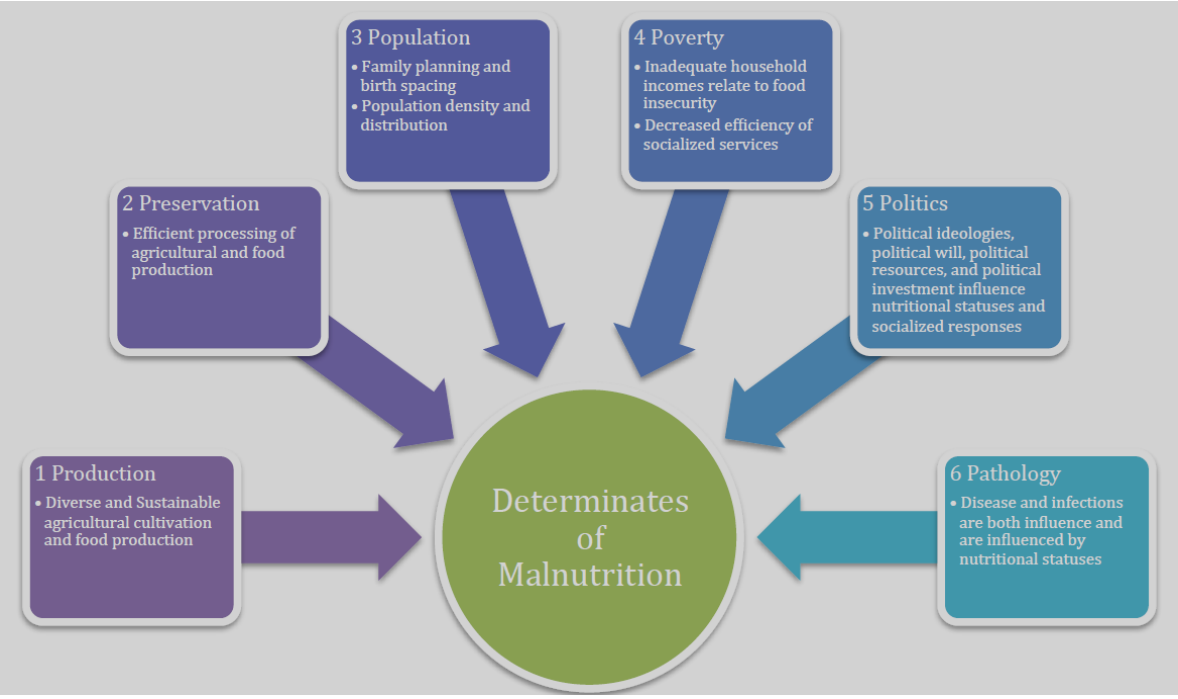


Figure 2.2. The six P's that predispose to malnutrition

Source: Harvard Medical School (2015)

2.3 Cassava production and utilization

Cassava (*Manihot esculenta* Crantz) is a drought-tolerant, staple food crop that is grown in tropical and subtropical areas (Chalaem *et al.*, 2017). In addition to serving as an essential raw material for the production of starch, bioethanol, and other bio-based goods like feed, medicine, cosmetics, and bio-polymers, cassava is a crucial food source for developing nations (Li *et al*., 2017). An estimated 800 million people worldwide rely on cassava, a tropical root crop that is native to the Amazon region. It is one of the few staple crops that can be produced effectively on a small scale, without the need for automation or expensive inputs, and in marginal areas with poor soils and uncertain rainfall. It is virtually solely grown by low-income, smallholder farmers. After rice and maize, cassava is the third-largest source of calories in the tropics (Latif & Müller, 2015).

In Africa, Asia, and Latin America, cassava is a staple food for millions of people. The cornerstone for food security at the household level and a significant source of dietary energy is cassava's wide range of agro-ecological adaptation and its capacity to generate respectable yields in locations where most crops cannot thrive (FAO, 2016). Between 2015 and 2017, global cassava production climbed from 277 to 278 million tonnes, with sub-Saharan Africa producing the most (FAO, 2017). With an estimated annual production of 85,007 tonnes, the Democratic Republic of the Congo is the country that produces the most cassava (FAO, 2014). Both cassava roots and leaves are eaten in this nation and are practically equally important to the nutrition of the populace.

Around the 1960s, cassava was introduced to Ethiopia (Kebede *et al*., 2012). But until 1984, consumption was not widespread. Cassava has recently gained popularity in several areas of the country where it was previously unknown (EDHS, 2016). South west Ethiopia has a variety of cassava types. People categorise variations according to sweetness or bitterness, stem and leaf colour, leaf size, and tree height. To combat food instability and malnutrition, cassava is being distributed throughout Ethiopia. In the southern region, the average total area coverage and output were 4942 hectares and 54036 tonnes, respectively (Shonga *et al.*, 2012).

Depending on cultivars, plant age, plant density, soil quality, fertilization application, frequency of harvesting, and climate, the potential yield of cassava leaves varies greatly (Chalaem *et al*., 2017). In locations where cassava mosaic disease is endemic, excessive leaf harvesting will increase leaf susceptibility in addition to lowering root productivity. For the best all-around yields in 12-month cultivars, a recommended harvesting frequency of 2 to 3

months, commencing at 4 months, is used (Chalaem *et al.*, 2017). In a review by Bakrie (2002), it was further explained that cassava leaves are nutritionally rich products and that cassava plants might produce 7–15 tonnes of leaves per hectare, adding an additional tonne of good protein and 2.5 tonnes of carbohydrates.

2.4 Maize production and utilization

In many developing nations, maize is still a significant component of the diet, and wherever it is grown, white varieties of maize tend to be far more important for human consumption than yellow types (FAO/CIMMYT, 1997). Next to wheat, maize is the most widely grown cereal crop in the world. The majority of African countries' economic and social development is significantly influenced by the maize crop, which is also essential to smallholder livelihood and food security. Nearly 100 million hectares of land are used to grow maize in developing nations, with low- and lower-middle-income nations accounting for nearly 70% of the total production (FAOSTAT, 2010). By 2050, the developing world's need for maize is expected to treble, and maize is anticipated to become the crop with the highest global production (Rosegrant *et al*., 2008).

In many sub-Saharan African nations, maize is eaten in a variety of ways, including roasted green maize, boiling maize, porridge, drinks, bread, and snacks (Ekpa *et al*., 2019). In Africa, there are dishes made from maize, although the processing techniques, food items, and consumption styles vary from one country to the next. It is not uncommon for variations in the processing and eating of maize meals within the same sociocultural group to convey socio-economic class and a person's cultural identity. An average Ethiopian consumes 1,858 kilo-calories daily, of which four major portions of cereals; maize, *teff*, wheat, and sorghum account for more than 60%, with maize and wheat representing 20% each. In addition, legumes such as chickpeas, field peas, lentils, and broad beans are used in the diet as a source of protein. However, they are occasionally roasted whole (*kolo*) and eaten as a snack with coffee. These beans are used in the sauce (*wot*) whole, divided, or as flour (Suleiman, 2015).

2.5 Nutritional profiles of maize and cassava leaf

Among the group of cereals in the "agri-food" category, the maize kernel is an excellent choice due to its chemical make-up and nutritional value (Demeke, 2018). The growing agro-ecological conditions and extraction rates have a significant impact on the nutritional contents of the maize meals (Suleiman, 2015). The colour and degree of product refining have a considerably greater impact on consumer choice for different varieties of maize meal. The main

nutritional drawbacks of maize grain are the amount of lysine and tryptophan, two important amino acids (Table 2.1). However, methionine and cysteine, two amino acids that contain sulphur, are present in maize grains in a reasonable amount (Scott *et al*., 2006).

In order to ensure the safety of cassava leaf eating without compromising the nutrients, it is necessary to develop easy processing techniques (Latif & Müller, 2015). According to their chemical make-up and results from *in-vitro* fermentation, Oni *et al*. (2010) assessed the nutritional content of the leaves of four kinds of cassava: *MS* 6, *TMS* 30555, *Idileruwa*, and *TMS* 30572. The highest crude protein (CP) concentrations were discovered in *TMS* 30555, with cassava leaves having a CP content of 240 g/kg dry matter (DM), ranging from 177 to 240 g/kg DM. Cassava leaves contained between 418 and and 546 g/kg DM and between 596 and 662 g/kg DM of neutral detergent fibre and acid detergent fibre, respectively. The concentrations of hydrocyanic acid (HA) and condensed tannin (CT) ranged from 58.5 to 86.7 mg/kg DM and 1.0 to 3.8 g/kg, respectively. The quantity of Na, K, Ca, and P composition of the cassava leaf declined with an increase in crop age, with mean values of 0.10, 0.32, 0.37, 0.94, and 147.67%, respectively. This was revealed by the mineral composition of the cassava leaf defoliated during different phenological stages (Dada & Oworu, 2010).

Table 2.1. Essential amino acid profiles of maize and cassava leaf flour

Essential amino acids	**Maize (mg/100g)**	**Cassava leaf (mg/100g)**
Histidine	0.26	2.30
Isoleucine	0.21	5.90
Leucine	0.76	9.70
Lysine	0.20	6.70
Methionine	0.23	1.30
Phenylalanine	0.52	3.30
Threonine	0.38	4.80
Tryptophan	0.13	0.80
Valine	0.74	3.35
Biological value (%)	32.1[a]	67-80[b]

Sources: [b]Bokanga (1994); [a]FAO (1992); Montagnac *et al.* (2009)

Cassava leaves come in many types, and they include nutrients and phytochemicals that are vital for human growth. According to Bokanga (1994), pregnant women take cassava leaves to

boost breast milk production and reduce stomach worms in various West African nations like Sierra Leone and Liberia. On account of their rich protein, mineral, and vitamin content, cassava leaves are also a preferred vegetable in Indonesia. Various cassava leaf variants can be considered healthy and acceptable for use in human and animal nutrition, particularly in regions with poor access to food (Ogbuji & Chukwu, 2016). Boiling undamaged leaves for 10 to 20 minutes or longer will lessen the toxicity level of many cassava kinds after being pounded for about 15 minutes. When paired with chopping and wilting, sun drying can eliminate up to 90% of HCN (Kebede *et al.*, 2012). Sun drying is a cheap way to preserve foods high in micro-nutrients, although depending on the weather, it can take several days. The processing of cassava leaves can be done using solar drying in conjunction with the appropriate pretreatments. Cassava leaf is a strong candidate for potential interventions to improve malnutrition, including anaemia, vitamin A and protein deficits in millions of people, when processed properly (Bentil, 2011). In human diets, cassava leaves can be consumed as a vegetable or prepared snack (Bentil, 2011).

2.6 Anti-nutrients in cassava leaves

The free HCN that is released during the hydrolysis of the cyanogenic glycosides, linamarin and lotustralin, which together make up 96% and 4% of the total glucosides, respectively, is often linked to the poisonous qualities of cassava roots and leaves. The natural enzyme linamarase, which acts in damaged plant tissues, or -glucosidases, which are found in the digestive tract, cause the release of free HCN. Healthy cassava leaves do not have any contact between the linamarase and the glucosides; nevertheless, when the tissues are mechanically harmed or when the physiological integrity is destroyed, as in the case of wilted leaves, contact does occur. Boiling intact leaves for at least 30 minutes or pounding and boiling in water are two processes for processing cassava leaves to eliminate cyanogens (Bradbury & Denton, 2011). In contrast to cyanide, which behaves in the opposite way, nitrate levels dropped as the cassava plant grew older (Wobeto *et al.*, 2007). Different scientists have determined the cyanide concentration in cassava leaves. The usual range of cyanide level is 20 to 80 mg HCN per 100 g of fresh leaves, however samples as low as 8 mg/100 g or as high as 186 mg/100 g have sporadically been observed for cassava leaves (Anbuselvi & Balamurugan, 2014). Another significant element affecting the stage of leaf development is what causes changes in cyanide levels. Additionally, cassava includes anti-nutrients such phytate, tannin, nitrate, polyphenols, oxalate, and saponins that can lower the bioavailability of nutrients (Anbuselvi & Balamurugan, 2014) (Demeke, 2018; Ogodo *et al.*, 2018).

2.7 Mechanisms to reduce cyanides in cassava

Cassava roots and leaves are high in calories and high in protein, respectively (Kobawila *et al.*, 2005). By causing the HCN in the cassava tubers to volatilize and lower the cyanide levels as a result, cassava processing enables the decrease of hazardous endogenous cyanogens that are present in varying amounts in cassava tubers (Ogbonnaya, 2016). The effectiveness of various drying techniques in lowering cyanide levels in cassava tubers was examined, and the findings revealed that the rate of cyanide reduction is 34.9% when drying in an oven and 93.14% when drying in the sun as opposed to tray drying (Atlaw, 2018). This is due to the fact that sun drying achieves longer exposure to the linamarase enzyme on linamarin detoxification than other drying methods. It was also advised to apply additional processing techniques, such as fermentation and boiling, to reduce the cyanide content to the desired amount (Atlaw, 2018). Cassava cyanide is transformed into hydrogen cyanide (HCN), which either dissolves easily in water or is discharged into the atmosphere, by the enzyme linamarase, -D-glucosidase. Linamarase, which is similarly created by microbes, is present in the leaves and roots of plants like cassava, lima beans, and flax. The internal glycoside of the cyanogenic plant is made accessible to the extracellular enzyme linamarase when the cellular structure of the plant is disturbed. Before being converted to acetone and hydrogen cyanide, linamarin is first hydrolyzed by linamarase to create D-gluco-pyranose and 2-hydroxyisolentyronotrite or acetone-cyanohydrin (Figure 2.3).

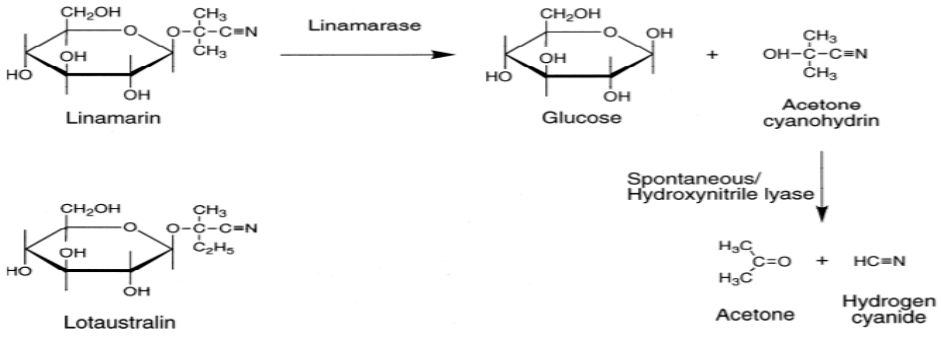


Figure 2.3. Breakdown of cyanogenic glucosides of cassava by linamarase

Source: Ogbonnaya (2016)

The potential to use fermentation to reduce cyanides to their lowest possible level is highly encouraging (Aro, 2009). One of the most researched species and a common probiotic bacterium and/or microbial starter in the food business is Lactobacillus plantarum, a member of the genus *Lactobacillus*. Utilizing *L. plantarum* strains, which have a long history of use in food fermentation, is an emerging field that contributes to the development of foods with added

value. They were also employed to create unique, conventional, or functional foods and drinks with enhanced technological and nutritional features (Sudhanshu *et al*., 2018). Through the actions of the bacterially generated linamarase, the cyanide concentration of the fermented cassava roots and leaves is reduced by 70%, enabling the hydrolysis of cyanogenic glycosides (Kobawila *et al.*, 2005). Researchers looked at how different factors, including fermentation time, inoculum level, and starter cultures' effects on pH, free cyanide, crude protein, and moisture content of fermented cassava roots using single starter cultures including *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* (Tefera *et al.*, 2014). The results showed that after fermentation, the pH value of the cassava flour declined from 6.68 to 3.70, the free cyanide level decreased by 97.92% and 97.76%, and the crude protein content increased from 0.71% to 4.58%.

2.8 Microbial fermentation

Enzymes, biomolecules, and proteins are produced by industries using micro-organisms as a source. The following are a few instances of the sources of micro-organisms: For the manufacture of enzymes and alcohol, *Saccharomyces cerevisiae* and *Aspergillus niger* are frequently utilized in businesses (Vittaladevaram, 2017). Biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids, and vitamins, improvement of aroma, flavour, and textural properties of foods, preservation of various foodstuffs through the production of lactic acid, acetic acid, alcohol, and alkali as a result of fermentation, and reduction in cooking time and fuel consumption are just a few of the many uses for microbial fermentations (Aro, 2009).

Further research into fermentation as a method of producing these substances has been motivated by the discovery of the beneficial activity of certain secondary metabolites (bioactive compounds) produced by microbes. Solid substrates like bran, bagasse, and paper pulp are used in solid-state fermentation. Utilizing these substrates has the primary benefit of making it simple to recycle waste materials rich in nutrients. The substrates are used very slowly and steadily in this fermentation method, allowing the same substrate to be used for extended fermentation times. Therefore, this method encourages the gradual release of nutrients. For fermentation methods involving fungi and micro-organisms that demand reduced moisture content, solid-state fermentation is the optimum option. However, it is ineffective when employed with species like bacteria that require high water activity (a_w) for fermentation to occur. Free-flowing liquid substrates, such as molasses and broths, are used in submerged fermentation. The fermentation broth is where the bioactive substances are released. Because

the substrates are used up so quickly, nutrients must be constantly supplied or added. For micro-organisms like bacteria that need a high moisture content, this fermentation method works well. The ease with which items can be purified is another benefit of this method. The primary function of submerged fermentation is the extraction of secondary metabolites that must be utilized in liquid form. (Subramaniyam & Vimala, 2012).

2.9 Cassava based products

One of the most significant staple root crops, cassava provides more than half a billion people with a robust source of carbohydrates (Karri & Nalluri, 2016). Bread consumption is constantly rising and there is a growing reliance on imported wheat in many emerging nations. Spaghetti and instant noodles are both high in carbohydrates and were traditionally manufactured from wheat flour. However, over the past three decades, the use of cassava as a preferred alternative to wheat in their production has dramatically increased due to a sharp rise in the consumption of instant noodles and spaghetti in Africa, which sparked a demand for other easily accessible, less expensive, carbohydrate-rich alternatives or complements for wheat. A sustainable and economical solution to the protein and micro-nutrient deficits that millions of people experience could be the fortification of everyday foods with protein- and nutrient-rich cassava leaves (Latif & Müller, 2015).

Cassava leaf flour offers a number of advantages as a component in extruded goods. The most significant factors impacting the dependent variables are the amount of moisture and the proportion of cassava leaf flour in the blends. It is possible to manufacture cassava-based snacks with suitable physical features under conditions of low content of cassava leaf flour (2 to 4%) in the mixture of cassava flour, low moisture level (12 to 14%), and intermediate values of extrusion temperature (100°C) and screw speed (230rpm) (Salata *et al*., 2014).

Traditional foods made from cassava that are conventionally prepared, such as *fufu* and *agbelima* (stiff porridge type), can be created. Extrusion cooking increases the textural features of cassava porridge for improved mastication and swallowing by young children. Cassava flour is combined with full fat or de-fatted soy flour. Additionally, it intensifies flavour while lessening the apparent drawbacks of normally cooked cassava porridge, such as stickiness, excessive viscosity, and a translucent, jelly-like appearance (Muoki *et al.*, 2014). The influence of extrusion parameters on the physical properties of blends of extruded cassava leaf flour and starch were examined in a different investigation. The results demonstrated that low screw speed (189 rpm) and extrusion temperature (90oC) with moisture (20%) and integration of

cassava leaf flour at an intermediate percentage (7.5%) may be used to produce materials with low final viscosity characteristics (Trombini *et al.,* 2016).

2.10 Extrusion applications

Extrusion is a method for giving a mixture of raw materials the desired shape. Extrusion processing is the result of a set of unit activities working together synergistically. The raw material is fed into the feeding chamber, and the finished product emerges through the die opening. Extrusion is a technique in which a piece of machinery called an extruder uses pressure and heat to form the material into the desired shape. To improve the product's look, flavour, and taste, additional dressings like dusting vegetable oils with various spices may be applied in specific circumstances (Salata *et al*., 2014).

Foods and feeds that are extruded are created from a wide variety of source components. These substances have many characteristics with those found in all other kinds of diets and feeds. They contain components with various functional roles that are crucial for the stabilisation and production of extruded goods, as well as for providing preferred colour, flavour, and nutritional properties in various products (Pathak & Kochhar, 2018). Extrusion cooking is initially distinguished from traditional baking or dough processing by a relatively low moisture input, often between 10 and 30 percent on a wet weight basis. Despite this low moisture content, the mass of raw materials (free-flowing or low-moisture powder or flour) is turned into a fluid that is then put through a number of processes to combine and change the original constituents into new functional forms (Ramachandra & Thejaswini, 2015).

The extrusion process factors' impact on the functional properties of snack products made from brown rice grits was successfully explained using the response surface methodology (RSM), which proved to be a useful technique. The extrusion procedure had an impact on each functional property. More so than screw speed, the changes in humidity and temperature had an impact on all of the responses (Pardhi *et al*, 2017). Another study found evidence that a flaxseed-sour snack made from cassava starch was impacted by the extrusion process factors and flaxseed flour (Mesquita *et al.,* 2013). The extrusion process is crucial for the oxidation of lipids, gelatinization of starch, destruction of anti-nutritional components, and increase in soluble dietary fibre (Nayak *et al.,* 2011). Complete gelatinization is a key factor in starch digestibility. For specific nutritional diets, such as newborn and weaning foods, high starch digestibility is necessary. Resistant starch produced by extrusion might be useful in products with less calories (Singh *et al.,* 2015). In general, mild extrusion cooking conditions boost the

nutritional value of vegetable protein because they increase digestibility due to protein denaturation, the inactivation of enzyme inhibitors contained in raw materials, and the exposure of additional active sites for enzyme assault (Ramachandra & Thejaswini, 2015). Using a single screw extruder, protein-rich extruded products were made from various soy-Kodo mixes, and their physical characteristics were assessed and linked to process variables. The following optimal level for preparation of the most nutrient-dense extrudates was determined from the results obtained for each response at various temperatures in relation to blend ratio and moisture content: snack prepared with 10% blending of soybean at 15% moisture content and 95°C temperature (Singh *et al.*, 2015). The antioxidant properties of the raw formulations were preserved by extrusion cooking in the extruded products, either in their original forms or as degraded products with radical scavenging activity (Nayak *et al.*, 2011).

2.11 Research

This research work is divided into six chapters. The first chapter (3) is about the effect of solid-state fermentation on proximate composition, anti-nutritional factors and *in vitro* protein digestibility of maize flour. The second chapter (4) deals with the effect of microbial fermentation on the nutritional and anti-nutritional contents of cassava leaf flour. The third chapter (5) is about the optimization of extrusion cooking variables for the production of protein-enriched maize-cassava leaf composite extruded instant porridge flour. The fourth chapter (6) is about optimization of functional properties, *in vitro* protein digestibility and mineral contents of extruded flour developed from maize-cassava leaf composites. The fifth chapter (7) deals with descriptive sensory profiling and consumer acceptability of maize-cassava leaf composite extruded instant porridge. The final chapter (8) is about the effect of packaging materials and storage durations on the shelf life of maize-cassava leaf composite extruded porridge flour.

CHAPTER THREE

EFFECT OF SOLID-STATE FERMENTATION ON PROXIMATE COMPOSITION, ANTI-NUTRITIONAL FACTORS AND *IN VITRO* PROTEIN DIGESTIBILITY OF MAIZE FLOUR

Abstract

Cereals including maize generally have limiting amino acids, particularly lysine. In most cases, spontaneous fermentation is used to improve the nutritional profiles of maize-based products. However, in such fermentation, biological risks including the presence of pathogenic microorganisms, chemical contaminants, and toxic compounds of microbial origin such as mycotoxins pose a health risk. The aim of this study was, therefore, to improve the nutritional properties of maize flour by reducing anti-nutritional factors through microbial fermentation by strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, and their co-cultures. A factorial experimental design was used to evaluate the effect of fermentation set-ups and time on proximate composition, anti-nutritional factors and *in vitro* digestibility of proteins in maize flour. During 48 hours of fermentation, protein content was improved by 38%, 55%, 49% and 48% whereas, *in vitro* protein digestibility improved by 31%, 40%, 36% and 34% for natural, *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and their co-cultures fermented maize flour, respectively. The highest improvement in protein content and digestibility was observed for *Lactobacillus plantarum* strain fermented maize flour. Phytate, tannin and trypsin inhibitor activity were reduced significantly ($p < 0.05$) for natural, *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and co-cultures fermented maize flour. The highest reduction of phytate (66%), tannin (75%) and trypsin inhibitor (64%) was observed for co-culture fermented maize flour. The two strains and their co-cultures were found feasible for the fermentation of maize flour to improve its nutritional profiles more than the conventional fermentation process.

***Keywords*:** Anti-nutritional factors, *In vitro* protein digestibility, *Lactobacillus plantarum*, Proximate composition, *Saccharomyces cerevisiae*

3.1 Introduction

Maize (*Zea mays* L.) is the most-produced cereal crop in the world and a major source of calories for most of the world's population. It accounts for 40% of the total cereal production in sub-Saharan Africa and provides about 30% of the total calorie intake of more than 4.5 billion people in developing countries (Chaves-López *et al*., 2020; Michel-Michel *et al*., 2020). However, the nutritional profile of maize is inferior compared to other cereal crops, specifically, maize is low in the essential amino acid, lysine. The over-reliance on starch-dense staples such as maize in sub-Saharan Africa results in widespread dietary micro-nutrient deficiency and protein energy malnutrition (PEM). Due to this, a number of processing techniques have been applied on maize based products to ameliorate the nutritional qualities of maize based products. These processing methods however, have their own limitations with regard to nutritional profile enhancement and anti-nutritional factors reduction. In the evolving functional food era, new sophisticated technological tools are leading to significant transformations in the field of food and nutrition (Tsafrakidou *et al*., 2020).

Fermentation technology is one of the forefront tools in food technology since it provides a solid foundation for the development of safe food products with better nutritional and functional attributes. It is recognized as a natural way to preserve and safeguard foods and beverages, enhancing their nutritional value, improving their digestibility and reducing anti-nutritional factors. Several pieces of research have been carried out about fermentation to get fermented maize-based products (Amankwah *et al*., 2009; Anaemene & Fadupin, 2020; Asiedu *et al.*, 1993; Cui *et al*., 2012; Ejigui *et al*., 2005; Forsido *et al*., 2020; Irtwange & Achimba, 2009; Ogodo *et al*., 2018; Ogodo *et al*., 2017). However, most of these studies are merely focused on spontaneous fermentation processes even though microbial fermentation such as the use of lactic acid bacteria is more efficient in improving the nutritional profile of foods and promoting human health beneficial properties.

Microbial fermentation, especially lactic acid bacteria has been used extensively for a variety of food products as they are confirmed as generally regarded as safe (GRAS) (Petrova, 2020). Some strains of lactic acid bacteria, mainly *Lactobacilli*, inhabit the gastrointestinal tract (GIT) of humans possessing probiotic effects, in addition to making the food easily digestible, decreasing the level of high-chain carbohydrates and some indigestible poly and oligosaccharides (Turpin *et al*., 2011). It has been also explained that the combination of lactic acid bacteria and yeasts in the fermentation of sourdough improves the nutritional properties and increases the volume of the subsequent bread, and softens its texture significantly (Katina

& Poutanen, 2013). However, there are few or no reports regarding the use of pure strains of *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and their co-cultures for the fermentation of maize flour. Hence, in this study, these two strains were evaluated with regard to their effects on proximate, anti-nutritional factors, and *in vitro* protein digestibility of maize flour during fermentation.

3.2 Materials and methods

3.2.1 Sample collection and preparation

Maize grain (BH 543) variety was collected from Hawassa Agricultural Research Centre, Southern Ethiopia. The grains were sorted and cleaned to remove foreign matter. Then the grains were washed with distilled water and dried in an oven (Binder, Germany) at 70^{o}C for 7 hours (Ogodo *et al.* 2018). The dried kernels were milled into flour using a laboratory disk miller (Alvan Blanch, Britain). The flour was sieved using a 100 µm mesh size and packed in a polyethylene bag and stored in a desiccator until the fermentation process was carried out. Starter cultures; *Lactobacillus plantarum* (Lp) and *Saccharomyces cerevisiae* (Sc), previously isolated from fermented maize flour dough were collected from Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia with a 5 ml plastic vial container and transported to the experimental site in an ice box.

3.2.2 Inoculum preparation

Lactobacillus plantarum (Lp) inoculum was developed following the method of Ogodo *et al.* (2017) with slight modifications. A standard culture of *L. plantarum* inoculum was prepared on MRS agar (Becton, Dickinson and Co., Sparks, Md., USA) from stock cultures frozen in MRS broth, from which isolated colonies were selected for further propagation. The *L. plantarum* bacterium in the frozen cultures was first activated in MRS broth by adding 0.1 ml of frozen culture to a test tube which contained 9 ml of MRS broth and incubated for 48 hours at 37^{o}C. Then, one loopful was taken and spread plated on Man Rogosa Sharpe (MRS) agar, after which, incubation was done anaerobically at 37^{o}C for 48 hours. The cells were harvested by centrifugation at 5000 x gravity for 10 minutes and washed with distilled water. Inoculum development of *Saccharomyces cerevisiae* (Sc) was performed according to Vilela *et al*. (2020) with slight modification. A stock culture of *S. cerevisiae* was activated by inoculating the cells into a freshly prepared yeast extract peptone dextrose (YPD) broth containing 2% glucose, 2% peptone and 1% yeast extract. The culture was incubated overnight

at 37ºC to achieve significant growth of population and one loopful was taken and spread plated on YPD agar and incubated at 30ºC for 3 days.

3.2.3 Fermentation of maize flour

The maize flour samples were fermented with four fermentation set-ups according to the method described by Ogodo *et al*. (2018) with slight modifications. The flours were mixed with distilled water in the ratio of 1:0.5 (w/v) in Erlenmeyer flasks. The samples were then sterilized in an autoclave at 121 ºC for 10 minutes to minimize the risk of contamination and allowed to cool for 30 minutes at room temperature (25 ± 2ºC). The samples were then inoculated with 7 mL of 1 $\times 10^6$ cells/ml of *L. plantarum* and *S. cerevisiae* strains each and 3.5 ml of 1 $\times 10^6$ cells/ml each for co-cultures and allowed to ferment in a solid state fermentation type. The flasks were covered by aluminium foils. The natural fermentation process was prepared using the same procedure without sterilization and the addition of starter cultures. All the fermentation processes were carried out within an incubator (Wagtech, Britain) set at 37ºC for 48 hours. Samples were withdrawn at 12 hours intervals for analyses. Before analysis, the fermented maize flour samples were dried in an oven (Binder, Germany) at 60ºC for 8 hours. The overall flow of sample preparation for analysis in this research was as indicated in Figure 3.1.

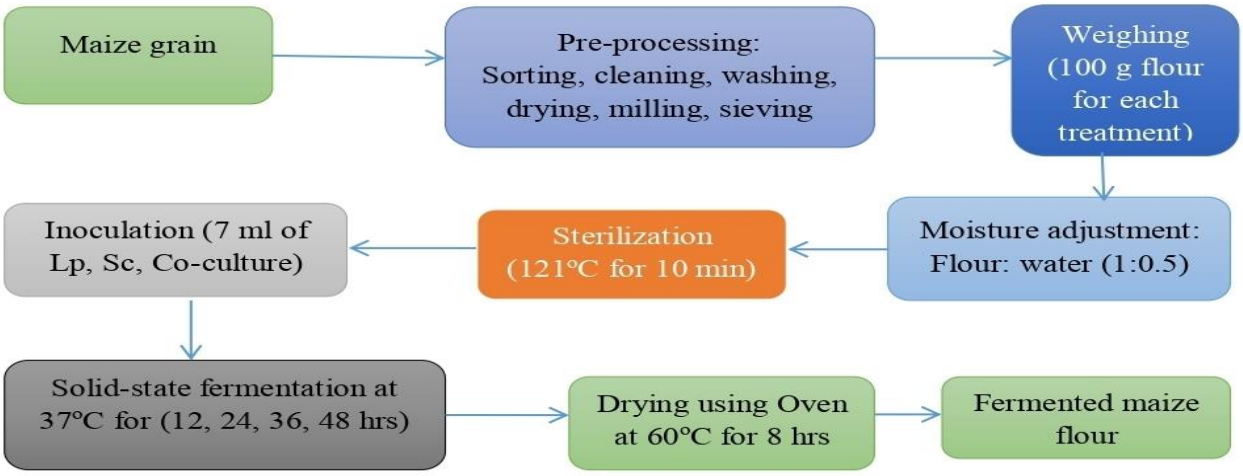


Figure 3.1. Schematic presentation of maize flour sample preparation for fermentation

3.2.4 Proximate composition analysis

All proximate composition parameters were determined using AOAC (2005) methods. The moisture content was determined by drying in an oven at 105ºC until a constant weight was reached (Method 925.09). Crude protein was done by micro-Kjeldahl method with an acid

(sulfuric acid) digestion of the sample and then an alkaline (sodium hydroxide) distillation and nitrogen to a protein conversion factor of 6.25 used (Method 979.09). Crude fat was determined using hexane extraction in a Soxhlet extraction system (Method 920.39). The crude fibre was determined as the combustible and insoluble organic residue obtained after the sample was subjected to acid (H_2SO_4) digestion and then alkaline (NaOH) distillation (Method 962.09). Ash content was quantified as the inorganic residue remaining after incineration of the sample at 550°C until loss of organic matter (Method 923.03). Carbohydrate content was estimated by difference (Ojokoh*et al*., 2020).

3.2.5 Anti-nutrients analysis

Phytate and trypsin inhibitors in maize flour were determined according to Ogodo *et al.* (2018) with slight modifications. Phytate in the sample was determined using UV-VIS Spectrophotometer. The quantity of phytic acid was measured using an absorbance of molybdenum blue at 655 nm. Trypsin enzymatic activity was assayed using casein as substrate and inhibition of the activity was measured in the extract. Then absorbance metrics were plotted against the volume of extract. Trypsin inhibitor activity was then measured as the number of trypsin units inhibited (TIU). The amount of tannin in the sample was determined as the percentage of catechin equivalents (% CE) according to Onyango *et al*. (2013).

3.2.6 Determination of in vitro protein digestibility

The method described by Galal *et al*. (2013) was used for the determination of *in vitro* protein digestibility of maize flour with slight modifications. Exactly 0.2 g maize flour was placed in a 50 ml centrifuge tube and incubated with 1.5 mg of pepsin in 15 ml of 0.1 N HCl at 37ºC for 3 hours and neutralized with 7.5 ml of 0.2 M NaOH. This was followed by the addition of 4 mg pancreatin in 7.5 ml phosphate buffer and incubated at 37ºC for 24 hours. Then, 5 mg Trichloro acetic acid was added to stop the reaction and centrifuged at 5000 × g for 10 minutes. The mixture was filtered through Whatman No. 1 filter paper. The supernatant was dried at 50ºC and followed by assaying for nitrogen using a micro-Kjeldahl method and the *in vitro* protein digestibility was calculated using the following formula.

$$In\,vitro\,protein\;\;digestibility\,(\%) = \frac{X - Y}{X} x100$$

Where; X is the percentage of protein in the sample before digestion and Y is the percentage of protein in the sample after enzymatic digestion.

3.2.7 Experimental design and data analysis

A 4 × 5 factorial experimental design was used to evaluate the effect of fermentation types (4 levels) and time (5 levels) on proximate composition, anti-nutritional factors and *in vitro* protein digestibility of maize flour (Table 3.1). All measurements were done in triplicate. Data analysis was carried out using SAS JMP pro13.0 (Richard Boulton, USA). The data obtained were analyzed for mean differences with analysis of variance (ANOVA) using Tukey's Honestly Significant Difference (HSD) test at a 5% level of significance.

Table 3.1. Factors (2) with level combinations of fermentation

Fermentation setups	**Fermentation time**
Natural	0, 12, 24, 36 and 48 hours
Lactobacillus plantarum inoculum	0, 12, 24, 36 and 48 hours
Saccharomyces cerevisiae inoculum	0, 12, 24, 36 and 48 hours
Co-cultures (*L. plantarum* and *S. cerevisiae*)	0, 12, 24, 36 and 48 hours

3.3 Results

The results for the proximate composition of maize flour fermented for 48 hours are presented in Figure 3.2. The moisture content of maize flour increased significantly ($P < 0.05$) from 10.25% of the unfermented maize flour to 13.94%, 13.70%, 13.11% and 13.06% for natural, *L. plantarum, S. cerevisiae* and co-culture fermented maize flours, respectively, at 48 hours of fermentation (Figure 3.1.2a). The moisture content was found to increase with an increase in the fermentation time. The increase in moisture content of naturally fermented flour was linear compared to flour fermented by the two strains and their co-cultures. However, *L. plantarum, S. cerevisiae* and their co-cultures inoculated fermented flour showed low increments after 36 hours of fermentation time. In all the fermentation set-ups, protein content of maize flour significantly ($p< 0.05$) increased from 9.03% to 12.49%, 14.06%, 13.44%, and 13.38% for natural, *L. plantarum,S. cerevisiae,* and their co-cultures fermented maize flours, respectively (Figure 3.1.2b). The highest increase in protein content was observed for *L. plantarum* strain fermented maize flour, while the lowest was with natural fermentation. In this study, fibre contents decreased significantly ($p< 0.05$) from 3.45% to 1.09%, 0.79%, 1.01% and 0.59% for natural, *L. plantarum, S. cerevisiae* and their co-cultures fermented maize flour, respectively (Figure 3.1.2c). The highest decrease in fibre content was observed for co-cultures fermented

maize flour, while the lowest decrease was observed with natural fermentation. Fat contents decreased significantly ($p< 0.05$) from 4.34% to 2.98%, 2.54%, 2.82% and 2.12% for natural, *L. plantarum*, *S. cerevisiae* and their co-cultures fermented flour, respectively (Figure 3.1.2d). The highest decrease in fat content was observed for co-cultures fermented maize flour while the lowest was with natural fermentation. Ash contents in this study showed a slight increment from 2.12% to 3.07%, 3.15%, 3.40% and 3.73% for natural, *L. plantarum*, *S. cerevisiae* and their co-cultures fermented flour respectively (Figure 3.1.2e). However, the ash content decreased after 36 hours of fermentation during *L. plantarum* and *S. cerevisiae* strains inoculated fermentation conditions. Carbohydrate content decreased significantly ($p< 0.05$) from 70.80% to 66.43%, 65.59%, 66.21% and 67.11% for natural, *L. plantarum*, *S. cerevisiae* and their co-cultures fermented flour respectively (Figure 3.1.2f).

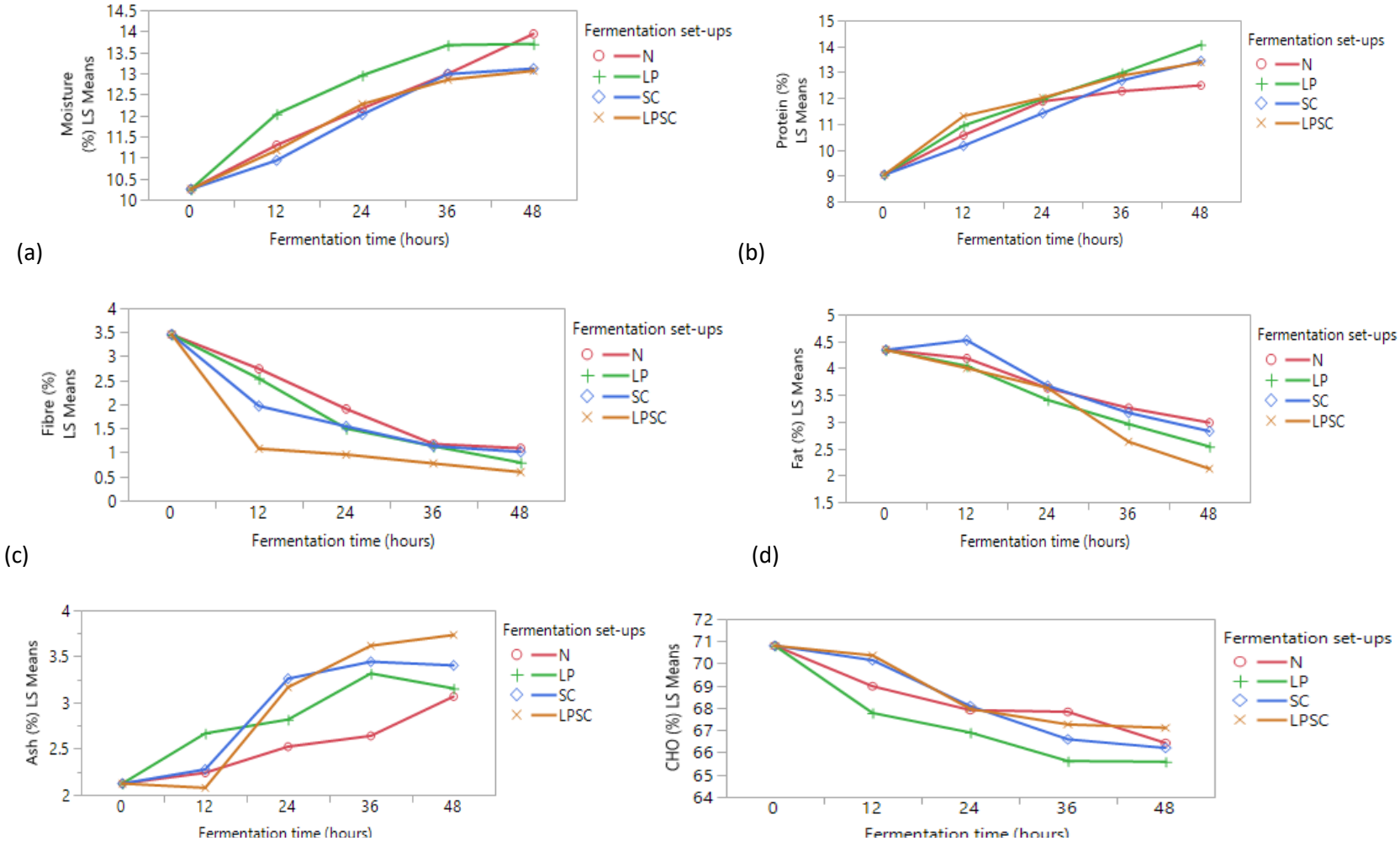


Figure 3.2. Interaction plots (fitted means) of proximate composition of maize flour fermented for up to 48hours. Moisture (a); Protein (b); Fibre; (c);Fat (d);Ash (e) and Carbohydrate (CHO) (f) contents, N, natural fermentation; LP, *Lactobacillus plantarum*; SC, *Saccharomyces cerevisiae*; LPSC, mixed co-culture of LP and SC.

Phytate, tannin and trypsin inhibitor contents decreased significantly ($p < 0.05$) with an increasing fermentation time in all fermentation set-ups (Figure 3.1.3). Phytate content decreased from 311.35 mg/100g flour at the start of fermentation to 179.41 mg/100g, 110.25 mg/100g, 119.38 mg/100g and 105.52 mg/100g for natural, *L. plantarum*, *S. cerevisiae* and their co-cultures fermented flour, respectively at 48 hours of fermentation (Figure 3.1.3a). The tannin content decreased from 49.84% CE at the start of fermentation to 12.3% CE for the mixed co-cultures that had the highest decrease at 48 hours of fermentation (Figure 3.1.3b). Trypsin inhibitor content decreased as well from 55.56 mg/100g at the start of fermentation to 19.94 mg/100g at 48 hours of fermentation with the mixed co-culture that had the highest decrease (Figure 3.1.3c).

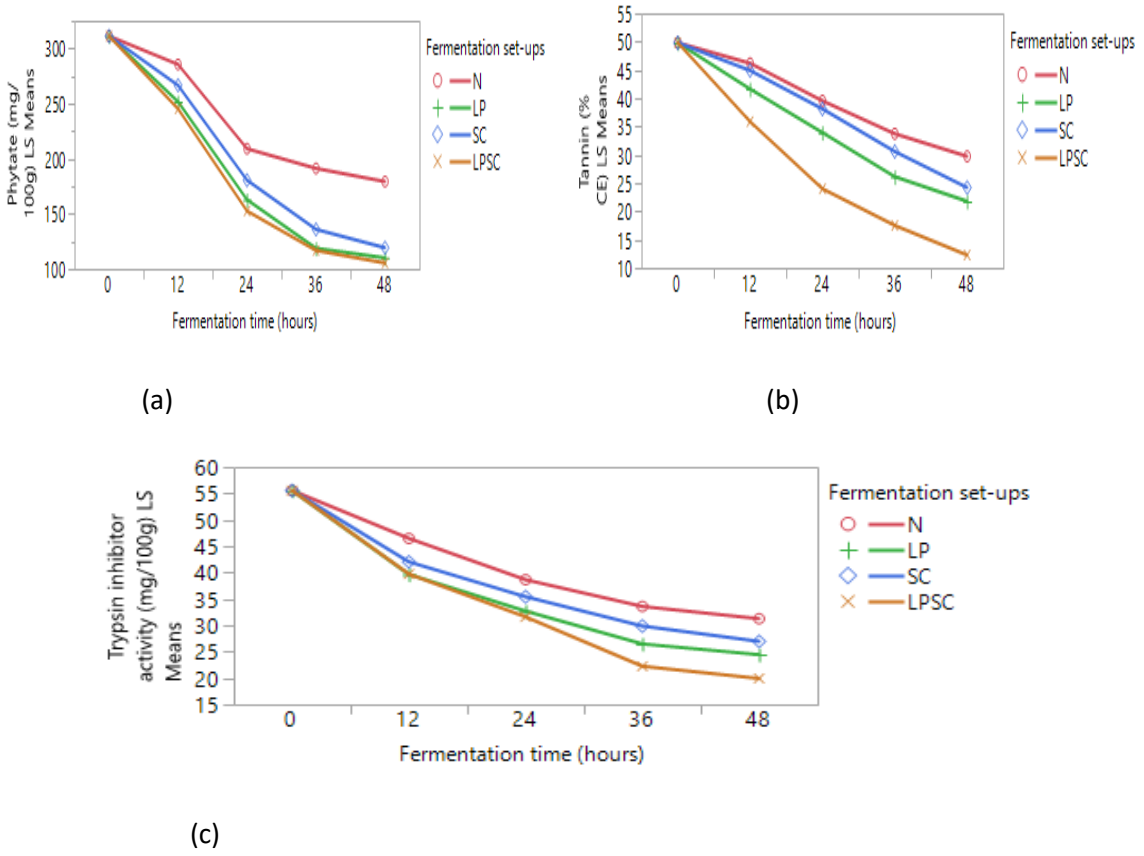


Figure 3.3. Interaction plots (fitted means) of anti-nutritional factors of fermented maize flour for 48 hours. Phytate (a); tannin (b) and trypsin inhibitor (c) contents. N, natural fermentation; LP, *Lactobacillus plantarum*; SC, *Saccharomyces cerevisiae*; LPSC, mixed co-culture of LP and SC.

The *in vitro* protein digestibility values increased significantly ($P < 0.05$) with an increase in fermentation time (Figure 3.1.4). The highest value (88.91%) was observed for *L. plantarum* strain fermented maize flour at 48 hours of fermentation, while the lowest (83.26%) was obtained with natural fermentation at 48 hours.

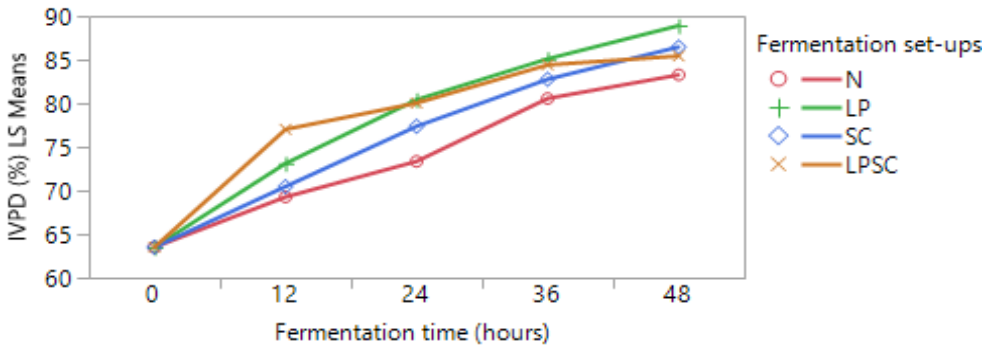


Figure 3.4. Interaction plots (fitted means) of *in-vitro* protein digestibility of fermented maize flour fermented for 48 hours. N, natural fermentation; LP, *Lactobacillus plantarum*; SC, *Saccharomyces cerevisiae*; LPSC, mixed co-culture of LP and SC.

3.4 Discussion

Maize and its derived fermented products are fundamental for human nutrition for a great proportion of the global population (Chaves-López *et al*., 2020). In fermentation, mixed cultures of lactic acid bacteria, yeasts, and sometimes moulds are involved in transforming the food thereby improving its nutritional value and shelf life. In most cases, spontaneous fermentation is used for the production of fermented maize-based products. However, in such fermentation, biological risks including the presence of pathogenic micro-organisms, chemical contaminants, and toxic compounds of microbial origin such as mycotoxins, biogenic amines, and cyanogenic glycosides pose a health risk (Capozzi *et al*., 2020), it is therefore important to understand the role of different micro-organisms in fermenting maize to optimize the final quality, improve food safety of the products, and understand their effect on the nutritional composition.

The moisture content of naturally fermented maize flour in this study was higher (13.94%) than the 10.82% obtained by Ogodo *et al.* (2017) but less than 14.2% reported by Assohoun *et al.* (2013). However, the values of moisture content of naturally fermented maize flour in this study were in agreement with that obtained by Mbata *et al.* (2009), who reported 13.20% moisture content in fermented maize flour. The moisture content of lactic acid bacteria consortium including *L. plantarum* fermented maize flour increased from 9.66% to 10.82%,

similar to that reported by Ogodo *et al.* (2017) which is less than 12.03% to 13.94% increment of the present finding. The increase in moisture content could be attributed to the addition of a calculated amount of water to the substrate before solid-state fermentation. Moreover, during natural fermentation, a consortium of microbial strains initiates the fermentation process to continue for a long period, which could lead to the moisture content of fermented maize flour increasing throughout the fermentation period (Sharma *et al*., 2020). However, during *L. plantarum*, *S. cerevisiae* and their co-cultures inoculated fermentation process, uninterrupted increase in moisture content continued only up to 36 hours of fermentation. This might be because micro-organisms actively utilize the substrate during logarithmic growth until the nutrients are depleted and produce products such as lactic acid, acetic acid and water produced depending on the ingredients used for fermentation (Sharma *et al*., 2020).

The protein content of maize flour naturally fermented for 48 hours was higher (12.49%) than the 10.44% reported by Anaemene and Fadupin (2020) but lower than the 18.4% reported by Mbata *et al.* (2009). The protein content of 14.06 % of *L. plantarum* strain fermented maize flour in this study is higher than the 12.97% reported by Ogodo *et al*. (2017). After 48 hours of fermentation, the increase in protein content in *L. plantarum* fermented maize flour was higher than that of natural fermentation, *S. cerevisiae* strain, and co-cultures fermented maize flour. This could be due to the ability of lactic acid bacteria like *L. plantarum* to secrete some extracellular enzymes which are proteins (Oseni & Akindahunsi, 2011). It was also reported that *L. plantarum* can produce different enzymes and biomolecules, which are proteinaceous during fermentation, hence increasing the protein content of the products (Siezen *et al.*, 2011). The protein content (13.44%) of *S. cerevisiae* strain fermented flour in this study is in agreement with that of Banik *et al*. (2020), who reported 13.68% of protein for multi-grain based food after fermentation (4 days) by *S. cerevisiae*. The increase in the protein contents of naturally fermented maize flour with an increase in fermentation time could be attributed to the logarithmic growth of different strains of micro-organisms during fermentation, which produces proteolytic enzymes, increasing the protein content (Ojokoh *et al.*, 2020). Moreover, the increase in protein content of maize flour after fermentation could also be attributed to a decrease in carbon ratio in the total mass and an increase in cell biomass and productions of non-protein nitrogen compounds like ammonia, amines, amino acids and peptides which are all included in the crude protein content (Onyango *et al.*, 2013). During fermentation, micro-organisms utilize carbohydrates as an energy source and produce carbon dioxide as a by-

product and this causes the nitrogen in the fermented product to be concentrated and thus, the proportion of protein in the total mass increases (Nasseri *et al*., 2011).

The fibre content of naturally fermented maize flour in this study decreased from 3.45% to 1.09%, which is in agreement with the finding of Anaemene and Fadupin (2020) who reported a crude fibre content of 1.18% in maize flour after 72 hours of fermentation. However, the fibre content in this study is much lower than the 5.20% reported by Mbata *et al*. (2009). More decrease in the fibre content (from 3.45% to 0.59%) was observed for co-culture fermented maize flour. The trend in fibre content decrease in this study is similar to the reported values of the fibre content of lactic acid bacteria consortium fermented maize flour by Ogodo *et al*. (2017). The decrease in the crude fibre content of fermented maize flour in this study could be attributed to the secretion of extracellular enzymes by micro-organisms that hydrolyze and metabolize insoluble polysaccharides. It has been also reported that the enzyme ß-D-glucosidase is produced by bacteria such as *L. plantarum* and this enzyme can hydrolyze terminal, non-reducing parts of the polysaccharide chains (Minnaar *et al*., 2017). The fermentation process usually decreases the soluble dietary fibre more than the insoluble dietary fibre content and this makes the total crude fibre content of the fermented products decrease (Brennan *et al*., 2013; Bunzel *et al*., 2001; Comino *et al*., 2018). The decrease in fibre contents after fermentation is an indication of softening of fibrous tissues and increased digestibility due to activities of micro-organisms which are known for the bioconversion of carbohydrates and lignocellulose into protein (Adegunloye & Oparinde, 2017; Igbabul *et al*., 2014).

The fat content of naturally fermented maize flour decreased from 4.34% to 2.98% in this study. The value of this decrease in this study is lower than the 5.2% to 3.76% reported in other studies (Amankwah *et al*., 2009; Gernah *et al*., 2011; Mbata *et al*. 2019; Ogodo *et al*., 2017). This might be due to the initial fat composition of maize grain as the fat content varies with variety and growing conditions. However, the present finding is consistent with the reported value of 2.77% by Opeifa *et al*. (2015) and 2.48% by Irtwange and Achimba (2009). The fat content decreased to 2.54%, 2.82% and 2.12% for *L. plantarum*, *S. cerevisiae* strains and their co-cultures fermented maize flour, respectively. The decrease in fat content by co-cultures was more than that with *L. plantarum*, *S. cerevisiae* strains and naturally fermented maize flour. The value of fat decrease was lower than the 4.08 % reported by Ogodo *et al*. (2017) for LAB consortium fermented maize flour after 48 hours of fermentation. The decrease in fat content might be attributed to the utilization of fat as an energy source by microorganisms for their metabolic activities during fermentation. Moreover, the reduction in fat content might be a

result of the oxidation process that could happen during fermentation (Fasasi *et al*., 2007; Li *et al*., 2020).

The ash content showed a slight increment from 2.12% to 3.73% in this study. However, the difference in ash content across the four types of fermentation medium was not significant ($p > 0.05$) except for 36 and 48 hours of fermentation time. A similar trend was observed by Ogodo *et al*. (2017) and Oluwamukomi *et al*. (2005) who reported an increment in ash content of fermented maize flour from 1.88% to 3.14% and 2.37% to 2.75%, respectively. The highest increment (3.73%) of ash content in this study was observed for co-cultures followed by *Saccharomyces cerevisiae* strain (3.40%) fermented flour. The slight increment in ash content could be attributed to the loss of organic matter and accumulation of inorganic matter caused by the activities of enzymes and microorganisms during fermentation (Uvere *et al*., 2010).

The highest decrease (65.59%) in carbohydrate content of fermented maize flour in this study was observed for *L. plantarum* strain fermented maize flour whereas, the lowest decrease (67.11%) was observed for co-cultures fermented maize flour. The current finding is in agreement with the finding of Ogodo *et al*. (2017) who reported a 70.82% to 68.01% decrease in carbohydrate content of LAB consortium fermented maize flour. A similar trend was reported by Ojokoh *et al*. (2014) who found a 74.2% to 66.66% decrease in carbohydrate content of breadfruit and cowpea blended fermented flour respectively. The change in carbohydrate content might be due to the increasing or decreasing values of other chemical compositions like moisture, protein, fibre, fat and ash by the effect of the fermentation process since the carbohydrate content was determined based on different methods. Moreover, the decrease in carbohydrate content could be attributed to the use of carbohydrates as a source of energy by microorganisms during fermentation (Nasseri *et al*., 2011).

Anti-nutritional factors are the major limiting components in cereals for nutritional bioavailability and they aggravate nutrition-related problems in humans. In this study, after 48 hours of fermentation of maize flour with four fermentation set-ups, the contents of phytate, tannin and trypsin inhibitor activity were reduced significantly ($p < 0.05$). The phytate content in the fermented maize flour was reduced from 311.35 mg/100g to 105.52 mg/100 g. A higher reduction in phytate was observed for co-cultures followed by the *L. plantarum* strain, *S. cerevisiae* strain and naturally fermented flour. This could be because micro-organisms are the source of phytase enzymes which can degrade phytate (Handa *et al*., 2020; Sandberg & Andlid, 2002). The amount of phytate reduction in this study is higher than the reduction from 296.10 mg/100g to 76.76 mg/100g for LAB consortium fermented maize flour as reported by Ogodo

et al. (2018). This might be due to the difference in the initial contents of the ingredient and the fermentation set-ups used in this study. Phytic acid is the major storage form for phosphorus in cereal grains and exists in the form of mixed salts of Ca–Mg–K (phytate), and occurs in many locations within the kernel(Wu *et al.*, 2009). It forms complexes with dietary minerals and causes mineral-related deficiency in humans and it also negatively affects protein and lipid utilization (Coulibaly *et al*., 2010; Kumar *et al*., 2010). The reduction in phytate which is found in the form of *myo*-inositol Hexa-phosphate (IP_6) in cereals, could be attributed to the production of phytase enzyme during the fermentation process that facilitates the degradation process (Selle *et al*., 2000; Troesch *et al*., 2013). Phytase can be produced naturally (endogenous phytase) or by microorganisms (exogenous phytase). The optimal temperature for phytase activity has been known to range between 35ºC and 45ºC (Sindhu & Khetarpaul, 2001). In this study, the temperature used during fermentation was 37ºC which favours an effective phytate reduction process by phytase enzyme. Phytases can dephosphorylate phytate in a step-wise manner to a series of lower inositol phosphate esters (Myo-inositol penta-phosphate to myo-inositol mono-phosphate) and ultimately, to inositol and inorganic Phosphorus (Selle *et al.,* 2000). This enzyme breaks down the phosphate bond and further reduces the most reactive inositol Hexa-phosphate into the least reactive inositol mono-phosphate. Therefore, de-phosphorylation of phytate is a prerequisite for improving the nutritional value of foods with various processing methods because the removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate.

The tannin content was reduced significantly ($P < 0.05$) from 49.84% CE to 12.3% CE. The highest reduction was observed for co-cultures followed by the *L. plantarum* strain, *S. cerevisiae* strain and naturally fermented flour. This finding is in agreement with Ogodo *et al.* (2018) who reported a reduction of 43.64% to 31.38% for LAB consortium fermented maize flour. Tannins are a chemically diverse group of water-soluble phenolic compound which binds proteins to form soluble or insoluble complexes and alter their structural and functional properties (Girard *et al*., 2018). The dietary proteins form complexes with phenolic compounds via non-covalent or covalent interactions. Both reaction mechanisms could affect the chemical structures of interacted proteins and phenolics, thereby inducing changes in their nutritional, functional and biological characteristics, as well as product qualities (Zhang *et al.*, 2020). Hence, the reduction might be attributed to the degradation of tannin by microbial enzymes produced during fermentation (Dlamini *et al*., 2007). The enzyme called tannases and mono

and dioxygenases is capable of hydrolyzing complex tannins, hydrolysable tannins and condensed tannins (Kuddus, 2018).

Trypsin inhibitors are protein inhibitors that limit the action of the enzyme trypsin (Cristina Oliveira de Lima *et al*., 2019). They inhibit the proteolytic enzyme trypsin that is secreted by the pancreas and thus affect the digestibility and bioavailability of protein (Sindhu & Khetarpaul, 2001). In this study, trypsin inhibitor activity was decreased significantly ($P < 0.05$) after 48 hours of fermentation with all fermentation set-ups. The highest reduction was observed for co-cultures followed by the *L. plantarum* strain, *S. cerevisiae* strain and naturally fermented flour. Similar observations have been reported by other researchers (Adeyemo & Onilude, 2013; Dordević *et al*., 2010; Ogodo *et al*., 2018; Osman & Gassem, 2013; Rahman & Osman, 2011). The reduction in trypsin inhibitor during fermentation might be attributed to microbial degradation of the trypsin inhibitor taking place throughout the fermentation process (Rahman & Osman, 2011).

Protein digestibility is a measure of the susceptibility of a protein to proteolysis and depends on the protein structure, thermal processing intensity, and presence of some compounds that are prejudicial to protein digestion, the so-called anti-nutritional factors (Sá *et al*., 2019). It is also affected by other parameters such as pH, temperature, and ionic strength, all of which are directly related to proteolytic activities (Joye, 2019). In this study, the protein digestibility of maize flour increased significantly ($p< 0.05$) at all fermentation set-ups after 48 hours of fermentation. The highest increase was observed for the *L. plantarum* strain followed by the *S. cerevisiae* strain, co-cultures and naturally fermented maize flour. This might be because microorganisms such as lactic acid bacteria have the potential to produce proteolytic enzymes which could be responsible for increased protein digestibility (García-Cano *et al*., 2019). Moreover, the reduction of anti-nutritional factors during fermentation indirectly increases the accessibility of proteins by enzymes and this in turn increases protein digestibility. The finding agrees with the finding of Ogodo *et al.* (2018) who reported a 61.28% to 88.70% increase in protein digestibility of LAB consortium fermented maize flour.

3.5 Conclusion

This research showed that fermentation with *L. plantarum, S. cerevisiae* and their co-cultures resulted in the improved nutritional value of maize flour. Fermentation with these strains significantly increases the contents of protein and the digestibility of maize flour. However, fat, fibre and carbohydrate contents were decreased. A significant reduction was also observed

for phytate, tannin and trypsin inhibitor activity of maize flour after fermentation with natural, *L. plantarum*, and *S. cerevisiae* strains and their co-cultures. Fermented maize flour was higher in nutritional profiles compared to its unfermented counterparts due to the activation of endogenous and exogenous enzymes that could be able to degrade anti-nutritional factors.

CHAPTER FOUR

EFFECT OF MICROBIAL FERMENTATION ON NUTRITIONAL AND ANTI-NUTRITIONAL CONTENTS OF CASSAVA (*Manihot esculenta* Crantz) LEAF

Abstract

Cassava leaves serve as a source of alternative proteins for people in developing countries who could not easily access the available protein sources. However, its widespread use is limited by the presence of toxic compounds particularly cyanogenic glycosides. The objective of this study was therefore to evaluate the effect of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* strains, and their co-culture on nutritional contents and anti-nutritional factors of cassava leaf during fermentation. A 4x5 factorial experimental design was used to determine the effect of fermentation set-ups and time on chemical composition, anti-nutritional contents, *in vitro* protein digestibility and mineral contents of cassava leaf. During 48 hours of fermentation, a significant change *(*$p < 0.05$*)* in moisture, protein, fibre, fat and ash content was observed. Protein content was improved by 34.91% while *in vitro* digestibility of protein was improved by 28.07% during 48 hours of *Lactobacillus plantarum* fermentation. Cyanide, oxalate, tannin and phytate contents were decreased significantly ($p < 0.05$) for all fermentation set-ups. The highest reduction in cyanide (97.17%) and oxalate (86.44%) was achieved under *Lactobacillus plantarum* fermentation. The highest reduction in tannin (93.25%) and phytate (91.11%) was achieved under the co-culture fermentation of cassava leaf. A significant ($p < 0.05$) reduction of mineral contents except iron was observed during 48 hours of fermentation. A significant ($p < 0.0001$) strong negative correlation was found between a protein with cyanide (-0.8164), oxalate (-0.7991), phytate (-0.7851) and tannin (-0.6906). *In vitro* digestibility of protein also showed a strong significant ($p < 0.0001$) negative correlation with phytate (-0.9628), oxalate (-0.9407), cyanide (-0.9305) and tannin (-0.8493). Application of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in cassava leaf fermentation showed a significant improvement in nutritional qualities. Hence, fermentation of cassava leaf using these strains ascertain utilization of cassava leaf for human consumption to tackle protein energy malnutrition.

Keywords: Anti-nutrients, cassava leaf, fermentation, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*

4.1 Introduction

Cassava (*Manihot esculenta*, Crantz) is grown in many tropical and subtropical developing countries for its roots while its leaves are considered a by-product (Latif *et al*., 2020). However, cassava leaves are a rich source of protein, minerals and vitamins and are consumed as a vegetable in many sub-Saharan African countries (Latif & Müller, 2015). Cassava is considered an "all sufficient crop'' in Congo because people get 'bread' from the roots and 'meat' from the leaves (Achidi *et al.*, 2005). Cassava leaves contain 17% - 38% protein on a dry weight basis (Latif *et al*., 2020). It has also a high amount of macro-minerals such as calcium (542.80 – 904.20 mg/100g), potassium (1120.11 – 2234.01 mg/100g) and magnesium (183.75 – 406.11 mg/100g) depending on the varieties (Jamil & Bujang, 2016). Cassava leaves also contain iron in the ranges of 61.5 mg/kg to 151 mg/kg on a dry weight basis (Chavez *et al*., 2000).

However, cassava leaves contain anti-nutrients such as cyanide, oxalate, tannin and phytate which limit their nutritional bioavailability and digestibility. Among these anti-nutrients, cyanogenic glycosides are present in the leaves in higher amounts compared to the roots and this makes it unhealthy for consumption without prior application of appropriate processing methods to reduce the levels (Latif & Müller, 2015). Cassava leaves have 5 to 20 times more cyanogenic potential and 200 times more linamarase activity than roots (Bradburry *et al*., 1994). Young leaves have the highest level of linamarin which decreases to 50-70% in mature leaves while senescent (yellow) leaves contain insignificant amounts (Nambisan, 2011). Cyanogens are found in three forms; Cyanogenic glycosides (95% linamarin and 5% lotaustralin), cyanohydrins, and free cyanide (Montagnac *et al*., 2009). The enzymes (linamarase and hydroxy nitrile lyase) which are responsible for the hydrolysis of this toxic substance are localized in the cell wall of cassava leaves and released during processing when the tissue is disrupted (Mcmahon *et al*., 1995). Linamarase catalyses the hydrolysis of cyanogens to glucose and cyanohydrins while hydroxy nitrile lyase catalyses the hydrolysis of cyanohydrins to hydrogen cyanide (HCN) and ketone. The HCN released during the hydrolysis of cyanogens is highly toxic for all aerobic organisms including humans as HCN binds to cytochrome oxidase which is the last step in mitochondrial respiration and hence prevents oxygen uptake (Cressey & Reeve, 2019). Therefore, cassava leaves should be processed adequately before consumption to reduce cyanogenic glycosides below 10 ppm, which is a safe limit recommended by the World Health Organization (Simeonova & Fishbein, 2004).

In common practices so far, cassava leaves are processed by grinding, pounding, boiling and cooking (Achidi *et al*., 2005). Pounding diminishes cyanogen content by 63% to 73% while pounding/crushing followed by boiling eliminates 97% of the cyanogenic glucosides and completely removes cyanohydrin and free cyanide (Msangi, 2017). However, all these processing methods affect the nutritional profiles of cassava leaves. Fermentation technologies by inoculation with different probiotic microorganisms play a significant role in reducing anti-nutritional factors and improving the nutritional profiles of cassava leaves (Hawashi *et al.*, 2019; Kobawila *et al*., 2005). However, the use of pure strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* and their co-cultures as a means of reducing anti-nutritional factors and improving nutritional profiles is scanty. Therefore, in this study, the two strains were evaluated for their effect on proximate composition, anti-nutritional factors, *in vitro* protein digestibility and mineral contents on cassava leaves during 48 hours of fermentation time.

4.2 Materials and methods

4.2.1 Sample collection and preparation

Cassava leaves (*Kello* variety) with 12 months maturity stages were collected from *Areka* Research Centre, Southern Ethiopia. The leaves were first wilted using open sun drying for 3 hours. Then, the wilted leaves were chopped into small pieces. Starter cultures; *Lactobacillus plantarum* (Lp) and *Saccharomyces cerevisiae* (Sc), were collected from Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia with a 5 ml plastic vial container and transported to the experimental site in an ice box.

4.2.2 Inoculum preparation

The *Lactobacillus plantarum* inoculum was prepared as shown in section 3.2.2. However, inoculum development of *Saccharomyces cerevisiae* was performed according to Hawashi *et al*. (2019) for cassava leaf fermentation. A stock culture of *S. cerevisiae* was activated by inoculating it into a freshly prepared yeast extract peptone dextrose (YPD) broth containing 2% glucose, 2% peptone and 1% yeast extract. The culture was incubated overnight at 37^{o}C to achieve maximum growth of population and one loop full was taken and spread plated on YPD agar and incubated at 30^{o}C for 3 days (Kiayi *et al*., 2019).

4.2.3 Fermentation of cassava leaf

The cassava leaf sample was fermented with four fermentation setups according to Hawashi *et al*. (2019) with slight modifications. The sample was weighed (100 g) and put into each fermentation beaker. The sample was mixed with distilled water in the ratio of 1:0.5 w/v in a

500 ml beaker. The beakers were covered by aluminium foils. Then the sample was sterilized in an autoclave at 121^{o}C for 10 minutes and allowed to cool for 30 minutes at room temperature (25 ± 2^{o}C). Nutrient sources (1% sucrose and 0.5% urea concentrations) were added to the mixture before starter culture inoculation. These percentage of nutrient sources were used based on previous optimization studies by Hawashi *et al*. (2019). The mixture was then inoculated with 7 mL of 1 ×10^{6} cells/ml of *L. plantarum, S. cerevisiae* strains and their co-cultures and the beakers were covered by aluminium foil. The natural fermentation process was prepared using the same procedure without sterilization and the addition of starter cultures. All the fermentation processes were carried out within the incubator (Wagtech, Britain) set at 37^{o}C for 48 hours. Samples were withdrawn at 12 hours intervals for analyses.

4.2.4 Chemical composition analyses

The proximate composition analyses were done as described in section 3.2.4.

4.2.5 Determination of in vitro protein digestibility

The *in vitro* protein digestibility of cassava leaf was determined as indicated in section 3.2.6.

4.2.6 Anti-nutrients analysis

Determination of cyanide in cassava leaves was done using picrate paper coupled with UV-Vis spectroscopy method according to Vinet and Zhedanov (2010) and Haque and Bradbury (2002). A round filter paper disc loaded with buffer at a pH of 6 was placed in a flat-bottomed plastic bottle. One hundred milligrams (100 mg) of cassava leaf were weighed and placed on the filter paper disc and 1 ml of distilled water was added using a 1 ml plastic pipette. A yellow picrate paper attached to plastic baking was then exposed to the plastic bottle ensuring that the paper does not touch the liquid in the bottle. The bottle was then closed and allowed to stand at room temperature for 24 hours. A blank procedure was done as described above without the addition of the sample. The bottles were then opened and the colour of the picrate papers matched against the picrate colour chart. The total amount of cyanide in cassava leaves was read in ppm from the colour chart and recorded. The picrate paper was then employed for the exact determination of cyanide using UV – Vis spectroscopy. Five milliliter (5 ml) of distilled water was measured into a beaker. The plastic baking strip was removed from the picrate paper used in the above procedure. The picrate paper was then immersed in water in the beaker then allowed to stand for 30 minutes with occasional gentle shaking. The same procedure was done for the blank. The absorbance of the resulting picrate solution was measured at 510 nm against

the blank as a reference. The linear calibration curve for cyanide was used to determine mg of HCN equivalent Kg^{-1} on a dry weight basis.

Determination of oxalate was done according to Manano *et al.* (2018). Two grams (2 g) of cassava leaf was digested with 10 ml of 6 M HCL for 1 hour. The filtrate (125 ml) was measured into beakers and 4 drops of methyl red indicator were added. Then, concentrated NH_4OH solution was added to the test solution until the colour changed from pink to yellow. Each portion was heated to 90^oC for 4 hours, cooled and filtered to remove the precipitate. The filtrate was again heated at 90^oC and 10 ml of 5% $CaCl_2$ solution was added with continuous stirring. The solution was allowed to stand overnight and decanted. The precipitate was completely dissolved in 10 ml of 20% (V/V) H_2SO_4 solution in water. The filtrate was made to the 300 ml mark and an aliquot of 125 ml of the filtrate was heated until near boiling, which was then titrated against standardized (0.05 M) potassium tetra-oxomanganate to give pink colour at the endpoint. Oxalate content was calculated as;

$$Oxalate\,(mg/100g) = \frac{t\ x V\ x\ Df}{ME\ x\ MF}\ x 100 \qquad \text{(Eq. 3)}$$

Where; t = titre value of $KMnO_4$; V = volume – mass equivalent (1 ml of 0.05 $KMnO_4$ = 0.00228 g of anhydrous oxalic acid); Df = dilution factor *VTA* (2.4, where VT is the total volume of filtrate (300 mL) and A is the aliquot used (125 mL)), ME = Molar equivalent of $KMnO_4$ in oxalate concentration (g/dm^3); MF = Mass of sample.

Determination of tannin was carried out according to Hawashi *et al*. (2019). Exactly 200 mg of cassava leaf was transferred into a conical flask containing 10 ml of aqueous acetone solution. The solution containing the sample was thoroughly soaked for 15 hours. The tannin was collected as a supernatant in a flask using a Whatman number 1 filter paper. Immediately, 6 mL of butanol-HCl reagent was added to 1 ml of extract in a test tube. The tube was heated at 120^oC using a dry block heater for 1 hour and then cooled at ambient temperature. The absorption was then read at 550 nm with atomic absorption spectrophotometer.

The Phytate content of cassava leaf was determined according to Adane *et al*. (2013) with slight modifications. About 0.5 g of dried cassava leaf flour was extracted with 10 ml of 0.2 N HCl for 1 hour at ambient temperature and centrifuged at 3000 x gravity for 30 minutes. The clear supernatant was determined using UV-VIS Spectrophotometer. The quantity of phytic acid was measured using an absorbance of molybdenum blue at 655 nm.

4.2.7 Minerals analysis

The minerals (Ca, K, Mg and Fe) content of the fermented cassava leaf samples were analyzed using atomic absorption spectrophotometer according to AOAC (2005). Each mineral was analysed using its respective wavelengths as follows; Ca (422.7 nm), K (766.5 nm), Mg (285.2 nm) and Fe (371.9) according to Ozbek and Akman (2016).

4.2.8 Experimental design and data analysis

A 4x5 factorial experimental design was used to evaluate the effect of fermentation set-ups and time on proximate composition, *in vitro* protein digestibility, anti-nutritional factors and mineral contents of cassava leaf as shown in section 3.2.7 (Table 3.1).

4.3 Results

The effect of microbial fermentation on the proximate compositions of a cassava leaf is shown in Table 4.1. Moisture content increased significantly ($p < 0.05$) from 8.97% to 10.13% at the end of 48 hours of natural fermentation. However, moisture content was not significant ($p > 0.05$) for treatments with pure cultures or co-cultures fermentation. Protein content increased significantly ($p < 0.05$) from 17.79% at the end of fermentation to >20% after 24 hours of fermentation and it increased with fermentation time for all the treatments. The highest protein content (24%) was achieved in cassava leaf fermented with *Lactobacillus plantarum*. Crude fibre content decreased significantly ($p < 0.05$) from 10.49% to 7.39% during 48 hours of fermentation. The highest decrease was observed for treatments where *Saccharomyces cerevisiae* was involved and the decrease was time-dependent. Fat content also decreased significantly ($p < 0.05$) from 4.16% to 2.86% during 48 hours of fermentation. The lowest value (2.86%) was observed for co-cultures fermented cassava leaves. Total ash content increased slightly from 6.76% to 6.95% and 7.44% up to 24 hours for natural and *Lactobacillus plantarum* inoculum fermented cassava leaf, respectively. However, ash content decreased after 24 hours of fermentation for each treatment. The change in total carbohydrate content during 48 hours of fermentation was not significantly different for all the treatments ($p > 0.05$). The same trend was also observed for total energy values except for natural fermentation which had a significant decrease ($p < 0.05$). The same trend was also observed for total energy values except for natural fermentation which had a significant decrease ($p < 0.05$).

Table 4.1. Effect of fermentation types and time on proximate composition of cassava leaf (% dry weight basis)

Fermentation set-ups	Fermentation time	Moisture	Protein	Fibre	Fat	Ash	CHO	Calories
	0	8.97±0.08^{cd}	17.79±0.67^{g}	10.49±0.65^{a}	4.16±0.12^{a}	6.76 ± 0.36^{bc}	62.32±0.47^{a}	357.89±2.01^{a}
Natural	12	10.00±0.22^{b}	19.38±0.58^{efg}	10.06±0.08^{ab}	4.08±0.12^{ab}	6.92±0.06^{ab}	59.63±0.73^{a}	352.72±1.79^{b}
	24	10.13±0.15^{b}	20.92±0.59^{cde}	9.74±0.24^{abc}	3.63±0.08^{cde}	6.95±0.11^{ab}	58.38±0.68^{a}	349.83±0.55^{c}
	36	11.01±0.18^{a}	22.45±0.66^{abc}	9.10±0.15^{bcde}	3.49±0.04^{def}	6.65±0.09^{bcd}	56.40±0.55^{a}	346.79±0.48^{c}
	48	10.13±0.23^{b}	22.46±0.66^{abc}	8.97±0.19^{cde}	3.30±0.62^{fg}	6.86±0.26^{ab}	57.25±1.09^{a}	348.54±1.28^{c}
Lp inoculum	12	8.76±0.45^{de}	21.10±0.86^{cde}	9.95±0.25^{abc}	3.73±0.09^{cd}	7.03±0.04^{ab}	59.37±1.39^{a}	355.48±1.31^{b}
	24	9.46±0.21^{bc}	24.00±0.24^{a}	9.23±0.06^{bcd}	3.35±0.06^{ef}	7.44±0.08^{a}	55.74±0.38^{a}	349.15±1.31^{c}
	36	9.01±0.21^{cd}	23.01±0.17^{ab}	8.63±0.08^{def}	3.06±0.07^{gh}	6.14±0.08^{cdef}	58.79±0.53^{e}	354.69±0.81^{b}
	48	8.09±0.16^{ef}	20.96±0.58^{cde}	8.44±0.314^{def}	3.03±0.10^{gh}	6.11±0.13^{cdef}	61.81±0.76^{a}	358.35±0.39^{a}
Sc inoculum	12	8.89±0.12^{cd}	19.50±0.69^{efg}	9.92±0.12^{abc}	3.81±0.03^{bc}	6.79±0.13^{b}	61.01±0.75^{a}	356.31±0.46^{a}
	24	9.05±0.08^{cd}	22.47±0.81^{abc}	8.50±0.19^{def}	3.53±0.04^{cdef}	6.52±0.4^{bcd}	58.43±0.94^{a}	355.35±0.31^{b}
	36	8.95±0.20^{cd}	21.99±0.18^{bcd}	7.43±0.14^{g}	3.47±0.09^{def}	6.08±0.19^{def}	59.50±0.43^{a}	357.25±1.98^{a}
	48	9.09±0.17^{cd}	22.06±0.29^{bcd}	7.39±0.08^{g}	3.52±0.06^{def}	6.05±0.11^{def}	59.28±0.35^{a}	356.99±0.76^{a}
Co-cultures	12	7.79±0.33^{f}	18.78±0.44^{fg}	10.07±0.13^{ab}	3.52±0.09^{def}	6.42±0.17^{bcde}	63.49±0.32^{a}	360.72±0.66^{a}
	24	8.64±0.46^{de}	20.48±0.78^{def}	8.14±0.10^{efg}	3.03±0.14^{gh}	5.82±0.15^{efg}	62.04±1.08^{a}	357.32±3.03^{a}
	36	8.60±0.13^{de}	20.68±0.67^{cde}	7.81±0.13^{fg}	2.96±0.09^{h}	5.52±0.28^{fg}	62.23±0.49^{a}	358.31±1.12^{a}
	48	9.08±0.26^{cd}	21.82±0.42^{bcd}	7.65±0.07^{fg}	2.86±0.13^{h}	5.36±0.14^{g}	60.88±0.34^{a}	356.54±1.15^{a}

Values are mean ± SD for triplicate measurements. Values in the same column with different superscript letters differ significantly ($P < 0.05$); Lp: *Lactobacillus plantarum*; Sc: *Saccharomyces cerevisiae;* CHO, Carbohydrate

The anti-nutritional factors found in cassava leaves as affected by different fermentation treatments are presented in Table 4.2. The hydrogen cyanide (HCN) content decreased significantly ($p < 0.05$) after 48 hours of fermentation.

Table 4.2. Effect of microbial fermentation on anti-nutritional factors of cassava leaf

Fermentation set-ups	Fermentation time	HCN (mg/kg)	Oxalate (mg/100g)	Tannin (mg/100g)	Phytate (mg/100g)
	0	148.77 ± 4.08^{a}	102.18 ± 4.55^{a}	63.28 ± 3.03^{a}	49.67 ± 1.36^{a}
Natural	12	118.17 ± 0.29^{b}	73.05 ± 3.90^{b}	55.52 ± 3.12^{b}	36.17 ± 1.25^{b}
	24	94.42 ± 0.75^{d}	51.29 ± 2.49^{d}	45.62 ± 1.80^{de}	20.18 ± 2.29^{de}
	36	56.75 ± 0.57^{f}	38.17 ± 1.20^{ef}	33.77 ± 1.46^{f}	18.06 ± 0.61^{ef}
	48	21.46 ± 0.39^{i}	19.76 ± 1.12^{g}	11.92 ± 1.63^{hi}	7.51 ± 1.03^{hijk}
Lp inoculum	12	87.02 ± 0.31^{e}	63.57 ± 3.79	52.76 ± 2.09^{bc}	30.08 ± 1.69^{c}
	24	32.08 ± 0.34^{h}	41.61 ± 3.01^{e}	41.37 ± 1.55^{e}	17.91 ± 2.65^{ef}
	36	9.55 ± 1.26^{jk}	32.15 ± 0.93^{f}	21.96 ± 2.11^{g}	10.07 ± 1.00^{ghi}
	48	4.21 ± 1.08^{k}	13.86 ± 2.79^{g}	6.71 ± 1.26^{ij}	5.81 ± 0.41^{jk}
Sc inoculum	12	104.83 ± 0.82^{c}	64.35 ± 0.92^{c}	51.17 ± 2.56^{bcd}	26.65 ± 0.84^{c}
	24	47.58 ± 0.89^{g}	51.85 ± 2.04^{d}	26.84 ± 1.46^{g}	14.15 ± 2.20^{fg}
	36	12.98 ± 0.12^{j}	36.26 ± 0.96^{ef}	10.74 ± 0.25^{hij}	8.71 ± 1.49^{hij}
	48	7.62 ± 0.89^{jk}	17.24 ± 1.77^{g}	6.49 ± 2.14^{ij}	6.37 ± 1.41^{ijk}
Co-cultures	12	93.68 ± 0.49^{d}	60.85 ± 1.19^{c}	46.93 ± 1.53^{cde}	22.40 ± 1.19^{d}
	24	36.01 ± 0.23^{h}	44.19 ± 1.29^{de}	26.99 ± 1.67^{g}	11.53 ± 1.34^{gh}
	36	11.49 ± 0.46^{j}	30.63 ± 1.12^{f}	14.58 ± 2.08^{h}	6.25 ± 0.63^{ijk}
	48	8.82 ± 0.50^{jk}	14.22 ± 1.92^{g}	4.27 ± 2.17^{j}	4.42 ± 0.40^{k}

Values are mean ± SD for triplicate measurements. Values in the same column with different superscript letters differ significantly ($P < 0.05$); Lp: *Lactobacillus plantarum*; Sc: *Saccharomyces cerevisiae*; HCN: Hydrogen cyanide.

In vitro protein digestibility of cassava leaf for the four fermentation set-ups during 48 hours of fermentation is shown in Table 4.3. The mean score of in vitro protein digestibility increases from 66.58% to 82.34%, 85.27%, 84.61% and 83.42% after 48 hours for natural, *Lactobacillus plantarum, Saccharomyces cerevisiae* and co-cultures fermented cassava leaf, respectively.

Table 4.3. Effect of microbial fermentation on *in vitro* protein digestibility of cassava leaf

Fermentation set-ups	Fermentation time	IVPD (%)
	0	66.58 ± 1.82^{h}
Natural	12	74.37 ± 0.54^{g}
	24	77.65 ± 0.69^{fg}
	36	80.25 ± 0.99^{bcdef}
	48	82.34 ± 0.56^{abcd}
Lp inoculum	12	78.77 ± 0.96^{ef}
	24	82.74 ± 0.79^{abc}
	36	83.61 ± 0.61^{ab}
	48	85.27 ± 0.43^{a}
Sc inoculum	12	79.56 ± 0.71^{cdef}
	24	82.03 ± 1.55^{abcde}
	36	83.13 ± 0.38^{ab}
	48	84.61 ± 0.94^{a}
Co-cultures	12	79.18 ± 1.04^{def}
	24	83.51 ± 0.47^{ab}
	36	83.57 ± 1.44^{ab}
	48	83.42 ± 0.66^{ab}

Values are mean ± SD for triplicate measurements. Values in the same column with different superscript letters differ significantly ($P < 0.05$); Lp: *Lactobacillus plantarum*; Sc: *Saccharomyces cerevisiae;* IVPD, *In vitro* protein digestibility.

The results for mineral contents of fermented cassava leaf are indicated in Table 4.4. Calcium content increased slightly from 504.60 mg/100g to 515.93 mg/100g up to 12 hours during natural fermentation. Potassium and magnesium contents decreased significantly ($p < 0.05$) in all fermentation set-ups. However, iron content increased slightly after 48 hours in all fermentation set-ups.

Table 4.4. Effect of microbial fermentation on mineral contents of cassava leaf

Fermentation set-ups	Fermentation time	Calcium (mg/100g)	Potassium (mg/100g)	Magnesium (mg/100g)	Iron (mg/kg)
	0	504.60 ± 5.71^{ef}	897.25 ± 7.23^{a}	161.26 ± 2.19^{a}	98.24 ± 1.09^{g}
Natural	12	515.93 ± 5.29^{abcde}	415.83 ± 7.64^{b}	150.85 ± 3.38^{b}	110.48 ± 1.29^{ef}
	24	488.42 ± 1.93^{g}	241.11 ± 3.58^{e}	146.62 ± 1.46^{bc}	112.36 ± 0.47^{bcdef}
	36	473.16 ± 6.42^{h}	156.73 ± 8.26^{hi}	133.29 ± 2.76^{fgh}	113.14 ± 0.29^{abcde}
	48	467.63 ± 4.30^{h}	141.66 ± 4.56^{ijk}	131.70 ± 2.50^{fghi}	114.15 ± 0.23^{abc}
Lp inoculum	12	$523.\ 00 \pm 4.24^{abc}$	394.38 ± 4.40^{c}	139.05 ± 0.80^{de}	111.66 ± 0.76^{cdef}
	24	528.73 ± 4.88^{a}	179.60 ± 3.79^{g}	134.86 ± 1.09^{efg}	112.86 ± 0.94^{abcdef}
	36	527.86 ± 1.41^{a}	140.94 ± 1.32^{ijk}	130.24 ± 1.28^{ghij}	113.59 ± 0.62^{abcd}
	48	519.41 ± 1.72^{abcd}	128.73 ± 2.08^{k}	128.63 ± 0.76^{hij}	114.44 ± 0.76^{abc}
Sc inoculum	12	510.31 ± 3.21^{cdef}	410.10 ± 2.89^{bc}	142.66 ± 0.87^{cd}	110.64 ± 1.87^{def}
	24	519.54 ± 1.52^{abcd}	221.98 ± 5.19^{f}	136.22 ± 1.11^{ef}	113.40 ± 0.72^{abcde}
	36	518.24 ± 1.66^{abcd}	147.94 ± 5.63^{ij}	131.30 ± 1.08^{fghi}	114.23 ± 0.93^{abc}
	48	500.62 ± 7.27^{fg}	137.97 ± 1.61^{jk}	128.55 ± 1.39^{hij}	115.63 ± 0.81^{a}
Co-cultures	12	514.51 ± 3.76^{bcde}	358.26 ± 6.71^{d}	133.11 ± 1.52^{fgh}	110.10 ± 1.49^{f}
	24	525.79 ± 2.89^{ab}	168.77 ± 2.68^{gh}	$128..21 \pm 1.23^{hij}$	112.52 ± 0.49^{bcdef}
	36	521.52 ± 1.19^{abcd}	150.74 ± 1.50^{ij}	127.06 ± 1.32^{ij}	114.02 ± 0.39^{abc}
	48	509.17 ± 2.61^{def}	142.13 ± 3.05^{ijk}	124.86 ± 1.06^{j}	114.74 ± 1.09^{ab}

Values are mean ± SD for triplicate measurements. Values in the same column with different superscript letters differ significantly ($P < 0.05$);

Lp: *Lactobacillus plantarum*; Sc: *Saccharomyces cerevisiae*.

The coefficients of the correlation matrix for proximate composition and anti-nutritional factors of fermented cassava leaf are shown in Table 4.5. Protein content had a significant ($p < 0.001$) strong negative correlation with cyanide (-0.8164), oxalate (-0.7991), tannin (-0.6906) and phytate (-0.7851). However, fibre, fat and ash contents had significant ($p < 0.001$) positive correlations with all the anti-nutrients. Moisture and total energy contents had no significant correlation with anti-nutrients both at a 5% and 1% significance level.

Table 4.5. Correlation matrix for proximate composition and anti-nutritional factors of fermented cassava leaf

	Moisture	Protein	Fibre	Fat	Ash	CHO	Calories	Cyanide	Oxalate	Tannin	Phytate
Moisture	1.0000										
Protein	0.3251*	1.0000									
Fibre	0.0674	-0.6804***	1.0000								
Fat	0.1343	-0.6782***	0.7804***	1.0000							
Ash	0.3701*	-0.1356	0.7245***	0.6048***	1.0000						
CHO	-0.7287***	-0.8151***	0.2434	0.2065	-0.3649*	1.0000					
Calories	-0.8807***	-0.5393***	-0.0267	0.1105	-0.5031***	0.8618***	1.0000				
Cyanide	0.0317	-0.8164***	0.8982***	0.9005***	0.5554***	0.3920*	0.1602	1.0000			
Oxalate	-0.0699	-0.7991***	0.8383***	0.8751***	0.5064****	0.4272***	0.2496	0.9593***	1.0000		
Tannin	0.0458	-0.6906***	0.9213***	0.8474***	0.6584***	0.2626*	0.0630	0.9501***	0.9310***	1.0000	
Phytate	0.0078	-0.7851***	0.8681***	0.9064****	0.5463***	0.3737*	0.1864	0.9643***	0.9718***	0.9315***	1.0000

* Significant at $P < 0.05$, *** significant at $P < 0.001$

The *in vitro* protein digestibility had a significant ($p < 0.001$) strong negative correlation with anti-nutritional factors as shown in Table 4.6. Among the anti-nutritional factors, phytate (-0.9628) had a strong negative correlation followed by oxalate (-0.9407), cyanide (-0.9305) and tannin (-0.8493).

Table 4.6. Correlation matrix for anti-nutritional factors and *in vitro* protein digestibility of fermented cassava leaf

	Cyanide	Oxalate	Tannin	Phytate	IVPD
Cyanide	1.0000				
Oxalate	0.9593***	1.0000			
Tannin	0.9501***	0.9310***	1.0000		
Phytate	0.9643***	0.9718***	0.9315***	1.0000	
IVPD	-0.9305***	-0.9407***	-0.8493***	-0.9628***	1.0000

*** Significant at $P < 0.001$

4.4 Discussion

Cassava is mainly grown for its roots in most African countries whereas its leaves are mostly considered a by-product. However, cassava leaves are consumed as a vegetable in at least 60% of sub-Saharan and some Asian countries (Achidi *et al.*, 2005). Cassava leaves are a rich source of protein and minerals but the anti-nutritional factors present in the leaves make them difficult to utilize. Various processing techniques have been applied in cassava leaves for the reduction of these anti-nutritional properties. However, none of these processing techniques guaranteed the reduction of cyanide contents to the safest level (< 10 mg/kg) without affecting the nutritional profiles of cassava leaves. The application of microbial fermentation technologies has proved a vital reduction in anti-nutritional factors without compromising the nutritional profile of cassava leaves (Hawashi *et al.*, 2019).

Moisture content is an important parameter which determines the storage stability of a given product. In this study, the moisture content of cassava leaf was found to range from 8.97% to 10.13% after 48 hours of fermentation. The moisture content of unfermented cassava leaf (8.97%) is in agreement with Jamil and Bujang (2016) who reported a moisture content range of 5.05% to 11.6% for six cassava leaf varieties grown in Malaysia. The slight increment in moisture content of cassava leaf during fermentation in this study might be attributed to the combined activities of microorganisms which utilizes the substrates until depletion and

produces products such as lactic acid, acetic acid including water depending on the ingredients used for fermentation (Okpako *et al*., 2008; Sharma *et al*., 2020).

The protein content is a critical constituent of foods and a major source of energy as well as an essential constituent of amino acids which are very critical for human health. In this study, protein contents of cassava leaf ranged from 17.79% to 24.0% after 48 hours of fermentation which increase by 34.91%. The percentage of protein improvement during natural fermentation (26.25%) in this study is higher than the finding of Morales *et al*. (2016) who found a 10.84% improvement in cassava leaf protein after 3 days of solid-state fermentation. The maximum protein improvement (34.91%) was achieved during *Lactobacillus plantarum* inoculum followed by *Saccharomyces cerevisiae* (26.31%) fermentation. The increase in protein content during fermentation might be due to a decrease in carbon ratio in the total mass and an increase in cell biomass and production of non-protein nitrogen compounds like ammonia, amines, amino acids and peptides as these all are included in the crude protein content (Onyango *et al*., 2013). It could also be attributed to the possible secretion of some extracellular enzymes (proteins) such as linamarase and amylase by the fermenting microorganisms in an attempt to make use of the starch present in the cassava leaves (Oboh & Akindahunsi, 2003; Okpako *et al*., 2008).

Fibre is found in food in two forms namely, soluble and insoluble fibres (Hu, 2003). In this study, the fibre content of cassava leaf was found in the range of 10.49% to 7.39% during 48 hours of fermentation. The decrease in crude fibre might be attributed to the enzymatic degradation of the crude fibre as a result of the enzymes excreted by the microorganisms involved in the fermentation process (Adane *et al*., 2013).

The total fat content decreased significantly ($p< 0.05$) from 4.16% to 2.86% after 48 hours fermentation process. This might be due to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components into fatty acid and glycerol (Obadina *et al*., 2013).

Ash content refers to any inorganic materials (such as minerals) left in food after the removal of water and organic matter by heating (Ismail, 2017). In this study, ash content showed a slight increase from 6.76% to 7.44% at 24 hours of fermentation. However, ash content decreases when fermentation was beyond 24 hours. The slight increment in ash content at 24 hours of fermentation could be attributed to the loss of organic matter and accumulation of inorganic matter caused by the activities of enzymes and microorganisms during fermentation.

Thereafter, the depletion of nutrients might have caused a reduction in total ash content (Fadahunsi *et al*., 2013).

The total carbohydrate content in different food products consists of dietary fibre, sugars and starches and it is important for sufficient energy intake and overall health, although excessive intake like other macro-nutrients can have undesirable side effects (Lunn & Buttriss, 2007). Excess intake of carbohydrates impairs the body's metabolic activities and increases the risk of heart disease (Lunn & Buttriss, 2007). In this study, the total carbohydrate content decreased from 62.32% to 53.74% during 48 hours of fermentation. However, the decrease in carbohydrate content of cassava leaf was not significant ($p > 0.05$) during 48 hours of fermentation. The decrease in carbohydrate content might be attributed to the use of carbohydrates as a source of energy by microorganisms during fermentation.

The total energy content in this study varied between 357.89 kcal/100g to 348.54 kcal/100g during 48 hours of fermentation. A reduction in total energy content was observed across all fermentation set-ups. This might be due to the reduction of other proximate compositions such as fat and carbohydrate as the energy value is calculated using these nutrients.

Cassava leaves contain anti-nutritional factors which reduce nutrients' bio-availability and digestibility (Oresegun *et al*., 2016). Fermentation technology through microbial inoculation is very effective in lowering anti-nutritional factors in foods due to the hydrolytic activities of various enzymes produced during fermentation which breaks down anti-nutrients and their complexes (Ismaila *et al*., 2018).

Cyanide is an anti-nutritional factor which is found in various food products at different levels. It is poisonous because it binds cytochrome oxidase and stops its action in respiration, which is a key energy conversion process in the body (Tokpohozin *et al*., 2016). In this study, cyanide contents of cassava leaf decreased from 148.77 mg HCN/kg to 4.21 mg HCN/kg, representing a reduction of 97.17% during 48 hours of *lactobacillus plantarum* inoculum fermentation. The reduction percentage of naturally fermented (85.57%) cassava leaf in the current study is higher than the 64.8% and 70.67% reported by Morales *et al.* (2020) and Kobawila *et al*. (2005), respectively. The reduction in cyanide content of cassava leaf could be due to the production of linamarase and hydroxy nitrile lyase enzymes during fermentation degrades the linamarin and lotaustarlin into hydrogen cyanide which is volatile during further processing (Cressey & Reeve, 2019).

Oxalates are anti-nutrients that negatively affect calcium and magnesium bio-availability (Montagnac *et al*., 2009). In this study, the oxalate content of cassava leaf decreased from 102.18 mg/100g (unfermented cassava leaf) to 13.86 mg/100g (*Lactobacillus plantarum* inoculum fermented cassava leaf). The reduction in oxalate content during fermentation might be attributed to the effect of leaching and enzyme/acid hydrolysis of the starch granule occurred (Oke & Bolarinwa, 2012).

Tannin is a high molecular weight compound consisting of phenolic hydroxyl groups that forms strong complexes with protein (Bule *et al*., 2020). The condensed tannins found in cassava leaves decrease protein digestibility by forming tannin-protein complexes (Nascimento *et al*., 2021). During microbial fermentation, the production of the tannase enzyme reduces the total amount of tannin in the fermented product (Hawashi *et al*., 2019). In this study, tannin content decreased from 63.28 mg/100g (unfermented cassava leaf) to 4.27 mg/100g (co-cultures fermented cassava leaf).

Phytate (*Myo-inositol* Hexa-phosphate) is a regulator of intracellular signalling and a form of phosphate storage in plant seeds, but it can bind proteins and minerals in the gastrointestinal tract preventing absorption and utilization by the body. Specifically, phytate interferes with the absorption of divalent metals, such as iron and zinc, which are essential micro-nutrients (Montagnac *et al*., 2009). Phytate content decreased from 49.67 mg/100g (unfermented cassava leaf) to 4.42 mg/100g (co-cultures fermented cassava leaf). The decrease in phytate content during fermentation could be attributed to the production of phytase enzymes which are responsible for the cleavage of the phosphate group at the 3rd and 6th position of the phytate molecule (Montagnac *et al*., 2009).

The digestibility of protein is dependent on internal factors such as amino acid profile, protein folding and cross-linking and external factors such as temperature, pH and the presence of anti-nutritional factors (Joye, 2019). Isolates of proteins from sweet cassava leaves vary to possess up to 87.59% protein digestibility compared to 78.22% of bitter cassava leaves (Dawodu & Abdulsalam, 2015). In this study, *in vitro* protein digestibility of fermented cassava leaf increased from 66.58% (unfermented) to 85.27% (*Lactobacillus plantarum* inoculum fermented flour).

Calcium is the most important macro mineral which is essential for bone health and proper functioning of the cardiovascular, muscular and nervous systems of the body (Bushinsky & Monk, 1998). Cassava leaves are a good source of calcium (0.43 – 1.14 g/100g dry weight)

depending on the maturity level, the more mature the leaves, the higher the calcium content (Ravindran & Ravindran, 1988). In this study, calcium content varied between 467.63 mg/100g to 504 mg/100g, which is in agreement with Ravindran and Ravindran (1988), but slightly lower than Jamil and Bujang (2016). However, the calcium content in this study is higher than 300 mg/100g which was reported by Bokanga (1994). The calcium content during fermentation was increased up to 24 hours after fermentation. The same trend was observed by Adewusi *et al*. (1999) who found that 134 mg/kg to 288 mg/kg increment up to 2 days of fermentation.

Potassium is the most abundant inorganic cation which is required for the activation of many enzymes, asd a cellular osmoticum for rapidly expanding cells, and as a countercation for anion accumulation and electrogenic transport processes (White & Karley, 2010). Cassava leaves contain 1.38 g/100g to 2.26 g/100g of potassium depending on the level of maturity, the more mature the leaves the lower amount of potassium (Ravindran & Ravindran, 1988). In this study, potassium content varied between 128.73 mg/100g to 897.25 mg/100g during 48 hours of fermentation. The potassium content of cassava leaves (897.25 mg/100g) for unfermented leaves in this study is lower than 1292.2 mg/100g reported by Umuhozariho *et al*. (2014).

Magnesium is an important mineral which helps to keep normal blood pressure, strong bones and a steady rhythm of the heart (Verma & Kumar, 2010). According to Ravindran and Ravindran (1988), cassava leaves contain 0.26 g/100g to 0.37 g/100g magnesium depending on maturity levels, the more mature the leaves, the lower magnesium contents. In this study, magnesium content varied between 128.63 mg/100g to 161.26 mg/100g during 48 hours of fermentation. It decreased when the fermentation time increases. A similar trend was observed by Adewusi *et al*. (1999).

Iron is an essential micro-mineral which is an important component of many proteins that participate in vital metabolic functions such as oxygen transport, oxidative energy production, mitochondrial respiration, inactivation of harmful oxygen radicals and DNA synthesis (Moll & Davis, 2017). Cassava leaves contain a significant amount of iron in the ranges between 15.2 mg/100g to 26.6 mg/100g depending on maturity, the more mature the leaves the higher the iron contents (Ravindran & Ravindran, 1988). In this study, iron content (98.24 mg/kg) for unfermented cassava leaf is lower than 152 mg/kg reported by Ravindran and Ravindran (1988).

4.5 Conclusion

Application of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in cassava leaf fermentation has shown a significant improvement in nutritional qualities by reducing the anti-nutritional factors and increasing proximate components. A significant improvement in protein and its digestibility was found as a result of microbial fermentation. Therefore, the use of microbial fermentation in cassava leaves can ascertain the utilization of cassava leaves for human consumption to tackle protein energy malnutrition.

CHAPTER FIVE

OPTIMIZATION OF EXTRUSION COOKING VARIABLES FOR THE PRODUCTION OF PROTEIN-ENRICHED MAIZE-CASSAVA LEAF COMPOSITE INSTANT PORRIDGE FLOUR

Abstract

Protein-energy malnutrition is one of the major public health problems in developing countries. The objective of this study was to develop protein-enriched maize-cassava leaf composite instant porridge flour using an optimum level of extrusion temperature, feed composition and feed moisture. Response Surface Methodology (RSM) in Box-Behnken design was used for formulation and optimization of the process variables. A co-rotating twin screw extruder was used with the combination of the three variables; extrusion temperature (80-120^{o}C), cassava leaf flour proportion (5-15%) and feed moisture (14-18%) that could give porridge flour with optimum response variables. Results obtained showed that the protein and lysine contents were found in the ranges of 11.45 to 20.1% and 1.98 to 6.2 g/100g of protein, respectively. The coefficient of determination (R^2) was 99.13% and 99.87% for protein and lysine contents, respectively. There was a significant ($p< 0.001$) increase in the protein and essential amino acid contents of the extruded flour due to supplementation with cassava leaf flour. The optimum extrusion variables that could give optimum proximate composition and essential amino acid profiles were; extrusion temperature (118^{o}C), feed composition (8%) and feed moisture (14%) with composite desirability of 99.8 percent. The optimum value of protein after optimization was 16%. Therefore, 100 gram serving of maize-cassava leaf extruded instant porridge can provide 47% of the recommended daily allowance of protein (34 g/day) for children up to 12 years old with enhanced protein quality. Therefore, extrusion cooking can be used effectively for the production of maize-cassava leaf composite instant porridge flours that have enhanced nutritional quality.

Keywords: Cassava leaf, composites, extrusion cooking, instant porridge, maize, optimization

5.1 Introduction

Malnutrition is one of the major causes of mortality among children below five years in developing countries (Grace *et al*., 2012; Ijarotimi, 2013; Serdula, 2017). The development of nutrient-enriched food formulations using locally available crops has been proposed as a means to tackle this problem by many scholars. Among the locally available crops, maize (*Zea mays* L.) is an important cereal crop dominantly produced in developing countries being used as a major source of calories for most of the world population (Paesani *et al*., 2020). Maize grain contains a proximate composition in the range of protein (4.50 – 9.87%), fat (2.17 - 4.43%) fibre (2.10- 26.70%), ash (1.10 – 2.95%) and carbohydrate (44.60- 69.60%) depending on cultivars and growing conditions (Enyisi *et al*., 2014). However, its overall nutritional composition is low compared to other crops as it particularly lacks essential amino acids such as lysine (0.2 mg/100g protein) and tryptophan (0.13 mg/100g protein) (Fapojuwo *et al*., 1987). Therefore, there is a need to blend maize flour with other locally available ingredients to fill the lacking nutritional components. In this context, cassava leaves can be used as an important candidate for such interventions to reduce malnutrition, such as anaemia, vitamin A and protein deficiencies in millions of people by being introduced as ready-to-eat snacks or extruded product in human diets. This is due to the fact that, cassava leaves contain high contents of crude protein (17.7 to 38.1% dry weight basis) and high contents of vitamins; B_2 (0.33 to 0.51 mg/100g fresh leaves), vitamin C (231 to 482 mg/ 100 g fresh leaves), and minerals such as phosphorous (180 to 420 mg/100g fresh leaves), magnesium (260 to 370 mg/100g fresh leaves), potassium (1380 to 2000 mg/100g fresh leaves), and calcium (430 to 1071 mg/100g fresh leaves) depending on the cultivar and climatic conditions (Achidi *et al*., 2005; Awoyinka *et al*., 1995; Lancaster & Brooks, 1983; Omeire, 2012; Ravindran, 1993; Ravindran & Ravindran, 1988). Cassava leaves also contain essential amino acids such as leucine (9.7), lysine (6.7) (6.7), isoleucine (5.9), threonine (4.8), valine (3.35), phenylalanine (3.3), histidine (2.3), methionine (1.3) and tryptophan (0.8) mg/100g protein (Ravindran, 1993; Ravindran & Ravindran, 1988). This makes the cassava leaf a potential raw material for use in extruded products, considering that extrusion could also reduce the anti-nutritional components that are found abundantly in cassava leaves (Latif & Müller, 2015).

Extrusion cooking is preferred over other food processing techniques because it is a continuous, highly productive process that results in significant nutrient retention because of the high temperature and short time required (Ferrari *et al*., 2014). Extrusion combines several unit operations such as mixing, heating and shearing in one energy-efficient rapid continuous

process and can be used to produce a wide variety of products (Ferrari *et al*., 2014; Salata *et al.*, 2014). It is a versatile and state-of-art technology and provides an enormous opportunity for modifying the functionality of food materials for improved digestibility and high sensory quality (Patil & Kaur, 2018). Apart from producing a variety of products, extrusion is helpful to prolong the shelf life of cereal products by inactivating enzymes such as lipases and lipooxygenases responsible for rancidity and off-flavours occur in whole grain flours (Alam *et al.*, 2016; Gómez *et al*., 2011). The extrusion process also offers an excellent opportunity to modify hydration properties and to improve paste stability and functionality of food matrices, by tailoring the processing conditions (Patil & Kaur, 2018). Extrusion of whole-grain maize flour has a positive impact on both the shelf life of the flour by reducing rancidity development and cookie quality (Paesani *et al*., 2020). Under conditions of low moisture content (12 to 14%), low percentage of cassava leaf flour (2 to 4%), and intermediate conditions of extrusion temperature (100°C) and screw speed (230rpm), it was possible to obtain puffed snack products with desirable characteristics (Salata *et al*., 2014). An advantage of extrusion cooking for cassava leaves can be the destruction of anti-nutritional factors, especially trypsin inhibitors, tannins and phytates; all of which inhibit protein digestibility (Salata *et al*., 2014). The protein contents and their digestibility is the most important component in extruded products (Cecilia *et al.*, 2006; Joye, 2019). During the extrusion process, proteins begin to denature and change from soluble to insoluble by bonding and these bonds are then broken by the increasing heat and shear to form a concentrated solution or melt phase that can lead to the formation of covalent bonds at high temperatures (Msarah & Alsier, 2018; Omosebi *et al*., 2018).

Despite the wide use of extrusion in the development of food products, the use of maize-cassava leaf flour composites as raw material has not yet been conducted. Response surface methodology (RSM) is an effective statistical technique for optimizing extrusion process variables (Fan *et al*., 2008; Jorge *et al*., 2006; Liyana-Pathirana & Shahidi, 2005; Nahemiah *et al*., 2016). The objective of this study was to optimize extrusion variables for the production of protein-enriched maize-cassava leaf composite porridge flour. Response surface methodology with Box-Behnken design was used for formulation and optimization of process variables to achieve the required values of the response variables. Extrusion temperature, feed composition and feed moisture were considered process variables and proximate compositions and essential amino acid profiles were chosen as response variables.

5.2 Materials and methods

5.2.1 Sample collection and preparation

Maize (BH 543) variety (25 kg) was collected from Hawassa Agricultural Research Centre, Southern Ethiopia. The grains were sorted and cleaned to remove foreign matter. The dried kernels were milled into flour using a laboratory disk miller (Alvan Blanch, Britain). The flour was sieved using a 100 µm mesh size and packed in a polyethylene bag until the extrusion process was carried out. Cassava leaves (*Kello* variety) were collected from the *Areka* research centre located in the Wolayta Zone of Southern Ethiopia. The fermentation process of cassava leaves was carried out according to Hawashi *et al*. (2019) with slight modifications. Chopped cassava leaf (10 kg) was fermented using *Lactobacillus plantarum* (previously isolated from orange juice) at a concentration of 7 mL of 1 $\times 10^6$ cells/mL and a 1:0.5 ratio of cassava leaf to distilled water under incubation temperature at 37^{o}C for 48 hours. The fermented samples were dried using an oven (Binder, Germany) at 60^{o}C for 8 hours. Then the dried cassava leaf flour was packed in a polyethylene bag until the extrusion process was conducted.

5.2.2 Maize-cassava leaf composite flour preparation

Three (3) formulations were prepared which contain cassava leaf flour ranging from 5 to 15% based on the experimental layout in Table 5.1. The composites of maize and cassava leaf flour were mixed using a blender. The initial moisture content of the blends was analyzed by drying them in an oven at 105^{o}C until a constant weight was reached (Method 925.09) (AOAC, 2005). The average initial moisture content of composite 1 containing 5% cassava leaf flour was 10.94%, while composite 2 and 3 containing 10% and 15% cassava leaf flour were 11.35% and 12.03%, respectively.

5.2.3 Extrusion process

The extrusion process was performed using a co-rotating twin screw extruder (model BC-21, No 194, Clextral, Firminy, France). According to the design, the barrel temperature at zone three of the extrusion varied between 80^{o}C to 120^{o}C (Table 5.1). Adjustments of flour feed rate and water flow rate based on the required process parameters were done after a pre-trial experiment. Flour feed rate and screw speed were set at 34.62 g/min and 400 rpm, respectively, following a pre-trial experiment. However, the water flow rate was adjusted according to the predetermined moisture level (Table 5.2) by using the hydration equation (1) (Sadik, 2015). After the extrusion variables reach a steady state as designed, the extruded samples were collected and dried using an oven at 60^{o}C for 4 hours. Then the samples were milled into flour

and sealed in polyethylene bags and stored in a desiccator until nutritional analyses were conducted.

$$W_a = S_w[\frac{m - m_0}{100 - m}] \qquad \text{(Eq. 1)}$$

Where; W_a = weight of water added (g)

S_w = sample flour weight (g)

m_0 = original flour moisture content (% dry weight basis)

m = required dough moisture (% dry weight basis)

Table 5.1. Process variables with their levels used in the Box Behnken design

	Level of coded variables		
Independent variables	Low	Medium	High
Barrel temperature (°C)	-1(80)	0(100)	1(120)
Feed composition (%)	-1(5)	0(10)	1(15)
Feed moisture (%)	-1(14)	0(16)	1(18)

Table 5.2. Water flow rate for the respective feed moisture level

Pump stroke number	**Water flow rate (g/min)**	**Feed moisture level (%)**
14	5.58	14
17	6.57	16
18	7.95	18

Figure 5.1. The extrusion machine used for this study

5.2.4 Proximate composition analysis

Proximate composition analyses were done as described in section 3.1.2. Carbohydrate content was estimated by difference (Omwamba & Mahungu, 2014). Total energy (kcal/100g) was calculated using Atwater factors according to Nahemiah *et al*. (2016).

$$Total\ energy\ (kcal/g) = 9 \times crude\ \ fat\ (\%) + 4 \times crude\ \ protein\ (\%) + 4 \times Carbohydrate\ (\%)$$

(Eq. 2)

5.2.5 Amino acid analysis

Essential amino acid contents of the extruded samples were analysed according to Dai *et al*. (2014) and Ngudi *et al*. (2003). One gram of extruded flour (0.5 mm) sample was weighed into a screw-capped test tube and 2 ml of 6 N HCL was added. The samples were hydrolysed for 24 hours at 100^{o}C. After hydrolysis, the mixtures were cooled to room temperature and evaporated using a rotary evaporator under a vacuum. However, for tryptophan analysis, 4.2 M NaOH was used for hydrolysis of the samples and hydrolysed at 105^{o}C for 20 hours. Then the amino acid analysis was carried out using a reversed-phase C_{18} column (250 mm x 4.6 mm, 5 µm particle size) at 43^{o}C operating temperature using High-Performance Liquid Chromatography (1260 infinity). The values were reported by computing the ratio of the peak area of the target amino acid to its concentration in the sample which is proportional to that of a known amount of the target amino acid in the standard solution.

5.2.6 Experimental design and process optimization

Response surface methodology (RSM) was applied to determine the combination of extrusion variables which could produce the best quality extruded flour from maize and cassava leaf composites. Box-Behnken experimental design with three factors (ET, FC and FM) was used. Preliminary trials were conducted to select the number and range of process variables (Table 5.3). A second-order polynomial regression equation was modelled based on the experimental data and optimum parameters defined using Design-Expert version 13 Software and the following empirical model presents the relationships among process and response variables.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_{11}^{\ 2} + \beta_{22} X_{22}^{\ 2} + \beta_{33} X_{33}^{\ 2} + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23} + \varepsilon$$

(Eq. 3)

Where; Y = estimated response, β_0, β_i, β_{ii} and β_{ij} are coefficients of the constant term, the linear terms, the quadratic terms and the interaction terms, respectively; X_1 = extrusion temperature effect, X_2 = feed composition effect and X_3 = feed moisture effect and Ɛ = the random error.

Table 5.3. Outline of Box-Behenken experimental design matrix

Experimental runs	Independent variables		
	ET (°C)	FC (%)	FM (%)
1	+1(120)	0(10)	+1(18)
2	0(100)	+1(15)	-1(14)
3	0(100)	0(10)	0(16)
4	-1(80)	+1(15)	0(16)
5	0(100)	0(10)	0(16)
6	0(100)	-1(5)	+1(18)
7	+1(120)	-1(5)	0(16)
8	-1(80)	0(10)	-1(14)
9	0(100)	+1(15)	+1(18)
10	+1(120)	+1(15)	0(16)
11	-1(80)	0(10)	+1(18)
12	+1(120)	0(10)	-1(14)
13	0(100)	0(10)	0(16)
14	0(100)	-1(5)	-1(14)
15	-1(80)	-1(5)	0(16)

5.2.7 Fitted model validation

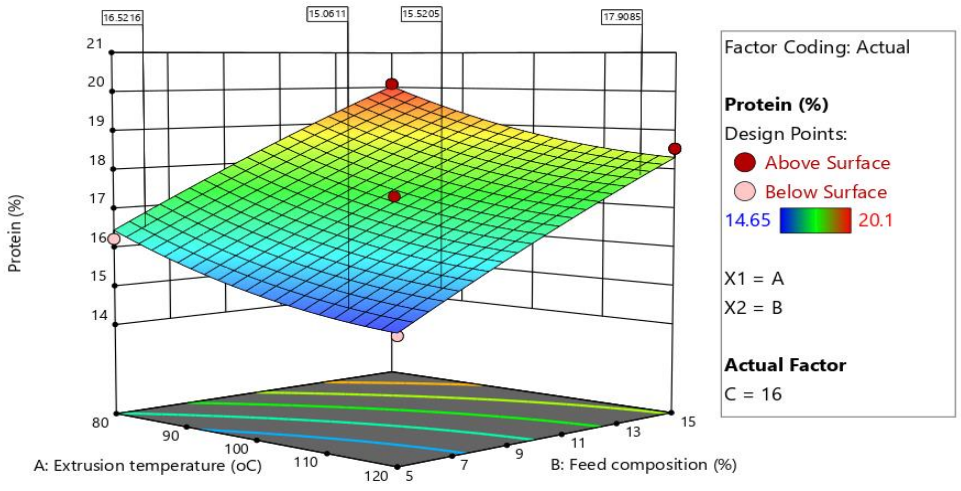
Coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), predicted coefficient of determination (R^2), lack of fit and coefficient of variation (C.V) were used to check if the fitted models provide an adequate estimation of the real system. The coefficient of determination (R^2) value close to unity and R^2_{adj} close to R^2 ensure satisfactory fitting of the model to the real system. When the difference between adjusted R^2 and predicted R^2 is less than 0.2, the model is fitted well. Adequate precision measures the signal-to-noise ratio and a ratio greater than 4 is desirable. A non-significant lack of fit is also considered. The probability value (P) of each response was also used to check for the significance of each factor and the interaction between the factors. The smaller the P value, the more significant the corresponding coefficients (Dhawane *et al*., 2015).

5.2.8 Statistical analysis

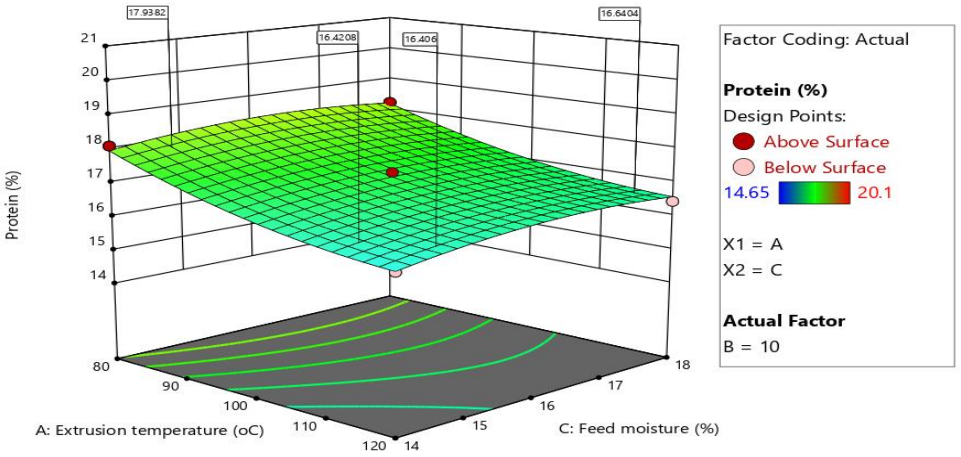
The response surface methodology procedure of Design-Expert (DX13, Stat Ease Inc. Minneapolis, MN, USA) software was used to analyse the experimental data. The significance and validity of regression model equations were analysed using analysis of variance (ANOVA).

5.3 Results

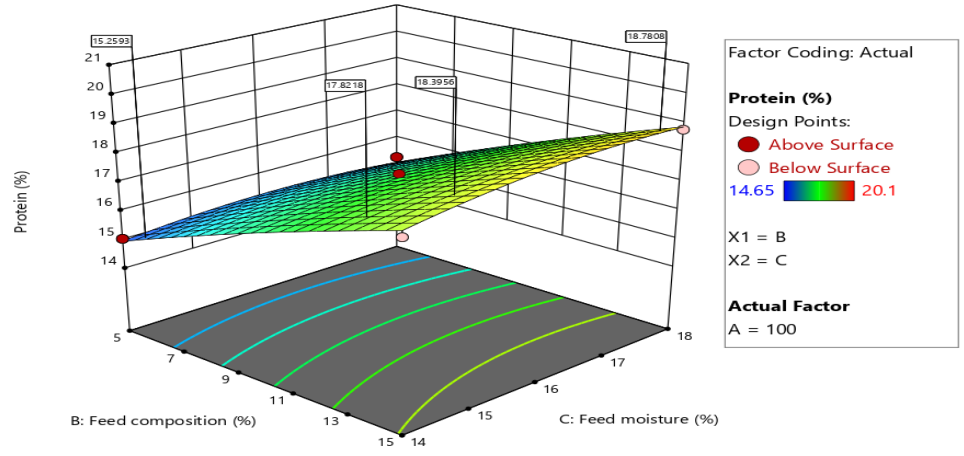
The mean values of the proximate composition of extruded maize-cassava leaf composite flour are shown in Table 5.4 (Appendix 5). Moisture, protein, fat, fiber, ash, carbohydrate and energy content ranged from 5.45% to 9.45%, 11.45% to 20.1%, 2.06% to 3.25%, 1.24% to 3.32%, 2.11% to 5.07%, 64.93% to 76.8% and 354.72 kcal to 382.25 kcal/100g, respectively. The mean values of the proximate composition of the extruded flour were significantly ($p < 0.05$) different from the control sample. The response surface plot (Figure 5.2a) shows the interactive effect of extrusion temperature and feed composition at constant feed moisture of 16% on protein content. Accordingly, an increase in extrusion temperature resulted in a decrease in protein content, whereas an increase in feed composition increased protein content. Figure 5.2b shows the interactive effect of extrusion temperature and feed moisture at a constant feed composition of 10% on protein content. Figure 5.2c shows the interactive effect of feed composition and feed moisture at a constant extrusion temperature of 100 ^{o}C on protein content. Thus, an increase in feed moisture increased protein content.



(a)



(b)



(c)

Figure 5.2. The 3D surface plots show the effects of extrusion temperature, feed composition and feed moisture on the protein content of maize-cassava leaf instant flour. (a) Feed moisture was held constant at 16% (b) feed composition was held constant at 10% (c) Extrusion temperature was held constant at 100^{o}C.

Essential amino acid profiles of maize-cassava leaf composite extruded flour are shown in Table 5.5 (Appendix 5). The mean score of lysine, isoleucine, leucine, valine, methionine, threonine, histidine, phenylalanine and tryptophan content ranged from 1.98 to 6.2, 1.98 to 5.21, 5.47 to 7.51, 3.39 to 4.55, 1.54 to 3.11, 2.15 to 2.77, 1.56 to 3.04, 4.26 to 7.90 and 1.23 to 2.30 g/100 g, respectively. The mean values of essential amino acid profiles of extruded flour were significantly ($P < 0.05$) different from the control sample. The response surface plot (Figure 5.3a) shows the interactive effect of extrusion temperature and feed composition at constant feed moisture of 16% on lysine content. Accordingly, an increase in extrusion temperature resulted in a decrease in lysine content, whereas an increase in feed composition increased protein content. Figure 5.3b shows the interactive effect of extrusion temperature and feed moisture at a constant feed composition of 10% on lysine content. Figure 5.3c shows the interactive effect of feed composition and feed moisture at a constant extrusion temperature of 100 ^{o}C on lysine content. Accordingly, an increase in feed moisture increased lysine content.

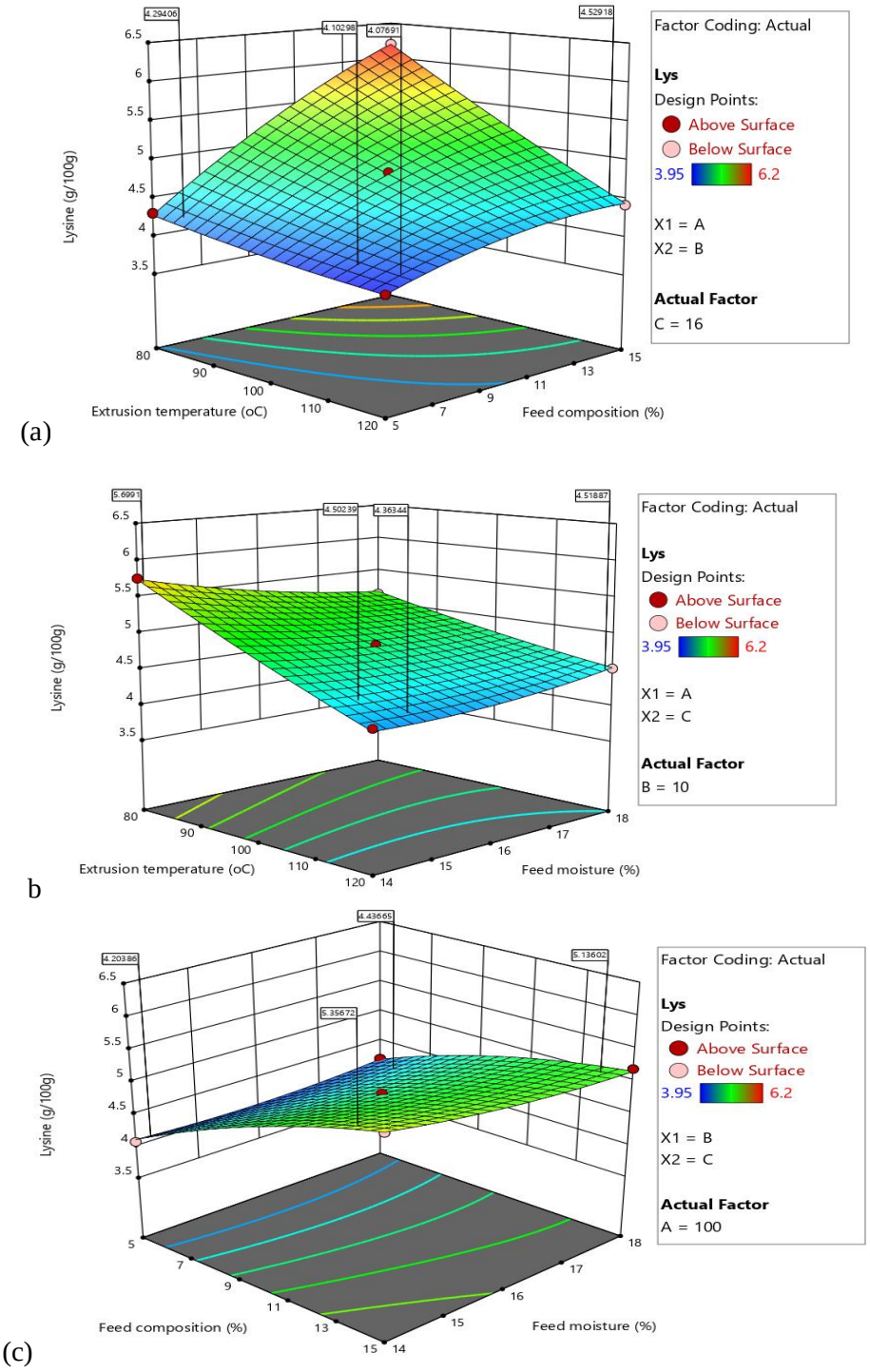


Figure 5.3. The 3D surface plots show the effects of extrusion temperature, feed composition and feed moisture on the lysine content of maize-cassava leaf instant flour. (a) Feed moisture was held constant at 16% (b) Feed composition was held constant at 10% (c) Extrusion temperature was held constant at 100ºC.

The predictive regression models developed for the relationship between the dependent (y) and independent (X) variables in terms of proximate composition and essential amino acid profiles

of extruded maize-cassava leaf composite instant flour are presented in Table 5.6 and Table 5.7, respectively. The independent and response variables were fitted to the second-order model equation (Eq. 3) and its goodness of fit was examined using analysis of variance (ANOVA). Accordingly, the coefficient of determination (R^2), adjusted R^2, predicted R^2 and adequate precision ranged from 0.9774 to 0.9936, 0.9368 to 0.9819, 0.8357 to 0.9056 and 17.5796 to 31.0702, respectively. The coefficients with single factor (X_1, X_2, and X_3) represent the independent effect of a particular variable, while coefficients with two of the factors (X_1X_2, X_1X_3, and X_2X_3) and the ones with second-order terms (X_1^2, X_2^2, and X_3^2) represent the interaction between the three factors and quadratic effects, respectively. A positive sign in front of the regression term is an indication of a synergetic relationship, while a negative sign indicates an antagonistic relationship. The value of the coefficient of variation in Tables 5.6 and 5.7 represents the variation of how far the data fall from the true response surface. The lower the value of the coefficient of variation (less than 10%), the better the model describes the response.

Table 5.6. Estimated regression coefficients for the proximate composition of maize-cassava leaf composite extruded instant flour

Term	Moisture	Protein	Fat	Fibre	Ash	CHO	Calories
Intercept							
$\beta 0$	8.35	17.17	2.28	2.80	4.48	67.72	360.09
Linear							
X_1	-0.7825^{***}	-0.8625^{***}	-0.0825^{**}	-0.3575^{***}	0.0075	1.72^{***}	2.69^{***}
X_2	1.24^{***}	1.80^{***}	-0.2188^{***}	0.6975^{***}	0.5150^{***}	-3.34^{***}	-8.12^{***}
X_3	0.4950^{***}	0.1663	-0.0488^{*}	0.0100	0.1150^{*}	-0.7275^{**}	-2.68^{**}
Quadratic							
X_1^2	-0.1800	0.3108	-0.1375^{**}	-0.0717	-0.0646	-0.0037	-0.0092
X_2^2	-0.5750^{*}	-0.1067	0.0500	-0.3567^{**}	-0.3846^{**}	1.02^{**}	4.09^{***}
X_3^2	-0.3450^{*}	-0.2617	0.0700^{*}	0.0133	0.2504^{*}	0.2862	0.7283
Interaction							
X_1X_2	0.2200	0.0025	-0.1625^{***}	0.0950	0.2975^{**}	-0.3575	-2.88^{**}
X_1X_3	0.5500^{**}	0.0425	-0.0325	-0.0650	-0.0725	-0.4875	-2.07^{**}
X_2X_3	-0.1800	0.1200	0.0500	0.0600	0.0675	-0.0575	0.7000
R^2	0.9919	0.9913	0.9886	0.9875	0.9774	0.9936	0.9934
Adjusted R^2	0.9773	0.9757	0.9682	0.9650	0.9368	0.9819	0.9815
Predicted R^2	0.8977	0.8765	0.9021	0.9056	0.8357	0.8977	0.9049
A. precision	25.9630	27.2119	30.6108	21.9718	17.5796	31.0702	28.0923
C.V	2.45	1.40	1.74	4.56	2.90	0.5825	0.2773

Model	0.0001	0.0001	0.0002	0.0003	0.0013	0.0001	0.0001
Lack of fit	0.3460	0.1848	0.6847	0.7602	0.7790	0.1470	0.1723

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$; X_1= Extrusion temperature; X_2= Feed composition; X_3= Feed moisture; β_0= Constant term; A= Adequate; C.V= Coefficient of variation, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Table 5.7. Estimated regression coefficients for the essential amino acid profiles of maize-cassava leaf composite extruded instant flour

Term	Lysine	Isoleucine	Leucine	Valine	Methionine	Threonine	Histidine	Phenylalanine	Tryptophan
Intercept									
β_0	4.82	4.01	6.90	3.67	3.00	2.53	2.41	7.63	2.05
Linear									
X_1	-0.5212^{***}	-0.5125^{***}	-0.1600^{**}	-0.1513^{***}	-0.0913^{***}	-0.0300^{*}	-0.2012^{***}	-0.1625^{**}	-0.0325^{**}
X_2	0.6050^{***}	1.04^{***}	0.8950^{***}	0.2000^{***}	0.1662^{***}	0.1912^{***}	0.2388^{***}	0.6275^{***}	0.1250^{***}
X_3	0.0813^{**}	-0.3112^{***}	-0.0875	-0.1063^{***}	-0.0725^{***}	-0.0262^{*}	-0.1000^{***}	-0.0500	0.0125
Quadratic									
X_1^2	0.0392	-0.0162	-0.0408	-0.0783^{**}	-0.0650^{**}	-0.0133	0.0342	-0.1729^{*}	0.0192
X_2^2	-0.1383^{***}	-0.7287	-0.4958	0.3192^{***}	-0.0450^{*}	0.0292	-0.0358	-0.3329^{***}	-0.0108
X_3^2	0.0792^{*}	-0.0038^{***}	-0.0308^{***}	0.0017	-0.2275^{***}	-0.0508^{*}	0.0717^{*}	-0.0929	-0.0858^{***}
Interaction									
X_1X_2	-0.3575^{***}	-0.3300^{**}	-0.1450^{*}	-0.3125^{***}	0.0300	0.0025	-0.1900^{***}	0.0775	-0.0850^{***}
X_1X_3	0.2000^{***}	0.1600^{*}	0.3200^{**}	0.0650^{**}	0.0375^{*}	0.0075	0.0225	-0.1525	0.0150
X_2X_3	-0.1175^{**}	-0.0225	0.0050	0.1075^{***}	-0.0275	-0.0200	-0.1475^{***}	-0.0475	0.0250

R^2	0.9987	0.9965	0.9929	0.9969	0.9925	0.9879	0.9929	0.9905	0.9901
Adjusted R^2	0.9964	0.9902	0.9802	0.9913	0.9789	0.9661	0.9801	0.9734	0.9723
Predicted R^2	0.9816	0.9714	0.9198	0.9680	0.9749	0.9273	0.9016	0.8792	0.9708
A. precision	70.5670	39.5131	24.8652	47.6978	25.6374	19.7928	26.8745	23.3385	29.9507
C.V	0.8134	2.74	1.63	0.7940	1.02	1.12	1.64	1.20	0.9890
Model	0.0001	0.0001	0.0001	0.0001	0.0001	0.0003	0.0001	0.0002	0.0002
Lack of fit	0.1652	0.7146	0.4619	0.5480	0.9779	0.8576	0.2267	0.3382	0.9885

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$; X_1= Extrusion temperature; X_2= Feed composition; X_3= Feed moisture; β_0= Constant term; A= Adequate; C.V= Coefficient of variation, * significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Numerical optimization of the extrusion and response variables was done using Design-Expert to identify the best combination of independent variables that could give the optimum values. The optimum extrusion variables were; extrusion temperature (118^{o}C), feed composition (8%) and feed moisture (14%) with overall composite desirability of 99.8% as shown in Table 5.8 and Figure 5.4. The bar graph in Figure 5.4 explains the desirability percentage for each response.

Table 5.8. Optimum conditions for extrusion and response variables of maize-cassava leaf composite extruded instant flour

Variables	Lower Limit	Upper Limit	Optimum	RDI*
Extrusion temperature (°C)	80	120	118	
Feed composition (%)	5	15	8	
Feed moisture (%)	14	18	14	
Lysine	3.95	6.2	4.17	35
Isoleucine	1.98	5.21	3.29	17
Leucine	5.38	7.51	6.12	38
Valine	3.39	4.55	3.62	22
Methionine	2.51	3.11	2.58	17
Threonine	2.3	2.77	2.38	18
Histidine	2.15	3.04	2.32	12
Phenylalanine	6.2	7.9	7.11	31
Tryptophan	1.81	2.3	1.91	5
Protein (%)	14.65	20.1	15.52	
Ash (%)	3.18	5.07	4.31	
CHO (%)	63.96	74.22	72.28	
Calories (kcal/100g)	354.72	377.98	372.79	
Moisture (%)	5.45	9.45	7.27	
Fat (%)	2.39	3.49	2.39	
Fibre (%)	2.3	2.84	2.15	

RDI= Recommended dietary intake, * Essential amino acid requirements (mg/kg /day) for children up to 13 years old according to the Institute of Medicine (2006).

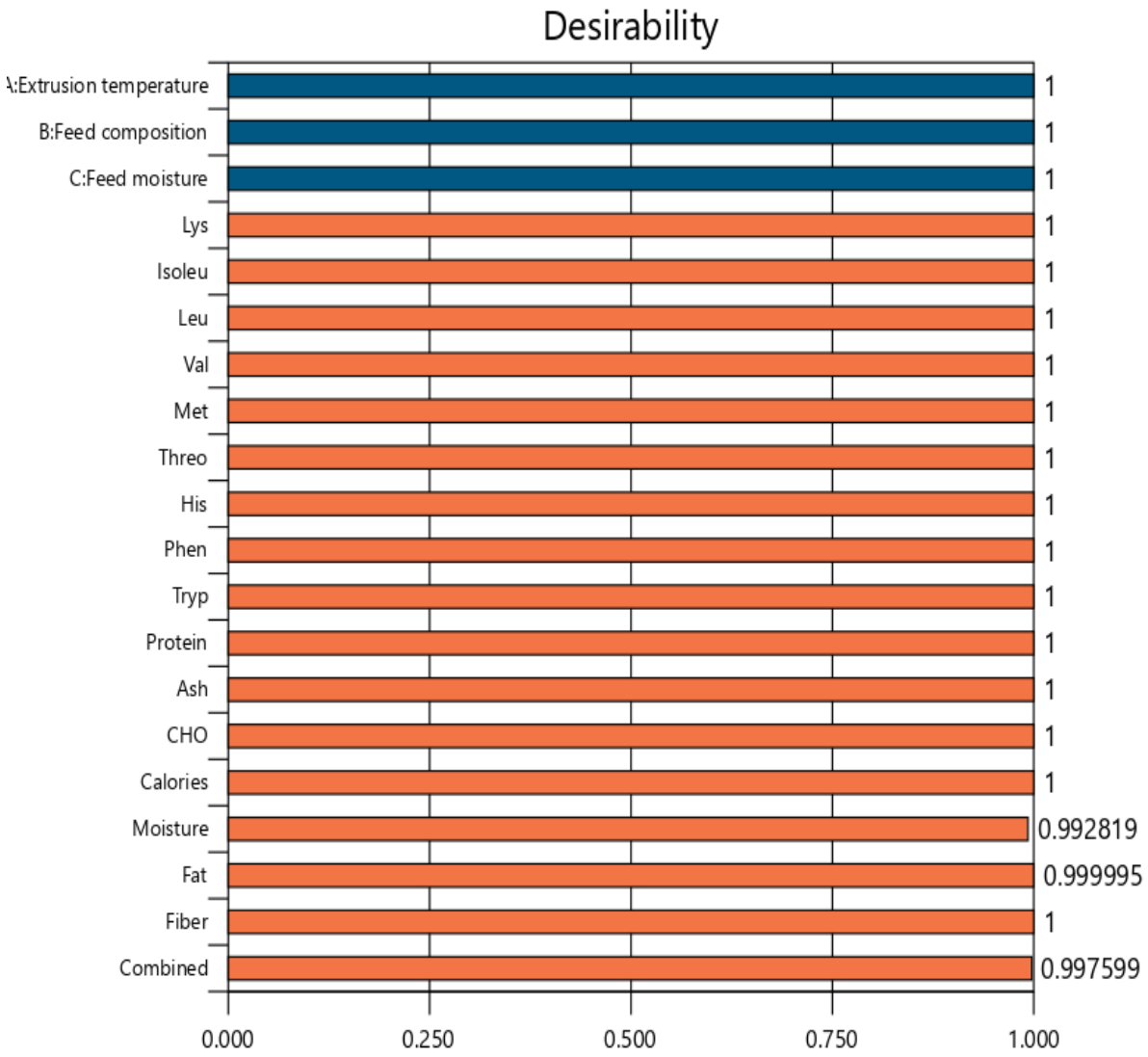


Figure 5.4. Bar graph for optimum extrusion and response variables with desirability

5.4 Discussion

Extrusion cooking technology offers the opportunity to reduce or eliminate natural toxins and anti-nutrients from a variety of products. However, during the extrusion process, several chemical changes occur due to several factors such as barrel temperature, feed composition, feed moisture, screw speed, screw configuration, feed rate, die geometry, product viscosity in the barrel, residence time that can cause thermal degradation, depolymerization and recombination of fragments (Mary, 1998).

In this study, extrusion cooking was used to produce blended instant flour with improved nutritional value. The Box Behnken design was used to optimize extrusion parameters using RSM. The response surface methodology (RSM) was found to be an effective tool for explaining the effect of extrusion process variables on proximate composition and essential

amino acid profiles of maize-cassava leaf composite instant porridge flour. About model validation, the coefficients of determination (R^2) for the proximate composition of the extruded flour were found in the range of 97.74%to 99.36%. This indicates that the model adequately predicts the response variables since the values of R^2 are very much close to 100%. This means the regression model adequately explains the variability in the response variables according to Nahemiah *et al*. (2016) and Zaibunnisa *et al*. (2009). The essential amino acid profiles of the extruded flour were adequately predicted by the model since the coefficient of determination (R^2) is found in the ranges 98.79% to 99.87% which is very close to 100%. According to Borror *et al*. (2002), a good predictive model should have an adjusted $R^2 \geq 0.80$, a significance level of $p < 0.05$, and coefficients of variance (CV) $\leq 10\%$. In this study, adjusted R^2 ranged from 0.9368 to 0.9964 and the coefficient of variation ranged from 0.5825 to 4.56, which indicates the goodness of fit. Furthermore, the non-significant lack of fit for all the proximate and essential amino acid contents is also an indication of the model goodness of fit.

In this study, the moisture content of the extruded flour ranged from 5.45% to 9.45%. Moisture content decreased significantly ($p < 0.001$) when the extrusion temperature increased. The decrease in moisture content was mainly dependent on the extrusion temperature. This is in agreement with Nahemiah *et al*. (2016) who found that the moisture content of extruded flour from broken rice and cowpea blends was highly dependent on the barrel temperature. However, when the proportion of cassava leaf flour in the composite and feed moisture was increased, the moisture content increased significantly ($p < 0.001$). This might be due to the hygroscopic nature of cassava leaf flour contributing to the increase in moisture content (Rodrigues & Pena, 2019). The moisture content of the extruded flour was explained by 99.19% by the model. The optimum moisture content of maize-cassava leaf composite extruded flour was about 7% with desirability of 99.3%. Moisture content is a very important parameter to influence the shelf life of the extruded product. The higher moisture content of the extruded product above 14% would require further drying (Gbenyi *et al*., 2016). This might be because of products having higher moisture content could have a possibility of high water activity which enhances microbial activity leading to spoilage. The range of moisture content in this study is within the recommended limit for extruded flour to have a prolonged shelf life (Asare *et al*., 2011).

The protein content of the extruded flour increased significantly ($p < 0.001$) from 11.45% to 20.1% when the proportion of cassava leaf flour in the composite increased up to 15%. The lowest value (11.45%) was recorded for the control sample. The protein content decreased significantly ($p < 0.001$) when the extrusion temperature increases. The increase in protein

content in this study was much more dependent on the level of cassava leaf flour proportion in the composite. The response surface plots showed that an increase in feed composition caused an increase in protein content. However, as the extrusion temperature increased, the protein content of the extruded flour decreases. This might be due to the occurrence of protein denaturation during extrusion (Akande *et al*., 2017). The response surface plot revealed that an increase in feed moisture also caused a reduction in the protein content of the extruded flour though it was not significant ($p > 0.05$). About 99% of the variation in protein content of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9913. The optimum protein content of maize-cassava leaf composite extruded flour was 16% with desirability of 100%. The recommended protein requirement for complementary foods to combat protein-energy malnutrition is 15% suggesting the adequacy of protein in the products (Gbenyi *et al*., 2016) and this is in agreement with the current finding.

The fat content of the extruded flour ranged from 2.06% to 2.32%. A highly significant ($p < 0.001$) reduction in fat content was observed when feed composition increased. It is also decreased significantly ($p < 0.01$) when extrusion temperature increases. This might be due to the formation of lipid starch complexes during extrusion cooking resulting in the crude fat content decreasing (De Pilli *et al*., 2007; Ferrari *et al.*, 2014). The reduction in fat content in this study is in agreement with Gbenyi *et al*. (2016) who observed a similar decrease in fat content during the extrusion of sorghum and cowpea blends. About 99% of the variation in fat content of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9886. The optimum fat content of maize-cassava leaf composite extruded flour was 2.39% with desirability of 99.99%. Low-fat foods are good for shelf-life stability because they are less prone to rancidity during storage.

Fibre contents in the extruded flour ranged from 1.24% to 3.32%. An increase in feed composition caused a significant ($p < 0.001$) increase in the crude fibre contents of the extruded flour. However, an increase in extrusion temperature caused a significant ($p < 0.001$) decrease in fibre content. This might be due to the high loss of insoluble fibre that can occur at higher extrusion temperatures and feed moisture (Mary, 1998). The decrease in fibre content in the current study is in agreement with the findings of Rinaldi *et al*. (2000) who observed a reduction of 25.5% of insoluble fibre during the extrusion of wheat extrudates enriched with wet okara (the residue left after soymilk production). About 99% of the variation in fibre content of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9875. The optimum fibre content of maize-cassava leaf composite extruded flour

was about 2% with desirability of 100%. Children require less fibre, ranging from 19 g/day for 1 to 3 years to 31 g/day for 9 to 13 years (American Society for Nutrition, 2011).

The ash content is an indication of the mineral contents of the extruded flour. The total ash in this study ranged from 2.11% to 5.07%. A significant ($p < 0.001$) increase in ash content was noted when feed composition increased. It was also observed that ash content increased significantly ($p < 0.05$) when feed moisture increases. The variation in ash content was majorly attributed to the feed composition. Minerals are generally stable since extrusion temperature is not expected to cause a significant change in their composition (Gbenyi *et al.*, 2016). The present result is in line with the finding of Gbenyi *et al*. (2016). About 98% of the variation in ash content of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9774. The optimum ash content of maize-cassava leaf composite extruded flour was about 4% with desirability of 100%.

The carbohydrate content of the extruded flour ranged from 64.93% to 74.22%. The carbohydrate content of the extruded flour significantly ($p < 0.001$) decreased as the amount of cassava leaf flour proportion and feed moisture increased. This could be due to the low amount of carbohydrates in cassava leaf flour which tends to lower the overall carbohydrate content of the extruded flour. However, the extrusion temperature had a significant ($p < 0.001$) effect on the carbohydrate content of the extruded flour. This might be due to starch gelatinization that could initiate the starch granules to swell and increase the available sugars (Mary, 1998). The carbohydrate content of the extruded flour was explained by 99.36% by the model. The optimum carbohydrate content of maize-cassava leaf composite extruded flour was about 72% with desirability of 100%.

The energy value of the extruded flour varied from 354.72 to 382.25 kcal/100g. An increase in feed composition resulted in a significant ($p < 0.001$) decrease in the energy value of the extruded product in this study. This indicates that the higher maize flour proportion in the composite can provide more energy value than the cassava leaf flour proportion. The energy value of the extruded flour was explained by 99.34% by the model. The optimum energy value of maize-cassava leaf composite extruded flour was 372.793 kcal/100g with desirability of 100%.

Essential amino acid profiles of maize-cassava leaf composite extruded instant flour was significantly ($p < 0.001$) improved as a result of cassava leaf flour proportion increment in the composite. However, extrusion temperature caused a significant ($P < 0.01$) reduction in all

essential amino acids. An increase in extrusion temperature reduced the lysine content of the extruded flour in this study. This might be due to the occurrence of the Maillard reaction at a higher temperature during extrusion leading to losses of the amino acids (Jiddere & Filli, 2015). Figure 5.2c shows the interactive effect of feed composition and feed moisture on lysine content. The increase in feed moisture resulted in a significant ($p < 0.01$) increase in the lysine content of the extruded flour. This finding is in line with Ilo and Berghofer (2003) who observed that feed moisture had a protective effect on the amino acid loss in extrusion cooking. Lysine is the most limiting and heat-labile amino acid in cereals and cereal-based products. Therefore, mild extrusion conditions (high feed moisture, low residence time and low temperature) are important to improve the nutritional quality of the extruded products, whereas high barrel temperature (> 200^{o}C), low moisture (<15%) and improper formulation (presence of high fat and reactive sugars) can affect the nutritional quality adversely (Singh *et al*., 2007).

5.5 Conclusion

Based on the findings of this study, it is possible to obtain protein-enriched instant porridge flour from maize-cassava leaf composites using extrusion cooking. Therefore, extrusion cooking can be used effectively for the production of maize-cassava leaf composite instant porridge flour that has enhanced nutritional quality. Increasing extrusion temperature leads to a decrease in protein content and essential amino acid profiles of the extruded flour. However, the increasing level of cassava leaf proportion in the composite had a positive effect on the protein and essential amino acid contents of the extruded flour. The optimum value of protein after optimization in this study was 16%. Therefore, 100 gram serving of maize-cassava leaf extruded porridge can provide 47% of the recommended daily allowance of protein (34 g/day) for children up to 12 years old with enhanced nutritional quality.

CHAPTER SIX

OPTIMIZATION OF FUNCTIONAL PROPERTIES, *IN VITRO* PROTEIN DIGESTIBILITY AND MINERAL CONTENTS OF EXTRUDED FLOUR DEVELOPED FROM MAIZE-CASSAVA LEAF COMPOSITES

Abstract

The application of extrusion cooking technology in product development is getting a high priority due to its effectiveness in quality improvement. Response surface methodology has been used extensively to optimize extrusion variables. The objective of this study was to optimize the functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite extruded instant porridge flour using response surface methodology. Box-Benkhen design was used for the formulation and optimization of the process variables. Extrusion variables; extrusion temperature (80^{o}C – 120^{o}C), feed composition (5% -15%) and feed moisture (14% - 18%) were used as input variables whereas, water absorption index (WAI), water solubility index (WSI), *in vitro* protein digestibility (IVPD), and minerals were used as responses. Results obtained showed that WAI and WSI increased significantly ($p < 0.05$) as a result of an increase in extrusion temperature and feed composition. WSI showed a significant ($p < 0.05$) positive correlation with IVPD and mineral contents. IVPD was significantly ($p < 0.05$) improved by extrusion temperature and feed composition. Calcium, potassium, magnesium and iron contents were significantly ($p < 0.001$) increased as a result of an increase in feed composition. Supplementation of cassava leaf flour during extrusion of maize-based products improves its functional properties, IVPD and mineral contents, hence suitable for the formulation of nutritious extruded food products.

Keywords: Cassava leaf, extrusion cooking, instant porridge flour, optimization, response surface methodology

6.1 Introduction

The acceptance of cereal flour by consumers are dependent on its functional properties and the degree of starch gelatinization (Adedeji *et al*., 2014). Maize (*Zea mays*) grains are used in the production of several traditional foods in developing countries (Mbata *et al*., 2009). Unfortunately, it lacks some micro-nutrients and its protein digestibility is low (Mbata *et al*., 2009). To alleviate the ever-increasing problems of malnutrition in developing countries, there is a need for supplementation of popularly consumed low-protein staple foods like maize flour with inexpensive locally-available sources of plant proteins such as cassava leaves. Cassava leaves are rich in proteins and minerals such as calcium, potassium and magnesium (Latif & Müller, 2015).

Maize can be processed/supplemented with cassava leaves for product diversity and broader acceptability. Extrusion cooking technology which is used in the food processing industry is attracting global attention due to its versatility and effectiveness in producing shelf-stable products. Extrusion is one of the most commercially successful technologies, escalating its demand in the diverse fields of food processing and digital food marketing (3-D printed food), and food packaging (Prabha *et al*., 2021), which can be deployed for maize-cassava flour product processing. In the extrusion process, raw ingredients are fed into the extruder barrel through the feed hopper and the screw conveys the food along with it, then products come out in different diameters of the die. Recent advancements in extrusion technology have made it possible to control the process parameters to achieve the desired characteristics of the final product (Shah *et al*., 2021). Product quality can vary considerably depending on the extruder type, screw configuration, feed moisture, temperature profile in the barrel, screw speed, feed composition, feed rate and die profile (Ding *et al*., 2006).

Response surface methodology consists of a group of mathematical and statistical techniques used in the development of an adequate relationship between a response of interest and several factors (Khuri & Mukhopadhyay, 2010). It is a powerful tool to optimize the process parameters in the manufacturing processes of various products (Kim *et al*., 2020). Response surface plots are used to understand the effect of process parameters on responses. The process parameters are optimized using the desirability approach of response surface methodology and confirmed by conducting confirmation tests (Chelladurai *et al*., 2020).

The objective of this study was to evaluate the functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite instant porridge flour.

Response surface methodology with Box-Behnken design was used for formulation and optimization of process variables to achieve the required values of the response variables. Extrusion temperature, feed composition and feed moisture were considered as process variables and water absorption index, water solubility index, in vitro protein digestibility, calcium, potassium, magnesium and iron contents were used as response variables. The results obtained are herein presented.

6.2 Materials and methods

6.2.1 Sample collection and preparation

The raw materials for the extrusion process were collected and prepared as shown in section 5.2.1.

6.2.2 Composite flour preparation

Three (3) formulations were prepared which contain cassava leaf flour ranging from 5 to 15% as described in section 5.2.2.

6.2.3 Extrusion process

The extrusion process was performed using a co-rotating twin screw extruder (model BC-21, No 124, Clextral, Firminy, France) as shown in section 5.2.3.

6.2.4 Determination of functional properties

Water absorption and water solubility indices were determined according to Yagci and Gogus (2008). Exactly 0.5 g sample was dispersed into a plastic centrifuge tube containing 10 ml distilled water. After standing for 30 min with intermittent shaking every 5 min, the sample was centrifuged at 1800 x g for 15 min. the supernatant was decanted into a tarred aluminium pan and dried at 105^{o}C until constant weight. The weight of the gel remaining in the centrifuge tube was weighed. The following formulas were used to calculate the water absorption and solubility indices.

$$WAI\ (g/g) = \frac{Weight\ gain\ of\ gel}{Dry\ weight\ of\ sample} \qquad \text{(Eq. 1)}$$

$$WSI\ (\%) = \frac{Weight\ of\ dry\ solids\ in\ \sup erna\tan t}{Dry\ weight\ of\ sample}\ x\,100 \qquad \text{(Eq. 2)}$$

6.2.5 Determination of *in vitro* protein digestibility

The *in vitro* protein digestibility of the developed instant flour was done as shown in section 3.2.6.

6.2.6 Mineral analyses

The minerals (Ca, K, Mg and Fe) content of the developed instant flour were determined as indicated in section 4.2.7.

6.2.7 Fitted model validation

Coefficient of determination (R^2), adjusted coefficient of determination (R^2), predicted coefficient of determination (R^2), lack of fit and coefficient of variation (C.V) were used to check if the fitted models provide an adequate estimation of the real system as described in section 5.2.7.

6.2.8 Optimization

Numerical optimization of the extrusion variables and responses was done using Design-Expert to identify the best combination of independent variables which could produce optimum responses of functional properties, in vitro protein digestibility and mineral contents of instant flour.

6.2.9 Experimental design and statistical analysis

Response surface methodology with Box-Behnken design was used as shown in section 4.1.2. The response surface methodology procedure of Design-Expert (DX13, Stat Ease Inc. Minneapolis, MN, USA) software was used as shown in section 5.2.6 to analyse the experimental data.

6.3 Results

The effect of extrusion cooking variables on the water absorption index (WAI) and water solubility index (WSI) of the resultant instant porridge flour is shown in Table 6.1. The water absorption index ranged from 4.7 to 7.3 g/g whereas the water solubility index ranged from 9.43 to 22.75%. A combination of feed composition, feed moisture and extrusion temperature had a significant effect on water absorption and water solubility indices of the resultant instant porridge flour as shown in Table 6.2.

Table 6.1. Effect of extrusion cooking variables on functional properties of maize-cassava leaf composites extruded flour

Runs	**Independent variables**			**Response variables**	
	X_1 (°C)	X_2 (%)	X_3 (%)	WAI (g/g)	WSI (%)
1	+1(120)	0(10)	+1(18)	6.37 ± 0.15^{bc}	19.15 ± 0.07^{b}
2	0(100)	+1(15)	-1(14)	6.07 ± 0.03^{cdef}	18.26 ± 0.04^{c}
3	0(100)	0(10)	0(16)	6.4 ± 0.10^{bc}	17.53 ± 0.07^{d}
4	-1(80)	+1(15)	0(16)	5.87 ± 0.16^{ef}	17.36 ± 0.04^{d}
5	0(100)	0(10)	0(16)	6.18 ± 0.12^{cde}	15.96 ± 0.09^{e}
6	0(100)	-1(5)	+1(18)	4.93 ± 0.08^{ij}	11.1 ± 0.17^{j}
7	+1(120)	-1(5)	0(16)	6.63 ± 0.15^{b}	13.36 ± 0.06^{h}
8	-1(80)	0(10)	-1(14)	5.77 ± 0.15^{fg}	16.15 ± 0.05^{e}
9	0(100)	+1(15)	+1(18)	5.37 ± 0.03^{h}	18.76 ± 0.24^{b}
10	+1(120)	+1(15)	0(16)	7.3 ± 0.10^{a}	22.75 ± 0.27^{a}
11	-1(80)	0(10)	+1(18)	4.7 ± 0.10^{j}	13.97 ± 0.09^{g}
12	+1(120)	0(10)	-1(14)	6.25 ± 0.11^{cd}	18.99 ± 0.10^{b}
13	0(100)	0(10)	0(16)	5.93 ± 0.10^{def}	17.33 ± 0.12^{d}
14	0(100)	-1(5)	-1(14)	5.43 ± 0.08^{gh}	14.98 ± 0.12^{f}
15	-1(80)	-1(5)	0(16)	5.12 ± 0.04^{hi}	12.79 ± 0.05^{i}
Control	100	0	16	7.06 ± 0.17^{a}	9.43 ± 0.43^{k}

Response variable values are mean ± standard deviation. Values with different superscripts in the column are significant at $p < 0.05$. **Key**: X_1 = Extrusion temperature, X_2 = Feed composition, X_3 = Feed moisture, WAI = Water absorption index, WSI = Water solubility index

Table 6.2. ANOVA for water absorption and water solubility indices

Source	WAI (g/g)	WSI (%)
Model	**0.0030**	**0.0021**
Linear terms		
X_1	**0.0003**	**0.0018**
X_2	**0.0077**	**0.0001**
X_3	**0.0140**	0.0674
Quadratic terms		
X_1^2	0.1336	0.3327
X_2^2	0.2741	0.1086
X_3^2	**0.0027**	0.4712
Interaction terms		
X_1X_2	0.8532	**0.0323**
X_1X_3	**0.0339**	0.2130
X_2X_3	0.6469	**0.0443**
R^2	0.9685	0.9731
Adjusted R^2	0.9119	0.9246
Predicted R^2	0.7234	0.7290
Adequate precision	15.4386	17.5302
C.V (%)	3.49	4.95
Lack of fit	0.6721	0.5746

Key: X_1 = Extrusion temperature, X_2 = Feed composition, X_3 = Feed moisture. All values represent the p-value except for coefficient of determinations (R^2, R^2 adj, Pred. R^2) and Adequate precision, P values in bold represent the significant terms at $p < 0.05$; WAI = Water absorption index, WSI = Water solubility index

The quadratic model equation for WAI is:

WAI (g/g) = $6.17 + 0.6362X_1 + 0.3125X_2 – 0.2687X_3 + 0.1913X_1^2 - 0.1312X_2^2 – 0.5888X_3^2 – 0.0200X_1X_2 + 0.2975X_1X_3 – 0.0500X_2X_3$ (Eq. 5)

It was observed from equation (5) that the coefficients of extrusion temperature and feed composition are positive, and that of feed moisture is negative. Therefore, the water absorption index increases significantly ($p < 0.05$) when extrusion temperature and feed composition

increase, whereas the water absorption index decreases significantly ($p < 0.05$) when feed moisture increases.

The quadratic model equation for WSI is:

WSI (%) = $16.94 + 1.75\ X_1 + 3.11X_2 - 0.6750X_3 + 0.4575X_1^2 - 0.8325X_2^2 - 0.3325X_3^2 + 1.21X_1X_2 + 0.5850X_1X_3 + 1.10X_2X_3$ (Eq. 6)

The quadratic equation (6) shows that the water solubility index increases significantly ($p < 0.05$) with an increase in extrusion temperature and feed composition. However, it decreases with an increase in feed moisture content though it was not significant at $p < 0.05$.

The response surface plot (Figure 6.1) shows an interactive effect of extrusion temperature and feed composition on the water absorption index. Accordingly, an increase in extrusion temperature and feed composition at constant feed moisture of 16% resulted in an increased water absorption index. Figure 6.2a shows that an increase in extrusion temperature and feed composition at constant feed moisture resulted in an increased water solubility index. It was also observed (Figure 6.2b) that an increase in feed composition and feed moisture at a constant extrusion temperature of 100°C increased the water solubility index.

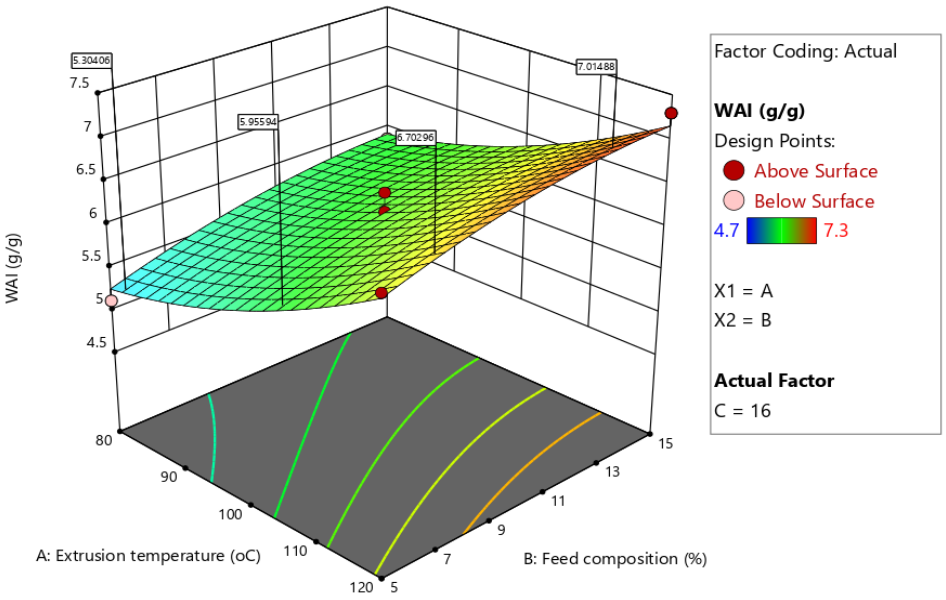
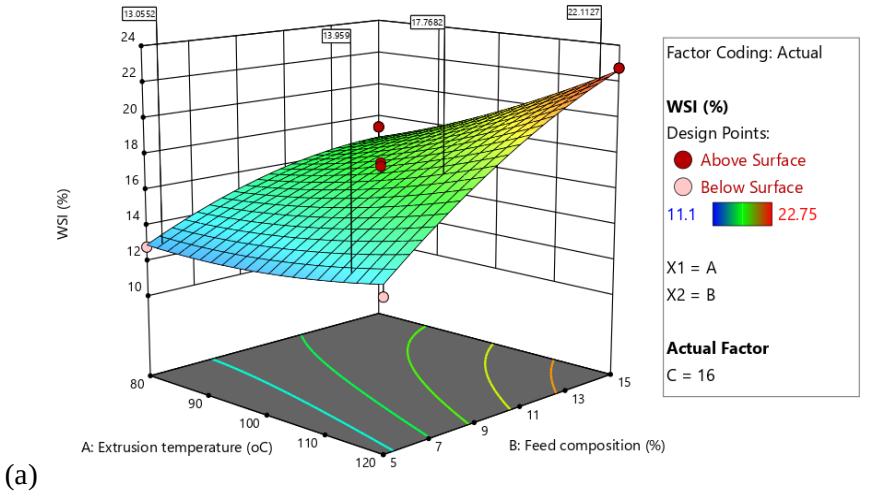
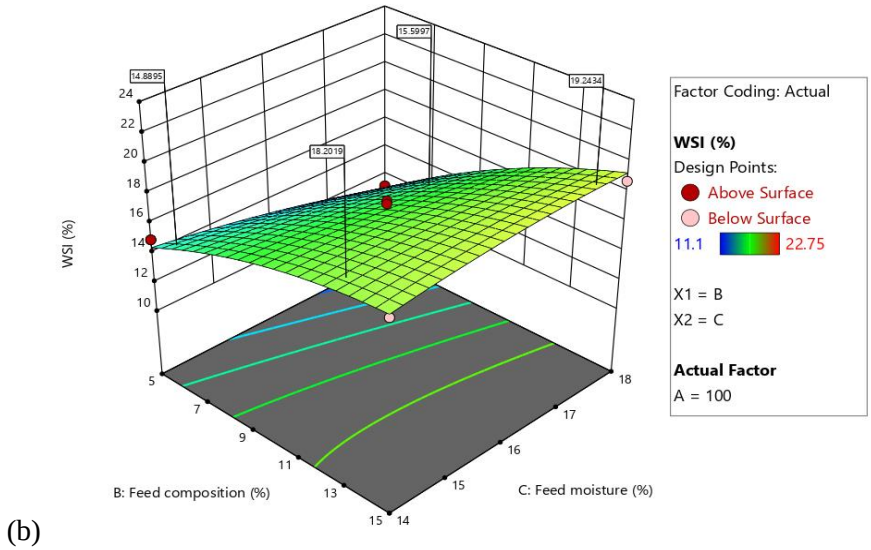


Figure 6.1. The 3D surface plot for the effect of extrusion temperature and feed composition on the water absorption index of extruded flour at constant feed moisture of 16%.



(a)



(b)

Figure 6.2. The 3D surface plots for the effect of extrusion variables on the water solubility index of extruded flour (a) feed moisture held constant at 16% (b) extrusion temperature held constant at 100ºC.

The effect of extrusion cooking variables on *in vitro* protein digestibility of maize-cassava leaf flour composite extruded instant porridge flour is shown in Table 6.3. The in vitro protein digestibility of the extruded flour ranged from 80.54 to 89.95%. Instant flour made at 100ºC extrusion temperature, 15% feed composition and 18% feed moisture had significantly ($p < 0.05$) the highest IVPD (89.95%), whereas the control sample had the lowest IVPD (80.54%). The ANOVA (Table 6.4) shows that *in vitro* protein digestibility significantly ($p < 0.05$) differs by the linear and interaction terms of extrusion temperature and feed composition.

Table 6.3. Effect of extrusion cooking variables on in vitro protein digestibility of maize-cassava leaf composites extruded flour

Runs	**Independent variables**			**Response variables**
	X_1 (°C)	X_2 (%)	X_3 (%)	IVPD (%)
1	+1(120)	0(10)	+1(18)	87.57 ± 2.81^{abcd}
2	0(100)	+1(15)	-1(14)	89.1 ± 1.21^{abc}
3	0(100)	0(10)	0(16)	87.44 ± 2.53^{abcd}
4	-1(80)	+1(15)	0(16)	89.17 ± 0.22^{ab}
5	0(100)	0(10)	0(16)	87.93 ± 0.39^{abcd}
6	0(100)	-1(5)	+1(18)	85.14 ± 1.64^{cde}
7	+1(120)	-1(5)	0(16)	85.35 ± 0.82^{bcd}
8	-1(80)	0(10)	-1(14)	84.8 ± 0.43^{cde}
9	0(100)	+1(15)	+1(18)	89.95 ± 0.98^{a}
10	+1(120)	+1(15)	0(16)	87.47 ± 0.18^{abcd}
11	-1(80)	0(10)	+1(18)	86.92 ± 0.93^{abcd}
12	+1(120)	0(10)	-1(14)	88.83 ± 1.04^{abc}
13	0(100)	0(10)	0(16)	85.97 ± 0.36^{abcd}
14	0(100)	-1(5)	-1(14)	83.97 ± 0.84^{def}
15	-1(80)	-1(5)	0(16)	81.19 ± 0.86^{ef}
Control	100	0	16	80.54 ± 2.00^{f}

Response variable values are mean ± standard deviation. Values with different superscripts in the column are significant at $p < 0.05$; X_1 = Extrusion temperature, X_2 = Feed composition, X_3 = Feed moisture, IVPD = *in vitro* protein digestibility

Table 6.4. ANOVA for in vitro protein digestibility (IVPD)

Source	IVPD (%)
Model	**0.0051**
Linear terms	
X_1	**0.0221**
X_2	**0.0003**
X_3	0.2084
Quadratic terms	
X_1^2	0.1426
X_2^2	0.1829
X_3^2	0.1864
Interaction terms	
X_1X_2	**0.0127**
X_1X_3	0.0800
X_2X_3	0.7179
R^2	0.9608
Adjusted R^2	0.8901
Predicted R^2	0.7495
Adequate precision	14.0641
C.V (%)	0.8894
Lack of fit	0.8351

Key: X_1 = Extrusion temperature, X_2 = Feed composition, X_3 = Feed moisture. All values represent the p-value except for coefficient of determinations (R^2, R^2 adj, Pred. R^2) and Adequate precision, P values in bold represent the significant terms at $p < 0.05$

The quadratic model equation for IVPD is:

IVPD (%) = $87.11 + 0.8925X_1 + 2.47X_2 + 0.3938X_3 – 0.6979X_1^2 – 0.6204X_2^2 + 0.6146X_3^2 – 1.47X_1X_2 – 0.8450X_1X_3 - 0.1475X_2X_3$ (Eq. 7)

The regression model in equation (7) shows that IVPD increased significantly with an increasing linear term of extrusion temperature and feed composition of the extruded flour. However, IVPD decreased significantly with an increasing interaction in terms of extrusion temperature and feed composition.

The response surface plot (Figure 6.3) shows an interactive effect of extrusion temperature and feed composition on *in vitro* protein digestibility. Thus, an increase in extrusion temperature and feed composition at constant feed moisture of 16% resulted in increased in vitro protein digestibility of extruded flour.

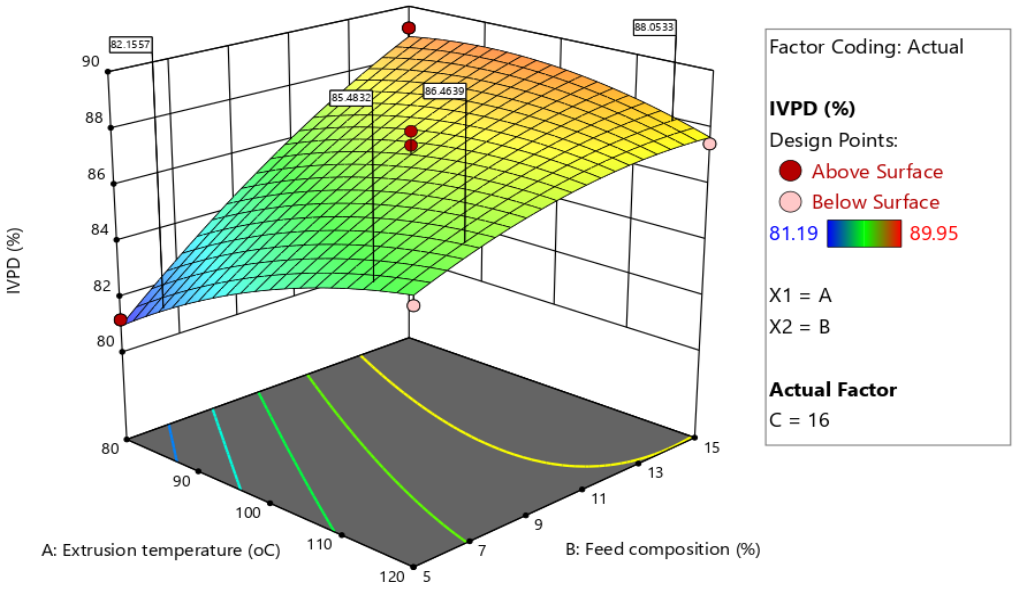


Figure 6.3. The 3D surface plot for the effect of extrusion temperature and feed composition on *in vitro* protein digestibility of extruded flour at constant feed moisture of 16%.

The effect of extrusion cooking variables on the mineral contents of extruded flour is shown in Table 6.5. Calcium, potassium, magnesium and iron contents of the extruded flour ranged from 81.19 to 346.25 mg/100g, 217.49 mg/100g, 79.10 to 148.7 mg/100g and 7.67 to 26.5 mg/100g, respectively. Instant flour prepared at an extrusion temperature of 80ºC, feed composition of 15% and feed moisture of 16% had significantly ($p < 0.05$) the highest mineral contents whereas the lowest values were observed for the control sample. The ANOVA (Table 6.6) shows that calcium and potassium contents significantly ($p < 0.05$) differ by the linear terms of feed composition and feed moisture, quadratic terms of extrusion temperature and feed moisture, and interaction terms of extrusion temperature and feed composition. Magnesium content significantly ($p < 0.05$) differs by all linear terms and quadratic terms. However, iron content was significantly ($p < 0.05$) by linear and quadratic terms of extrusion temperature and feed composition.

Table 6.5. Effect of extrusion cooking variables on functional properties of maize-cassava leaf composites extruded flour

Runs	Independent variables			Response variables			
	X_1 (^oC)	X_2 (%)	X_3 (%)	Ca (mg/100g)	K (mg/100g)	Mg (mg/100g)	Fe (mg/100g)
1	+1(120)	0(10)	+1(18)	315.18 ± 4.02^{e}	190.7 ± 0.89^{fg}	117.6 ± 1.03^{de}	16.37 ± 0.14^{bcd}
2	0(100)	+1(15)	-1(14)	332.3 ± 0.90^{b}	213.18 ± 0.38^{b}	143.59 ± 2.11^{ab}	24.17 ± 0.86^{a}
3	0(100)	0(10)	0(16)	304.87 ± 0.17^{f}	177.54 ± 0.34^{j}	112.84 ± 1.89^{ef}	14.83 ± 0.84^{bcdef}
4	-1(80)	+1(15)	0(16)	346.25 ± 0.94^{a}	217.49 ± 0.99^{a}	148.7 ± 5.75^{a}	26.5 ± 3.29^{a}
5	0(100)	0(10)	0(16)	306.74 ± 2.01^{f}	187.46 ± 1.13^{gh}	111.28 ± 0.71^{efg}	11.59 ± 0.15^{efg}
6	0(100)	-1(5)	+1(18)	286.83 ± 0.92^{h}	181.5 ± 0.33^{i}	97.85 ± 0.77^{hi}	10.87 ± 1.11^{fg}
7	+1(120)	-1(5)	0(16)	294.63 ± 3.26^{g}	193.95 ± 2.06^{ef}	95.44 ± 1.12^{i}	10.96 ± 0.05^{fg}
8	-1(80)	0(10)	-1(14)	325.77 ± 4.02^{cd}	205.8 ± 0.35^{d}	134.19 ± 1.17^{c}	17.44 ± 0.14^{bc}
9	0(100)	+1(15)	+1(18)	325.59 ± 1.00^{cd}	210.26 ± 0.37^{bc}	140.95 ± 0.99^{bc}	25.32 ± 0.82^{a}
10	+1(120)	+1(15)	0(16)	324.38 ± 0.93^{cd}	186.61 ± 1.23^{h}	137.4 ± 0.85^{bc}	23.41 ± 1.01^{a}
11	-1(80)	0(10)	+1(18)	321.8 ± 0.61^{d}	196.78 ± 0.27^{e}	121.62 ± 1.03^{d}	18.74 ± 1.06^{b}
12	+1(120)	0(10)	-1(14)	329.03 ± 0.23^{bc}	208.86 ± 1.12^{cd}	116.05 ± 2.97^{def}	15.62 ± 0.91^{bcde}
13	0(100)	0(10)	0(16)	302.34 ± 0.22^{f}	186.89 ± 0.49^{h}	104.98 ± 5.95^{gh}	13.78 ± 0.17^{cdef}
14	0(100)	-1(5)	-1(14)	295.59 ± 3.69^{g}	195.47 ± 2.31^{e}	109.28 ± 0.30^{fg}	12.75 ± 0.90^{def}
15	-1(80)	-1(5)	0(16)	290.09 ± 0.24^{gh}	170.21 ± 1.11^{k}	111.06 ± 0.02^{efg}	14.22 ± 3.32^{cdef}
Control	100	0	16	81.19 ± 2.66^{i}	47.46 ± 2.58^{l}	79.10 ± 1.35^{j}	7.67 ± 0.93^{g}

Table 6.6. ANOVA for mineral contents of the extruded flour

Source	Ca (mg/100g)	K (mg/100g)	Mg (mg/100g)	Fe (mg/100g)
Model	**0.0002**	**0.0028**	**0.0002**	**0.0008**
Linear terms				
X_1	0.0656	0.4176	**0.0016**	**0.0265**
X_2	**<0.0001**	**0.0007**	**<0.0001**	**<0.0001**
X_3	**0.0129**	**0.0122**	**0.0249**	0.7129
Quadratic terms				
X_1^2	**0.0010**	0.0999	**0.0069**	**0.0211**
X_2^2	0.3076	0.1298	**0.0049**	**0.0032**
X_3^2	**0.0065**	**0.0021**	**0.0080**	0.0529
Interaction terms				
X_1X_2	**0.0082**	**0.0011**	0.4755	0.9462
X_1X_3	0.1734	0.3123	0.0531	0.8275
X_2X_3	0.7553	0.2325	0.1774	0.2618
R^2	0.9888	0.9693	0.9902	0.9821
Adjusted R^2	0.9687	0.9140	0.9725	0.9500
Predicted R^2	0.8521	0.8254	0.9620	0.9012
Adequate precision	22.8041	14.7264	22.5281	15.6251
C.V (%)	0.7530	0.8221	1.27	7.00
Lack of fit	0.2861	0.8750	0.9599	0.8835

Key: X_1 = Extrusion temperature, X_2 = Feed composition, X_3 = Feed moisture. All values represent p value except for coefficient of determinations (R^2, R^2 adj, Pred. R^2) and Adequate precision; P values in bold represent the significant terms at $p < 0.05$

The quadratic model equation for mineral contents is:

Ca (mg/100g) = $304.65 – 2.59X_1 + 20.17X_2 - 4.16X_3 + 11.03X_1^2 -1.84X_2^2 +7.27X_3^2 - 6.60X_1X_2 -2.47X_1X_3 + 0.5125X_2X_3$ (Eq. 8)

The quadratic model equation (8) shows that calcium content increases significantly ($p < 0.05$) with an increase in feed composition. However, calcium content decreases significantly ($p < 0.05$) with an increase in feed moisture.

K (mg/100g) = $183.96 -1.27X_1 +10.80X_2 - 5.51X_3 + 4.27X_1^2 + 3.83X_2^2 + 12.30X_3^2 -13.65X_1X_2 – 2.29X_1X_3 +2.76X_2X_3$ (Eq. 9)

The quadratic model equation (9) shows that potassium content increases significantly ($p < 0.05$) with an increase in feed composition. However, potassium content decreases significantly ($p < 0.05$) with an increase in feed moisture.

Mg (mg/100g) = $109.70 - 6.14X_1 + 19.63X_2 - 3.14X_3 + 6.45X_1^2\ 7.00X_2^2 + 6.22X_3^2 + 1.08X_1X_2 + 3.53X_1X_3 + 2.20X_2X_3$ (Eq. 10)

The quadratic model equation (10) shows that magnesium content increases significantly ($p < 0.05$) with an increase in feed composition. However, magnesium content decreases significantly ($p < 0.05$) with an increase in extrusion temperature and feed moisture.

Fe (mg/100g) = $13.40 - 1.32X_1 + 6.32X_2 + 0.1650X_3 + 2.07X_1^2 + 3.30X_2^2 + 1.57X_3^2 + 0.0425X_1X_2 - 0.1375X_1X_3 + 0.7575X_2X_3$ (Eq. 11)

The quadratic model equation (11) shows that iron content increases significantly ($p < 0.05$) with an increase in feed composition. However, iron content decreases significantly ($p < 0.05$) with an increase in extrusion temperature.

The response surface plot (Figure 6.4) shows an interactive effect of extrusion temperature and feed composition on the calcium content of extruded flour. Accordingly, an increase in extrusion temperature and feed composition at constant feed moisture of 16% resulted in increased calcium content. The response surface plot (Figure 6.5) shows that an increase in extrusion temperature and feed composition at constant feed moisture of 16% resulted in increased potassium content.

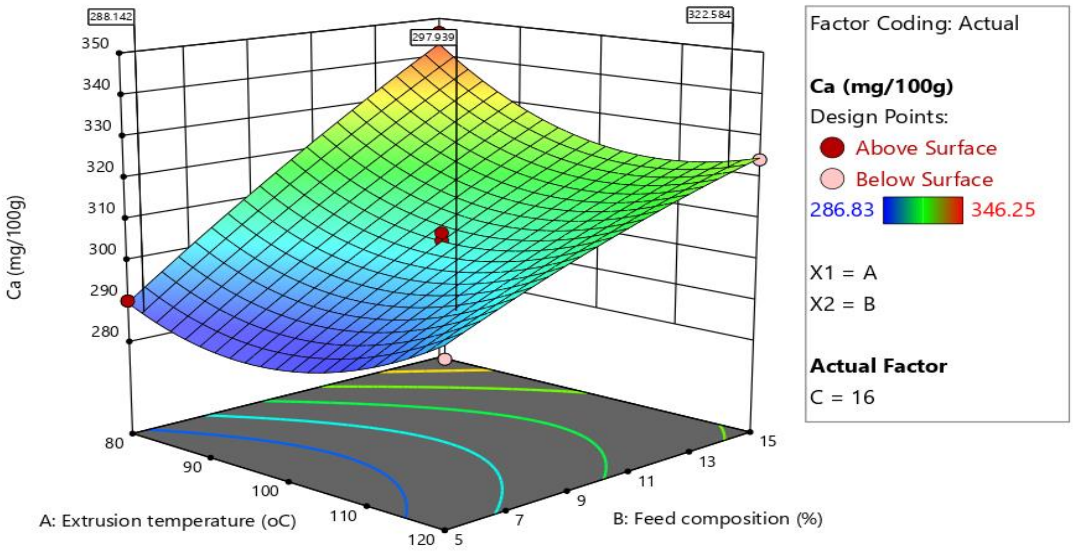


Figure 6.4. The 3D surface plot for the effect of extrusion temperature and feed composition on the Calcium content of extruded flour at constant feed moisture of 16%.

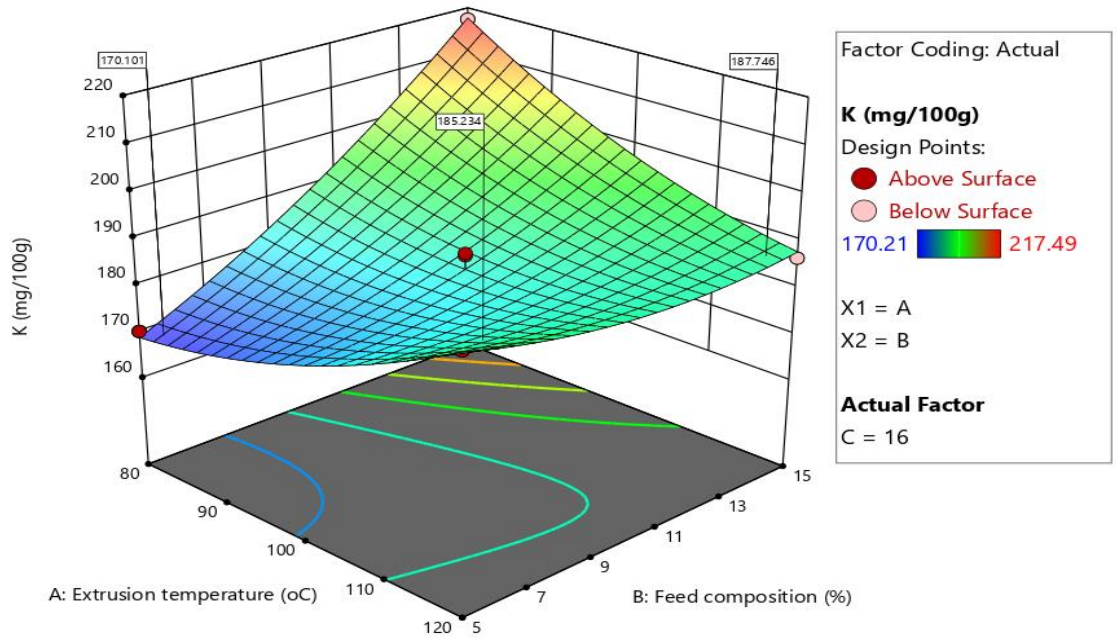


Figure 6.5. The 3D surface plot for the effect of extrusion temperature and feed composition on the Potassium content of extruded flour at constant feed moisture of 16%.

The correlation between functional properties, in vitro protein digestibility and minerals, is shown in Table 6.7. The water solubility index had a highly significant ($p < 0.0001$) strong positive correlation between minerals and in vitro protein digestibility. However, the water absorption index was not significant ($p > 0.05$) except for showing a weak significant ($p < 0.05$) correlation between the water solubility index, calcium and potassium.

Table 6.7. Correlation (Pearson correlation coefficients) matrix between responses

	WAI	WSI	Ca	K	Mg	Fe	IVPD
WAI	1.00000						
WSI	0.32329	1.00000					
	(0.0250)						
Ca	-0.32412	0.65842	1.00000				
	(0.0246)	(<.0001)					
K	-0.36877	0.59095	0.97893	1.00000			
	(0.0099)	(<.0001)	(<.0001)				
Mg	-0.12239	0.70896	0.71763	0.69276	1.00000		
	(0.4073)	(<.0001)	(<.0001)	(<.0001)			
Fe	-0.07029	0.67539	0.60260	0.57892	0.94352	1.00000	
	(0.6350)	(<.0001)	(<.0001)	(<.0001)	(<.0001)		
IVPD	0.03582	0.69631	0.66370	0.65746	0.63981	0.62836	1.00000
	(0.8090)	(<.0001)	(<.0001)	(<.0001)	(<.0001)	(<.0001)	

Values in bracket represent p values.

The optimum extrusion variables and responses are shown in Table 6.8. The optimum extrusion temperature, feed composition and feed moisture were 101^{o}C, 12% and 16%, respectively. Optimum value of WAI, WSI, IVPD, Ca, K, Mg and Fe; 6.245 g, 18.3%, 88.23%, 312.96 mg/100g, 189.14 mg/100g, 120.08 mg/100g and 12.21 mg/100g, respectively. Figure 6.6 shows a deviation of responses from the actual values as influenced by extrusion cooking variables.

Table 6.8. Optimum values for extrusion and response variables

Variables	Lower Limit	Upper Limit	Optimum
A: Extrusion temperature (°C)	80	120	101
B: Feed composition (%)	5	15	12
C: Feed moisture (%)	14	18	16
WAI (g/g)	4.7	7.3	6.25
WSI (%)	11.1	22.75	18.30
IVPD (%)	81.19	89.95	88.23
Ca (mg/100g)	286.83	346.25	312.96
K (mg/100g)	170.21	217.49	189.14
Mg (mg/100g)	95.44	148.7	120.08
Fe (mg/100g)	10.87	26.5	12.21

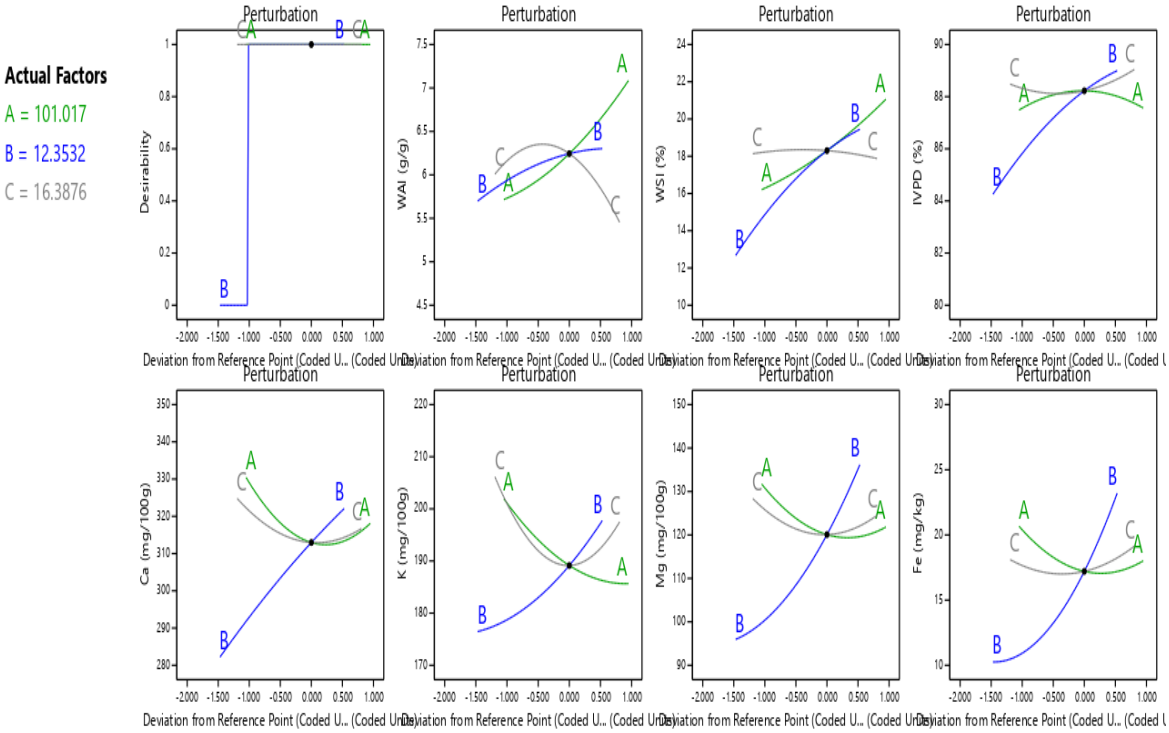


Figure 6.6. Perturbation plots for each response and extrusion variables

6.4 Discussion

The high utilization and consumption levels of maize in developing countries call for the application of different processing techniques and supplementation of other ingredients to help introduce variety as well as improve the functionality and nutrient quality of maize-based foods (Sefa-Dedeh *et al*., 2004).

In this study, extrusion cooking was used to produce a blended instant flour with improved functional properties, in vitro protein digestibility and mineral contents. The Box Behnken design was used to optimize extrusion parameters using RSM. The RSM was found to be an effective tool in explaining the effect of extrusion process variables on functional properties, in vitro protein digestibility and mineral contents of maize-cassava leaf composite instant porridge flour.

The water absorption index depicts the amount of water held by the extruded flour and this is triggered by the gelatinization and melting of molecules (Pasqualone *et al*., 2021). It is attributed to the dispersion of starch in excess water and the dispersion is increased by the degree of starch damage due to gelatinization and extrusion-induced fragmentation (Yagci & Gogus, 2008). In this study, all the linear terms, the quadratic term of feed moisture and interaction terms of extrusion temperature and feed moisture of the regression model of WAI were significant at $p < 0.05$. About 97% of the variation in WAI of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9685. The adequate precision; a signal-to-noise ratio greater than 4 indicates the goodness of fit (Banki *et al*., 2021), in this case, an adequate signal was obtained with a value of 15.44. Also, lack of fit which serves as a tool for measuring the failure of a model is expected not to be significant, in this study a p-value of 0.6721 was obtained. The quadratic model equation (4) shows that the coefficients of extrusion temperature and feed composition are positive, but that of feed moisture is negative. Therefore, an increase in extrusion temperature and feed composition may increase the water absorption index, whereas an increase in feed moisture may decrease the water absorption index of the extruded flour. The response surface plot (Figure 6.1) shows the interactive effect of extrusion temperature and feed composition at constant feed moisture of 16% on the water absorption index. Accordingly, with increasing extrusion temperature up to 120^{o}C and feed composition up to 15%, WAI increased. This might be due to an increase in extrusion temperature may have caused fibre structure to be more unfolded by releasing free hydroxyl groups from cellulose and increasing the ability to bind with water (Tabibloghmany *et al*., 2020). Moreover, the denaturation of proteins occurred during extrusion at higher

temperatures increases the availability of polar groups of amino acids, this enhances hydrophobicity and consequently increases the water absorption capacity of the extruded product (Altan *et al*., 2009). In the study conducted by Lazou and Krokida (2010) it was explained that protein denaturation occurring in high extrusion temperature conditions is the main phenomenon influencing the hydration properties of the extrudate and increases the product's characteristics to have more water affinity.

The water solubility index is often used as an index for the degradation of molecular components, indicating the number of soluble polysaccharides released from fibre components during extrusion (Tabibloghmany *et al*., 2020). In this study, the water solubility index ranged from 9.43 to 22.75%, which is lower than the finding of Seth *et al*. (2015) who reported a water solubility index in the range of 18.13 to 30.39 % for yam-corn-rice based extruded snack. However, the present finding is in agreement with the finding of (Kothakota, 2013) who reported a water solubility index of 11.2 to 20.8% for broken rice flour, dehydrated pineapple waste and red gram powder composite extruded flour, but slightly higher than the finding of Grossmann *et al*. (1998) who reported WSI in the range of 2.94 to 16.04% for malted and extruded corn flour. In the current study, the linear terms of extrusion temperature and feed composition, the interaction terms of extrusion temperature and feed composition as well as interaction terms of feed composition and feed moisture for WSI were significant at $p < 0.05$. Therefore, about 97% of the variation in WSI of the extruded flour could be linked to the extrusion cooking variables based on its R^2 value of 0.9731. Response surface plot (Figure 6.2a) indicates that an increase in extrusion temperature up to 120^{o}C and feed composition up to 15% at constant feed moisture of 16%, increased the water solubility index. This may be due to an increase in the amount of soluble starch because of gelatinization and lateral expansion of starch occurring at a higher temperature during extrusion (Yousf *et al*., 2017). The response surface plot (Figure 6.2b) also explains that an increase in feed composition and feed moisture at constant extrusion temperature increased the water solubility index of the extruded flour. This may be related to the modification of fibre coming from cassava leaf flour during extrusion causing increase in WSI. Similar behaviour was observed by Altan *et al*. (2009) for barley based extrudes from fruit and vegetable by-products.

Proteins are essential macronutrients for the human diet and their digestibility defines the quality (Qi *et al*., 2021). Digestibility specifies the protein quantity absorbed by an organism relative to the consumed amount and depends on the protein structure, previous processing, and the presence of compounds limiting the digestion (Kamani *et al*., 2021). The extrusion

cooking variables in this study contributed 96% of the variations in protein digestibility. The *in vitro* protein digestibility was significantly ($p < 0.05$) improved up to 89.95% when fermented cassava leaf flour is supplemented with maize flour during extrusion. This might be due to the increase in the availability of protein in cassava leaf flour resulting from the fermentation process in addition to the extrusion effects. The increase in protein digestibility with an increase in extrusion temperature and feed composition at constant feed moisture of 16% (Figure 6.3) might be due to the inactivation and/or reduction of anti-nutrients which limits the availability of nutrients (Batista *et al*., 2010; Omosebi *et al*., 2018). In addition, the denaturation of proteins during the extrusion process can expose sites that are susceptible to enzymatic hydrolysis (Bekele *et al*., 2021). A similar trend was observed by Ukeyima *et al*. (2021) who found an improvement of *in vitro* protein digestibility by 92.91% for maize flour, peanut flour and beetroots flour composite extruded flour.

Calcium content significantly ($p < 0.05$) increased from 81.19 to 346.25 mg/100 g when cassava leaf flour proportion in the composite increased. This might be attributed to the higher content of calcium in cassava leaf flour can contribute to calcium in the composite increase. Because cassava leaves are a good source of calcium (0.43 – 1.14 g/100g dry weight) depending on the maturity level, the more mature the leaves, the higher the calcium content (Ravindran & Ravindran, 1988). However, the calcium content of the extruded flour was not affected significantly ($p > 0.05$) by extrusion temperature. Extrusion temperature is not supposed to cause a significant change in the mineral contents of extruded products as they are stable at a higher temperature. This agrees with Nahemiah *et al*. (2018) who stated that minerals are heat stable and unlikely to be lost during extrusion cooking. The extrusion cooking variables contributed about 99% variations in calcium content of which the maximum contribution was by linear terms of feed composition followed by quadratic terms of feed moisture, interaction terms of extrusion temperature and feed composition, and linear terms of feed moisture. The response surface plot (Figure 6.4) also explains that the change in calcium content was much attributed to feeding composition. The calcium content of the extruded flour had a highly significant ($p < 0.0001$) positive correlation with water solubility index, potassium, magnesium, iron and in vitro protein digestibility.

Potassium content significantly ($p < 0.05$) increased from 47.46 to 217.49 mg/100g when cassava leaf proportion increases up to 15% in the composite. The increase in potassium content might be due to the contribution of cassava leaf flour in the composite. Cassava leaves contain 1.38 g/100g to 2.26 g/100g of potassium depending on the level of maturity, the more

mature the leaves the lower amount of potassium (Ravindran & Ravindran, 1988). About 97% of variations in the potassium content of the extruded flour were caused by the extrusion cooking variables. The maximum variation was due to linear terms of feed composition followed by interaction terms of extrusion temperature and feed moisture, quadratic terms of feed moisture, and linear terms of feed moisture. It was also observed in Figure 6.5 that an increase in extrusion temperature and feed composition at constant feed moisture of 16% resulted in increased potassium content.

Magnesium content was significantly ($p < 0.05$) increased from 79.10 to 148.7 mg/100g when cassava leaf proportion increases up to 15% in the composite. This could be attributed to the higher amount of magnesium (0.26 g/100g to 0.37 g/100g) in cassava leaves (Ravindran & Ravindran, 1988). A significant ($p < 0.05$) effect on magnesium content was observed by both linear and quadratic terms of extrusion temperature, feed composition and feed moisture. The quadratic model equation (Eq. 10) shows that magnesium content was negatively affected by linear terms of extrusion temperature feed moisture. The extrusion cooking variables contributed about 97% variations in magnesium content of which the maximum contribution was by linear term of feed composition followed by a linear term of extrusion temperature, quadratic terms of feed composition, extrusion temperature, feed moisture, and linear term of feed moisture. The variation in magnesium content during extrusion might be due to the reduction of anti-nutrients favours releasing of the bound minerals (Adebowale *et al*., 2017).
Iron is vital for erythropoiesis to thrive; it is an integral part of red blood cells (RBCs) which transports oxygen from the lungs to different organs of the body and it is a key constituent of haemoglobin (Suri *et al*., 2020). Iron content significantly ($p < 0.05$) increased from 7.67 to 26.5 mg/100g when cassava leaf proportion increases up to 15% in the composite. Cassava leaves contain a significant amount of iron in the ranges between 15.2 mg/100g to 26.6 mg/100g depending on maturity, the more mature the leaves the higher the iron contents (Ravindran & Ravindran, 1988). It can be observed from the regression model, that the linear effect of extrusion temperature and feed composition, as well as the quadratic effect of extrusion temperature and feed composition on iron content, were found to be significant ($p < 0.05$). The quadratic model equation (Eq. 11) shows that iron content was negatively affected by linear terms of extrusion temperature and interaction terms of extrusion temperature and feed moisture.

6.5 Conclusion

This study has evaluated the functional properties, in vitro protein digestibility and some mineral contents for the production of extruded instant porridge flour. Maize and cassava leaf flour can be combined to produce good-quality instant porridge flour. The results of this study showed that up to 15% cassava leaf flour could be incorporated into maize flour for the production of instant porridge flour with improved functional properties, in vitro protein digestibility and mineral contents. The response surface methodology optimized the response variables. The optimum extrusion variables that could give optimum functional properties, *in vitro* protein digestibility and mineral contents were; extrusion temperature (101^{o}C), feed composition (12%) and feed moisture (16%). The bioavailability of minerals, which is crucial for assessing mineral quality should be explored further.

CHAPTER SEVEN

DESCRIPTIVE SENSORY PROFILING AND CONSUMER ACCEPTABILITY OF MAIZE-CASSAVA LEAF COMPOSITE FLOUR EXTRUDED INSTANT PORRIDGE

Abstract

Integration of processing and food formulation in the development of novel protein-enriched products greatly influences their sensory properties. This study aimed to describe the sensory characteristics and evaluate the acceptability of instant porridge developed from maize flour enriched with fermented cassava leaf flour. The study had 14 samples and each of them was a treatment from a combination of cassava leaf flour substitution level (0- 15%), feed moisture (14-18%) and extrusion temperature (80-120°C). The descriptive panel developed a lexicon of 12 attributes to profile the product's appearance, aroma, flavour, taste and texture. A seven (7) point hedonic scale was used to evaluate the consumer acceptability of instant porridge developed from maize flour enriched with fermented cassava leaf flour. In comparison to the control, the inclusion of cassava leaf flour increased the intensity of bitterness by up to 59% and the number of specks by up to 46%. On the contrary inclusion of cassava leaf flour reduced intensities of brown colour by up to 53%, aroma by up to 46%, sweetness by up to 75%, corny flavour by up to 79%, starchy flavour by up to 73%, stickiness up to 64% and rough texture up to 73%. It was found that there were three major components out of the twelve descriptors, where component one accounted for 42%, component two 14.5% and component three 11.2%, giving a total of 67.7%. The control sample had the highest consumer acceptability score for all the attributes followed by instant porridge developed at a feed composition of 5%, feed moisture of 14% and extrusion temperature of 100^{o}C. This study demonstrates that extensive sensory characterization of maize-cassava leaf instant porridge flour can provide a tool for guiding development, improvement and quality control.

Keywords: Cassava leaf, instant porridge, descriptive sensory analysis, consumer acceptability

7.1 Introduction

Recent years have seen a rise in the use of extrusion technology in the production of instant flour, mostly from foods with a grain foundation. Incorporating extrusion technology with food blending has enormous potential for alleviating food insecurity, a major issue in the developing world (Tao & Li, 2018). There is relatively little information available on the sensory characteristics of the resulting instant flour from the extrusion processing of maize flour that has been enhanced with fermented cassava leaf flour. When creating unique food products, sensory characteristics are a crucial part of quality that are constantly taken into account (Vanga *et al*., 2017; Vivek *et al*., 2020). These characteristics are vital in establishing consumer acceptability and/or preference for the developed product in order to produce an acceptable and useful product with the greatest possible economic efficiency (Singh-Ackbarali & Maharaj, 2014).

Descriptive analysis is one technique that has been widely utilized to describe the sensory characteristics of novel foods. This is because descriptive sensory analysis provides a profile for flavour, texture, look, and taste and records a product's features in terms of its perceived attributes and intensities (Cherdchu *et al*., 2013; Varela & Ares, 2012). As a result, by providing both qualitative and quantitative measures of the intensities of each sensory attribute, the descriptive sensory analysis offers a more in-depth evaluation of the product's sensory profile. A food product's consumer acceptability is crucial since it measures the attitude or preferences of the intended user for particular qualities (Muoki *et al*., 2012). Though there is information on the descriptive sensory profile and consumer acceptability of instant flours developed through extrusion, none of the maize enriched with cassava leaves instant flour is available.

In order to combat malnutrition in underdeveloped nations, cassava leaf flour is becoming more and more popular for adding macro- and micro-nutrients to staple meals like maize (Karri & Nalluri, 2016). However, the presence of a toxic substance called cyanogenic glycoside at 192.47 - 301.04(mg/100 g) HCN is the limiting factor for cassava leaf consumption, though it can be reduced by different processing methods such as fermentation (Hawashi *et al*., 2019; Salata *et al*., 2014). Despite being one of the most popular cereals consumed worldwide, maize is notorious for having low-quality protein. Lysine and tryptophan are insufficient in maize protein, although methionine and cysteine, which contain sulphur, are present in reasonable proportions (Scott *et al.*, 2006). As a result, protein-energy malnutrition is high in areas where maize is the only option as a staple food. Hence, enriching maize flour with other nutrient-rich

ingredients such as cassava leaf flour is one of the possible solutions for tackling malnutrition in developing countries.

Therefore, the purpose of this study was to determine the influence of integrating extrusion processing and composite formulation on descriptive sensory characteristics and consumer acceptability of resultant instant porridge developed from maize flour enriched with fermented cassava leaf flour. It was hypothesized that the enriched instant flour will possess improved sensory properties as compared to the control. The control was instant flour from 100% maize. A trained panel was used to develop a lexicon of attributes to describe, score and compare the intensities among the developed instant porridges, while a semi-trained consumer panel was used to evaluate the acceptability of the instant porridge.

7.2 Materials and methods

7.2.1 Sample collection and preparation

The ingredients used for extrusion cooking were collected and prepared as shown in section 5.2.1.

7.2.2 Extrusion process

The extrusion process was performed using a co-rotating twin screw extruder (model BC-21, No 194 Clextral, 42702 Firminy, France) as shown in section 5.2.3.

7.2.3 Instant porridge preparation

Instant porridge was prepared from 14 runs of extruded flour samples independently (Chanadang & Chambers, 2019). A weighted instant flour (200 g) was mixed with hot water (400 mL) and stirred continuously until uniform mixing was achieved.

7.2.4 Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board of Hawassa University (IRB/269/12) (Appendix 2).

7.2.5 Sensory panel training

Panellists were requested to fill out the consent form before consecutive training and tests. Individual panellist's evaluated porridges developed at different extrusion conditions and generated the attributes describing the differences among the samples. This was followed by group discussions to fine-tune the attributes generated by individuals. A round table discussion was made to compile a consolidated master list of attributes generated by the panellists. The

training was conducted for 7 days in 2 hours sessions each day. The meaning and suitability of the attributes for the developed instant porridge were reviewed and compared to attributes from the literature before final selection. Reference materials that best represented each attribute definition were agreed upon, and scale anchor terms were generated for rating the intensities of the attributes. The left side of the scale corresponded to the absence or low intensity (0) and the right side to the higher intensity (10) of the attribute was used.

7.2.6 Descriptive sensory analysis

A trained descriptive panel (n = 7, 2 males and 5 females, aged between 25 to 40 years) was used to evaluate the sensory attributes of instant porridge developed from extruded flour using maize-cassava leaf composites. This panel were selected from an initial population of 15 people through a series of screening tests and observation of individual performance as stated in section 7.2.5. All 14 samples including the control sample were carried out using the developed lexicon attributes.

7.2.7 Consumer acceptability

A semi-trained consumer panel (n = 30, 11 males and 19 females) including children (under 12 years), were used. The panellists ranked their acceptability of the product attributes using a seven (7) points hedonic scale (7 = “like very much”, 6 = “like moderately”, 5 = “like slightly”, 4 = “neither like nor dislike”, 3 = “dislike slightly”, 2 = “dislike moderately”, 1 = “dislike very much”).

7.2.8 Data analysis

Data analysis was carried out using SAS JMP pro13.0 (Richard Boulton). The data obtained were analyzed for mean differences with analysis of variance (ANOVA) using Tukey’s honest significant difference (HSD) test at a 5% level of significance.

7.3 Results

The descriptive sensory panel generated a lexicon of 12 attributes for the description of maize-cassava leaf composite instant porridge provided in Table 7.1.

Table 7.1. The descriptive sensory attributes lexicon developed by the panel to test the porridge

Attributes	Definition	Rating scale	Reference products
Appearance			
Colour Whitish Dark brown	Degree of whiteness/brownness	0= light 10 = dark brown	White maize porridge
Specks (dark)	The presence of dark-coloured specks visible on the porridge	0 = No specks 10 = Many specks	 
Aroma			
Overall aroma	The overall aroma sensation of the porridge	0= not intense 10= very intense	
Rancid	Aroma associated with oxidized, old oil	aroma 0= not rancid 10= intense rancid	Sunflower oil aged 14 days at 60^{o}C
Taste			
Sweet	The basic taste associated with sucrose	0= not intense sweet 10= very intense sweet	10% sucrose in water
Bitter	Fundamental taste elicited by caffeine	0= not intense bitter 10= very intense bitter	0.15% caffeine in water
After taste			
Umami	Fundamental taste sensation	0= not	20% w/v

	associated with monosodium glutamate	intense umami 10= very intense umami	monosodium glutamate
Astringent	Dry sensation on the tongue and other surfaces of the oral cavity, associated with tannins	0= not astringent 10= very intense astringent	
Flavour			
Corny flavour	The flavour of sweet corn heated enough to caramelize sugars	0= not intense 10= very intense	Sweet corn
Starchy flavour	The flavour of undercooked raw starchy or flour	0= not intense 10= very intense	
Texture			
Stickiness	The extent to which material adhered to fingers during handling	0= not sticky 10= very sticky	Maize porridge Peanut butter and coarse sorghum porridge
Rough texture	The degree to which roughness could be perceived in the mouth while eating	0= smooth 10= rough	

The mean scores of descriptive sensory attributes of extruded maize instant porridge enriched with cassava leaves at different extrusion conditions are shown in Table 7.2 (Appendix 5). Cassava leaves substitution level, feed moisture and extrusion temperature had no significant ($p > 0.05$) effect on rancid aroma and astringent after-taste with a mean score range of 0.57 - 1.57 and 2.21 - 3.86, respectively, but other attributes were significantly ($p < 0.05$) affected. Instant flour of 100% maize only had the highest score for colour at 8.71 ± 0.32, which significantly ($p < 0.05$) decreased with an increase in cassava leaves substitution level and also a decrease in extrusion temperature.

On the contrary, instant flour of 100% maize only had the lowest score for specks at 1.71 ± 0.39, which significantly ($p < 0.05$) increased with an increase in cassava leaves substitution level and also an increase in extrusion temperature. The intensity of overall aroma score was significantly ($p < 0.05$) highest for instant flour of 100% maize at 8.07 ± 0.40 followed by instant flour with 15% cassava leaves, 16% moisture level and extruded at 120°C at 8.00±0.50. However, instant flour with 5% cassava leaves, 16% moisture level and extruded at 80°C scored significantly lowest at 4.36 ± 0.30. Instant flour developed from 100% maize scored significantly ($p < 0.05$) highest for sweetness at 7.57 ± 0.30 and significantly lowest for bitterness at 1.71 ± 0.32, while instant flour with 15% cassava leaf flour, 16% moisture level and extruded at 80°C scored significantly ($p < 0.05$) lowest for sweetness at 1.86±0.21, but significantly highest for bitterness at 4.57 ± 0.52. The intensity of umami flavour was significantly ($p < 0.05$) highest in instant flour with 15% cassava leaves, 14% moisture level and extruded at 100°C at 3.29 ± 0.21 and significantly lowest in instant flour with 5% cassava leaves, 18% moisture level and extruded at 100°C at 1.71 ± 0.32, but for each substitution level, umami decreased with increase in feed moisture. Mean scores for corny flavour, starchy flavour, stickiness and roughness were significantly ($p < 0.05$) highest in instant flour with 100% maize at 6.36 ± 0.43, 5.64 ± 0.71, 4.71 ± 0.43 and 5.71 ± 0.71 while significantly lowest in instant flour with 15% cassava leaves, 16% moisture level and extruded at 120°C at 1.29 ± 0.72, 1.50 ± 0.33, 1.71 ± 0.24 and 1.57 ± 0.28, respectively.

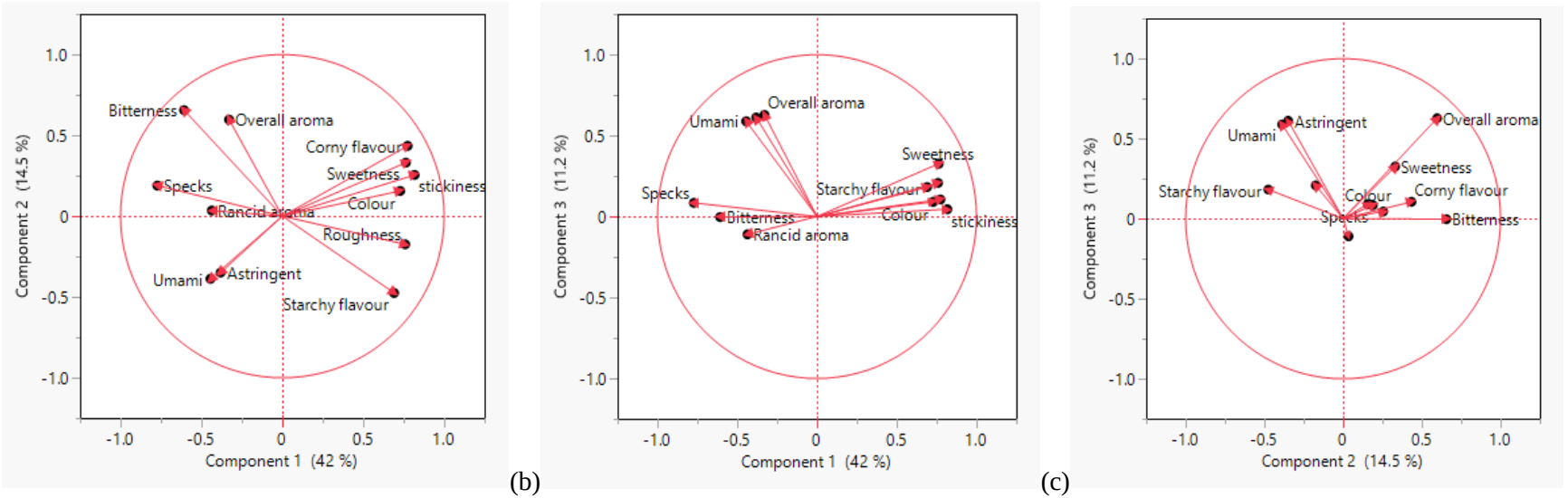
Descriptive sensory attributes coefficients loading matrix to principal components is shown in Table 7.3. It was found that there were three major components out of the twelve descriptors, where component one had eight, component two had one and component three had three. Component one had colour (0.73), specks (-0.77), rancid aroma (-0.44), sweetness (0.76), corny flavour (0.77), starchy flavour (0.69), stickiness (0.81) and rough texture (0.76). Component two had only bitterness (0.66) while component three had overall aroma (0.63), umami (0.59) and astringent after-taste (0.61).

Table 7.3. Loading matrix coefficients of the sensory descriptive attributes to principal components

Sensory attributes	PC 1	PC 2	PC 3
Stickiness	0.817338		
Corny flavour	0.774000		
Sweetness	0.762947		
Rough texture	0.759181		
Colour	0.728203		
Starchy flavour	0.690304		
Bitterness		0.655979	
Overall aroma			0.627853
Astringent after-taste			0.612261
Umami taste			0.588369
Rancid aroma	-0.435792		
Specks	-0.772426		

Key: PC= Principal component

A graphical representation of how descriptive attributes are loaded on principal components and the interaction between the components are shown in Figure 7.1. Component one accounted for 42% of the loading, component two accounted for 14.5% and component three accounted for 11.2%, giving a total of 67.7% accounted for by principal components. Interaction between components one and two shows that sweetness and stickiness have a positive influence on the sensorial properties of instant porridge while umami, rancid aroma and astringent aftertaste have a negative influence.



(a) (b) (c)

Figure 7.1. Pictorial representation of descriptive parameters loading on principal components (a) PC 1 vs PC 2 (b) PC 1 vs PC 3 (c) PC2 vs PC3

The mean consumer sensory acceptability scores of instant porridge developed from maize flour enriched with cassava leaf flour are shown in Table 7.4. The control sample (instant porridge from maize flour only) had significantly ($p < 0.05$) the highest mean score for all the attributes followed by instant porridge developed from feed composition of 5%, feed moisture of 14%, and extrusion temperature of 100^{o}C. However, instant porridge developed from feed composition of 15%, feed moisture of 16% and extrusion temperature of 80^{o}C had significantly ($p < 0.05$) the lowest mean score of overall acceptability.

Table 7.4. Mean scores of consumer acceptability of maize-cassava leaf composite extruded instant porridge

FC (%)	FM (%)	ET (°C)	Appearance	Taste	Aroma	Flavour	Texture	Overall acceptability
0	16	100	5.43 ± 1.14^{a}	5.43 ± 0.97^{a}	5.30 ± 1.12^{a}	5.50 ± 1.07^{a}	5.70 ± 0.99^{a}	5.63 ± 0.96^{a}
5	14	100	4.27 ± 0.83^{b}	4.73 ± 0.64^{ab}	4.67 ± 0.84^{ab}	4.83 ± 0.83^{ab}	4.57 ± 0.97^{b}	4.70 ± 0.79^{b}
5	16	80	4.00 ± 0.87^{bcd}	4.40 ± 0.72^{bc}	4.50 ± 1.01^{abc}	4.47 ± 1.11^{bc}	4.10 ± 1.12^{bc}	4.40 ± 0.86^{bcd}
5	16	120	4.13 ± 0.86^{bc}	4.50 ± 0.78^{bc}	4.47 ± 1.04^{abc}	4.50 ± 1.04^{bc}	4.27 ± 1.11^{bc}	4.50 ± 1.01^{bc}
5	18	100	3.90 ± 0.84^{bcd}	4.20 ± 0.89^{bcde}	4.23 ± 1.10^{bcde}	3.97 ± 1.24^{bcd}	3.87 ± 1.04^{bc}	4.03 ± 0.99^{bcdef}
10	14	80	4.03 ± 0.81^{bcd}	4.27 ± 0.78^{bcd}	4.37 ± 0.99^{abcd}	4.13 ± 1.07^{bcd}	4.27 ± 0.98^{bc}	4.27 ± 0.98^{bcdef}
10	14	120	3.73 ± 0.74^{bcd}	4.07 ± 0.91^{bcde}	4.23 ± 1.07^{bcde}	3.80 ± 1.06^{cd}	4.13 ± 1.07^{bc}	3.90 ± 0.99^{bcdef}
10	16	100	3.60 ± 0.62^{bcd}	3.90 ± 0.84^{cde}	3.97 ± 1.03^{bcde}	4.00 ± 1.14^{bcd}	4.03 ± 1.09^{bc}	3.87 ± 1.07^{bcdef}
10	18	80	3.30 ± 0.70^{d}	3.50 ± 0.94^{de}	3.43 ± 1.00^{de}	3.23 ± 1.01^{d}	3.53 ± 0.86^{c}	3.47 ± 0.94^{ef}
10	18	120	3.33 ± 0.84^{cd}	3.43 ± 1.04^{e}	3.40 ± 1.00^{e}	3.40 ± 1.04^{d}	3.47 ± 0.94^{c}	3.53 ± 1.04^{def}
15	14	100	3.37 ± 0.85^{cd}	3.50 ± 1.07^{de}	3.43 ± 1.01^{de}	3.43 ± 0.97^{d}	3.50 ± 0.90^{c}	3.47 ± 1.07^{ef}
15	16	80	3.27 ± 1.14^{d}	3.43 ± 1.10^{e}	3.30 ± 1.18^{e}	3.37 ± 1.09^{d}	3.50 ± 1.11^{c}	3.37 ± 1.16^{f}
15	16	120	3.73 ± 1.14^{bcd}	4.20 ± 1.13^{bcde}	4.13 ± 1.19^{bcde}	4.17 ± 1.18^{bcd}	4.23 ± 1.28^{bc}	4.30 ± 1.26^{bcde}
15	18	100	3.70 ± 1.44^{bcd}	3.90 ± 1.32^{cde}	3.63 ± 1.38^{cde}	3.97 ± 1.25^{bcd}	4.10 ± 1.32^{bc}	3.73 ± 1.23^{cdef}

Values are means ± standard deviation (n =30), and values with different superscript letters in the column are significant at $p < 0.05$. A 7 point hedonic scale was used (7= Like very much; 6= Like moderately; 5= Like slightly; 4= Neither like nor dislike; 3= Dislike slightly; 2= Dislike moderately; 1= Dislike very much)

7.4 Discussion

All the 12 attributes that were generated by the panel as shown in the lexicon of Table 7.1, significantly differentiated the instant porridges by bringing out the sensory profile of the products. For the development of maize-cassava leaf-enriched instant flour, quality control, product enhancement, and tracking changes over the course of a product's shelf life, this established sensory lexicon can be used as an efficient communication and guidance tool (Suwonsichon, 2019).

A food's appearance greatly depends on its colour. Many people frequently automatically connect colour to other aspects of food quality (Cardona *et al*., 2020). In this study, the substitution of cassava leaves for maize significantly reduced the level of brownness of instant flour, where the control sample had the highest score. On the contrary, the number of specks significantly increased with an increase in cassava leaf substitution level and increase in temperature within each level, where the instant flour from 100% maize had the lowest score for the number of specks while instant floor from composite with 15% cassava leaves and extruded at 120°C. This implies that cassava leaf flour contributed directly to the presence of the specks and also extrusion temperature made the specks to be more visible. Therefore, colour and specks, which constitute the appearance of the instant flour, were a result of both levels of formulation and temperatures of extrusion. According to Das *et al*. (2021), certain ingredients that possess health benefits used to enrich others can affect the overall appearance of a product and it is important to prime consumers and develop expectations before consumption.

The overall aroma of the instant flour was also influenced by both extrusion temperature and the composite ratio. Instant flour from 100% maize had the highest aroma intensity, which was not significantly different from instant flour with 15% cassava leaves, 16% moisture level and extruded at 120°C. According to studies, the chemicals that give extruded maize products their scent are linked to higher levels of the Maillard reaction (Smith & Peterson, 2020). The aroma intensity of extruded products may be increased by the fortification of the material to be extruded with reducing sugars and amino acids, which are the most important precursors of Maillard reactions (Mansour *et al*., 2001). Apart from the overall aroma, the rancid aroma was not significantly affected. This could be linked to the low-fat content of the ingredients used in making formulas.

In terms of taste, instant porridge from 100% maize had significantly the highest intensity of sweetness and lowest intensity of bitterness which was contrary to instant flour from 15%

cassava leaves, 16% moisture level and extruded at 80°C had the highest bitterness and lowest sweetness. The increase in the bitterness, which directly proportionally reduced sweetness could be linked to the substitution level of cassava leaves in the composites. Cassava leaves are known to contain phytochemicals (Linn & Myint, 2018), whose concentration increases with an increase in substitution levels.

Apart from sweetness and bitterness, umami taste intensity was significantly lowest in instant flour with 5% cassava leaves and highest in flour with 15% cassava, though it decreased with an increase in moisture within each substitution level. The "meaty" taste is the simplest way to describe umami flavor (Dermiki *et al*., 2013). Umami is a flavour that predominates in foods containing L-glutamate, such as chicken broth, meat extracts, and aged cheese, according to Vilela *et al*. (2016). L-glutamate, a relatively ubiquitous amino acid, thereby directs the ingestion of peptides and proteins, from which it is released via proteolysis (curing and decay). One of the main amino acids in corn is glutamate (Seebauer *et al*., 2004), and this could be the reason for the presence of this attribute in the products. However, the intensity of astringent after-taste was not significantly influenced by blending and extrusion temperatures. Phytochemicals such phenolic acids (ferulic acid, coumaric acid, and syringic acid), carotenoids, and flavonoids, as well as other compounds, are typically responsible for the astringent flavour found in items made from maize (anthocyanins) (Siyuan *et al*., 2018).

Flavour (corny and starchy) and textural (stickiness and roughness) attributes of the instant flavour intensity significantly decreased with an increase in substitution level of cassava leaf in the composites, where the flour from 100% maize had the highest intensity. These attributes are dependent on carbohydrate levels in the maize, which reduces upon blending, whereas cassava leaf flour inhibits reactions that favour flavour formation. According to Onyeoziri *et al*. (2021), the creation of pyrazines, a byproduct of Maillard reactions, and maybe other interactions between Maillard reactions and lipid degradation products, are likely responsible for some of the flavour development in extruded products. Reduced carbohydrate content, which is typically involved in dextrinization events during extrusion that give the goods stickiness, may have contributed to the decrease in stickiness (Gat & Ananthanarayan, 2015).

An example of a multivariate statistical analysis is principal component analysis (PCA), which is used to examine the correlation in a set of measurements of a certain number of variables for a predetermined number of assessors (Calviño *et al*., 1996). In this study, principal component analysis was performed to compare and explain the effect of maize enrichment with fermented cassava leaf and extrusion processing on instant porridge flour. There were three principal

components which described 67.7% of the total variance. Principal component one was highly correlated with stickiness, corny flavour, sweetness, rough texture, colour, starchy flavour and dark specks. This indicates that these attributes should be given priority during the development of maize-cassava leaf instant porridge flour. Component two was correlated with bitterness and this could be the second priority. Component three was correlated with overall aroma, astringent and umami after-tastes, and this could be considered a third priority during the development of maize-cassava leaf instant porridge flour.

7.5 Conclusion

The results from this study identified the effects of the extrusion process and feed composition used on the sensory properties of the instant porridge. The combination of formulation and extrusion temperature significantly influenced both descriptive sensory characteristics and consumer acceptability of instant porridge developed from maize enriched with fermented cassava leaves.

CHAPTER EIGHT

EFFECT OF PACKAGING MATERIALS ON PHYSICOCHEMICAL, MICROBIAL, AND DESCRIPTIVE SENSORY PROPERTIES OF MAIZE-CASSAVA LEAF INSTANT PORRIDGE FLOUR AT AMBIENT STORAGE CONDITIONS

Abstract

The inherent properties and environmental conditions under which a given food product is stored have a direct bearing on the quality attributes over a particular storage period. The purpose of this study was to determine the influence of packaging material on microbial, physicochemical, and sensory attributes of instant maize porridge flour enriched with cassava leaves during real-time storage conditions. Irrespective of the packaging type used, the study established that the total viable count, yeasts and moulds, moisture, water activity, and titratable acidity significantly increased with the storage period of the flour. At the end of the storage period, the increase of moisture, water activity, titratable acidity, total viable counts, and yeast and moulds was higher in flour that was stored in low-density polyethylene than in aluminium laminated paper bags by 5.2%, 9.8%, 13.6%, 17.8%, and 56.3%, respectively, Similarly, over the storage period, brownness, the number of specks, bitterness and rancid flavour intensities of the instant flour increased while overall aroma, sweetness, stickiness, and roughness decreased. Paper packaging material had a higher score for desirable attributes such as overall aroma and sweetness while low-density polyethylene had a higher score for undesirable attributes such as bitterness and rancid off-flavours. The low performance of low-density polyethylene bags could be linked to higher permeability to oxygen and moisture from the environment to the product than paper bags. The findings of this study show that paper bag packaging is better than low-density polyethylene in preserving the quality characteristics of instant flours.

Keywords: shelf life; maize-cassava leaf instant flour; physicochemical properties; descriptive sensory analysis; microbial properties

8.1 Introduction

According to Giménez *et al.* (2012) and IFT (1993), the shelf life of a food product is the amount of time that it has the desired sensory, chemical, physical, microbiological, and functional properties while still being safe for human consumption. Comparing the performance of various qualities simultaneously under fictitious but realistic storage situations can help in the selection of an indication that denotes the end of shelf life for any given food product (Corradini, 2018; Fikry *et al*., 2020). Another workable strategy for achieving a more accurate depiction of a product's actual shelf life is to combine multiple limiting criteria into a single index (Corradini, 2018). This suggests that a food product's sensory, chemical, physical, microbiological, and functional characteristics might be examined collectively to determine a more accurate shelf life. On the shelf-life examination of the instant porridge flour made from maize enhanced with cassava leaf flour, however, there is very little information currently accessible.

The shelf life of a food product after it has been developed is influenced by both intrinsic and external variables (Symmank, 2019). The initial quality of the food, which results from the use of high-quality components and low microbial loads, the inherent characteristics of the product, such as its level of perishability, and its formulation, which includes the use of preservatives, are examples of intrinsic elements (Awulachew, 2021). Extrinsic factors include consumer handling, transportation and storage conditions like high temperatures and relative humidity that can shorten shelf life, processing techniques like the intensity of heat treatment and pressure, barrier properties of the packaging, and processing methods like degree of heat treatment and pressure (Moschopoulou *et al.,* 2019). To prevent deterioration processes, effectively communicate a product's condition, and reduce food waste, distribution/storage conditions must be monitored and modelled properly (Annese *et al.,* 2015). In terms of mathematics, shelf-life is a function of time, environmental variables, and food product sensitivity, which directly affects both safety and quality elements, resulting in an acceptable shelf life (Taoukis *et al*., 1997).

Because it shields the product from oxygen, prevents moisture loss or gain, safeguards it from microbe contamination, and makes handling easier, food packaging has become crucial (Baranwal *et al*., 2014). Effective packaging serves two functions, primarily technical and presentational. Technical features of packaging work to better protect food during storage from all risks in order to reduce food spoiling (Konstantoglou *et al*., 2020). Hazards that result in physical, chemical, and biological changes that cause product deterioration may be connected

to food spoilage, and these changes may eventually jeopardize the product's nutritional, microbiological, and sensory quality (Abraha *et al*., 2018). Most perishable foods experience quality degradation due to the growth of spoilage micro-organisms, and the level of microbial growth also known as the spoilage level is frequently connected with sensory acceptability and used as a marker. Mesophilic microbe counts greater than 10^6 CFU/g or ml have been linked to the expiration of perishable products' shelf lives (Corradini, 2018).

It can often be challenging to pinpoint a single crucial characteristic that can clearly indicate when a product has reached the end of its useful life. Product-, consumer-, and market-specific characteristics determine when a product's shelf life ends (Manzocco, 2016; Sciortino *et al.* 2016). Additionally, several criteria are employed to pick the threshold level that denotes the end of the shelf life as well as the limiting quality attribute. Before any health risk to customers is realized, sensory features change in a lot of items (Giménez, 2012). When estimating the shelf-life of food goods, analytical and sensory methods can both be incorporated to support one another (Wibowo *et al.,* 2018). The shift in sensory qualities (such as odour, taste, look, and texture) described by descriptive analysis with expert panellists can point to consumer rejection. Although acceptance testing or the use of consumer panels for acceptability can be more accurate, these procedures are rarely used since they require a large number of panellists, which makes the process more expensive and time-consuming (Corradini, 2018).

Since there are not enough studies about the shelf-life analysis of instant porridge flour developed from maize enriched with cassava leaves, this study aimed to determine the influence of packaging material and storage time on the physicochemical, microbial, and sensory properties of this product. It was hypothesized that packaging material will not have a significant effect on the quality attributes considered in this study to monitor the shelf life of the developed product over 90 days. The control for the study was an instant porridge flour made from 100% maize.

8.2 Materials and methods

8.2.1 Sample preparation

Maize-cassava leaf composite flour was extruded at an extrusion temperature of 100^{o}C, feed composition of 12%, and feed moisture of 14% based on previous optimizations done. The extrusion process was performed using a co-rotating twin screw extruder (model BC-21, No 194 Clextral, 42702 Firminy, France). The control (maize flour) was extruded in the same procedure without the addition of cassava leaf flour.

8.2.2 Packaging treatment

The developed extruded samples (100 g) were packed using two different packaging materials, low-density polyethylene (0.15 mm) and aluminium laminated paper bags and kept under ambient storage conditions for up to 90 days. The stored samples were evaluated for their physicochemical properties, microbial quality, and descriptive sensory attributes at 30 days intervals for 90 days.

8.2.3 Determination of physicochemical properties

Moisture was determined according to AOAC (2005) method (925.09). A sample was dried in an oven at 105^{o}C. Then the moisture content was analyzed by subtracting the final moisture content from the initial and dividing it by the initial moisture content. Water activity during the storage period was estimated by using a water activity meter (Hygrolab, Retrogenic Company) (Nkubana & Dusabumuremyi, 2019). About 2 g of ground sample was put into the instrument and a_w was measured automatically. The pH of the extruded sample during storage was measured using a pre-calibrated pH meter. Ten gram (10 g) sample was suspended in 90 ml of sterile distilled water and homogenized. The mixtures were allowed to stand for 30 minutes before being filtered. Then pH values of the filtrates were determined by a combined glass electrode probe and a pH meter. To determine titratable acidity (TTA), a 10 g sample was blended with 100 mL of acetone: water (5:95 v/v) under constant agitation and 2-3 drops of phenolphthalein were added as an indicator. Then, it was titrated with 0.1 N NaOH until it turned to permanent pale pink colour, and the calculation of TTA was done.

8.2.4 Determination of microbial load

The media was prepared by weighing the manufacturer's specified quantity using a vacuum weighing balance and dissolving it in distilled water. This was then followed by sterilization at 121^{o}C for 15 minutes using an autoclave, cooling to about 40^{o}C. Plate count agar was used to determine total viable counts (TVC), whereas MacConkey and potato dextrose agars were used for coliforms, and yeast and moulds, respectively. The diluent was prepared by dissolving 20 g in 1 litre of distilled water, followed by pipetting 9 ml into the bottles. The diluent was then sterilized by autoclaving at 121^{o}C for 15 minutes. The samples were shaken to ensure homogeneity before pipetting 1 ml into the first bottle of serial dilution (10^{-1}). The bottle was then shaken and continuous pipetting was done up to six dilutions (10^{-6}). Using a micropipette, 1 ml of each sample was transferred to the labelled plates corresponding to dilution (1-6) in duplicates before pouring the respective media into the plates. Upon setting of the media, plates

for TVC and coliforms were incubated at 37ºC in the incubator for 48 and 24 hours, respectively. Yeasts and moulds were incubated at room temperature in a sterile environment for 72 hours. The colonies were counted using a digital colony counter and recorded.

8.2.5 Descriptive sensory characteristics

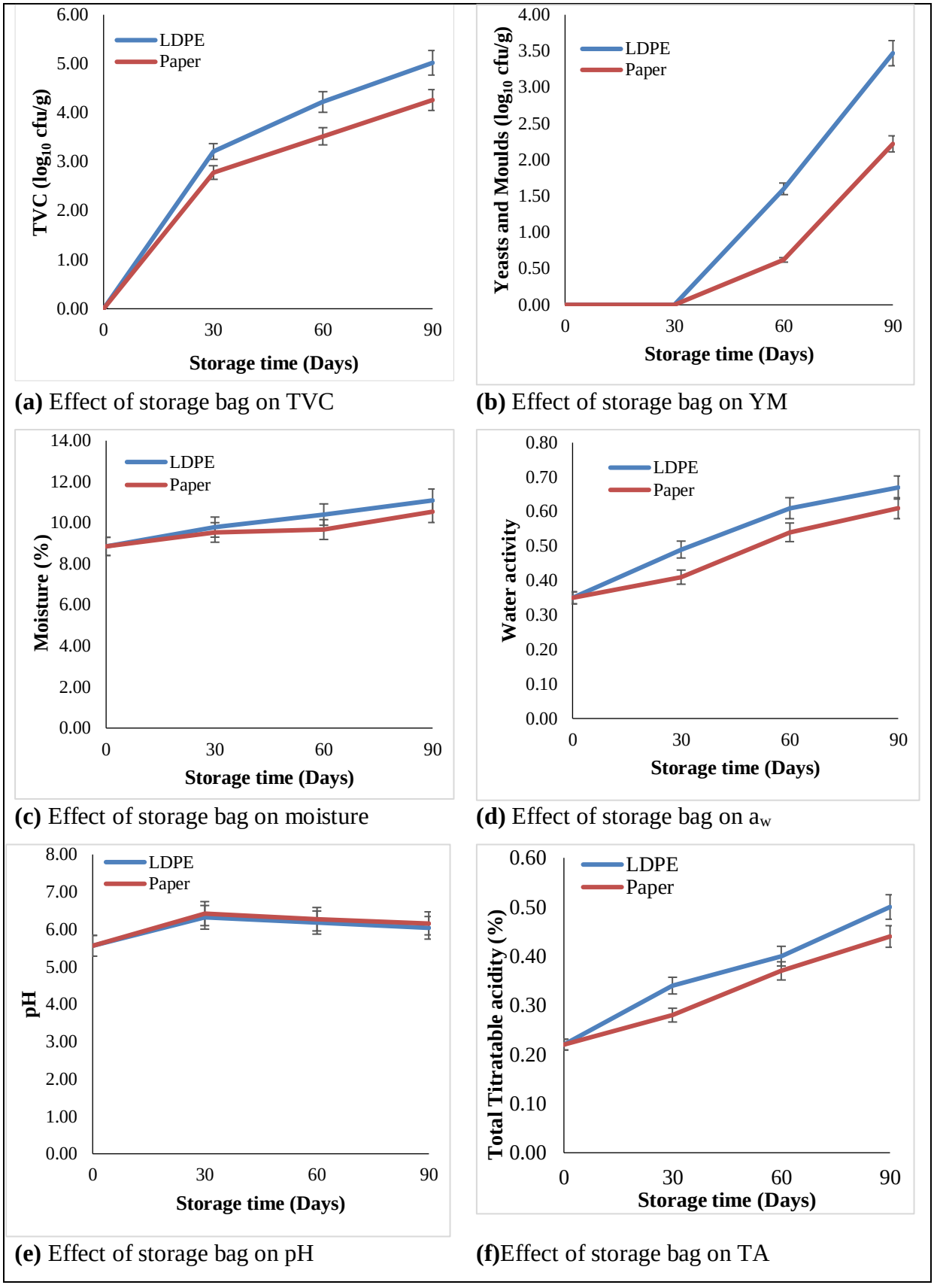
A trained descriptive panel (n = 7) was used to evaluate the sensory attributes of instant porridge developed from extruded flour using composite flour of maize and cassava leaf stored for 90 days in 30 days intervals.

8.2.6 Data analysis

The microbial counts were first transformed to $\log_{10}$ before analysis. All data obtained were analyzed using SAS software version 9.4. Analysis of variance (ANOVA) was used to test the experimental hypotheses and *posthoc* analysis was done using Tukey's honest significant difference (HSD) test at a 95% level of significance. The principal component analysis was carried out using SAS JMP pro 16 (Richard Boulton, USA).

8.3 Results

The effect of the type of packaging material used for the storage of instant porridge flour on microbial and physicochemical properties is shown in Figure 8.1. During the storage period, TVC, YM, moisture, water activity and titratable acidity of the instant flour significantly ($p < 0.05$) increased, where the increase in flour stored in LDPE bags was higher than flour stored in paper bags. There was no significant ($p > 0.05$) change in the pH of the flour during storage and between the two storage bags. During the storage period, both storage bags did not have any coliform growth.



(a) Effect of storage bag on TVC

(b) Effect of storage bag on YM

(c) Effect of storage bag on moisture

(d) Effect of storage bag on a_w

(e) Effect of storage bag on pH

(f) Effect of storage bag on TA

Figure 8.1. The overall effect of the type of packaging material on microbial and physicochemical properties of instant porridge flour

Key: TVC= Total viable count; YM= Yeasts and Moulds; TA= Titratable acidity

The influence of packaging material type on microbial growth of maize and cassava-leaf enriched instant flour during storage is shown in Table 8.1. During the storage period, there was no growth of coliforms in both packaging materials for maize and cassava-leaf enriched instant flours. TVC and YM count significantly increased in maize and cassava-leaf enriched instant flours stored in both packaging materials during the storage period. However, TVC and YM growth was higher in both maize and cassava-leaf enriched instant flours stored in LDPE in comparison to instant flours that were stored in paper bags.

Table 8.1. Influence of packaging material on microbial growth of instant flour during storage

Package	**Duration**	**TVC**		**YM**	
		Control	CLEF	Control	CLEF
LDPE	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	30	3.86 ± 0.59^{a}	2.55 ± 0.33^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	60	4.76 ± 0.54^{a}	3.68 ± 0.30^{ab}	2.11 ± 0.48^{a}	1.09 ± 0.47^{b}
	90	5.25 ± 0.30^{a}	4.78 ± 0.21^{a}	3.39 ± 0.50^{a}	3.56 ± 0.39^{a}
Paper	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	30	3.32 ± 0.59^{a}	2.24 ± 0.39^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	60	3.71 ± 0.43^{a}	3.32 ± 0.34^{a}	1.24 ± 0.55^{ab}	0.00 ± 0.00^{b}
	90	4.36 ± 0.48^{a}	4.15 ± 0.15^{a}	2.34 ± 0.41^{a}	2.10 ± 0.40^{a}

Key: LDPE= Low-Density Polyethylene; TVC=Total Viable Count; YM= Yeasts and Moulds; CLEF= Cassava-Leaf Enriched Flour; Means with the same letter along the columns within each packaging material are not significantly different.

The influence of packaging material on the physicochemical properties of maize and cassava-leaf enriched instant flour during storage is shown in Table 8.2. Moisture content, water activity, and titratable acidity of both maize-only and cassava-leaf enriched instant flour significantly ($p < 0.05$) increased during the storage period, while the pH of only cassava-leaf enriched instant flour that showed a significant ($p < 0.05$) increase during the same period. Increase in moisture content and titratable acidity, and also the decrease in pH was higher in cassava-leaf enriched flour than maize flour only during the storage period. On the contrary, water activity increase was higher in maize instant flour than in cassava-leaf enriched flour during storage. On other hand, moisture, water activity, and titratable acidity increase for both maize only and cassava-leaf enriched instant flours was higher in LDPE packaging material than in paper bags during the storage period.

Table 8.2. Influence of packaging material on physicochemical properties of maize and cassava-leaf enriched instant flour during storage

Package	Duration	Moisture		a_w		pH		TA	
		Control	CLEF	Control	CLEF	Control	CLEF	Control	CLEF
LDPE	0	8.24±0.13^{c}	9.46±0.06^{d}	0.32±0.05^{c}	0.38±0.01^{d}	6.61±1.98^{a}	6.51±0.06^{a}	0.17±0.01^{c}	0.27±0.02^{c}
	30	9.48±0.03^{b}	10.09±0.06^{c}	0.50±0.01^{b}	0.48±0.02^{c}	6.47±0.01^{a}	6.16±0.03^{b}	0.31±0.02^{b}	0.37±0.02^{b}
	60	9.82±0.05^{b}	10.98±0.04^{b}	0.64±0.02^{a}	0.57±0.01^{b}	6.27±0.01^{a}	6.08±0.02^{bc}	0.37±0.01^{ab}	0.42±0.01^{b}
	90	10.27±0.03^{a}	11.90±0.08^{a}	0.71±0.01^{a}	0.64±0.01^{a}	6.11±0.01^{a}	5.96±0.06^{c}	0.43±0.01^{a}	0.57±0.01^{a}
Paper	0	8.24±0.13^{c}	9.46±0.06^{d}	0.32±0.05^{b}	0.38±0.01^{d}	6.61±1.98^{a}	6.51±0.06^{a}	0.17±0.01^{b}	0.27±0.02^{c}
	30	9.19±0.11^{b}	9.87±0.09^{c}	0.40±0.03^{b}	0.42±0.01^{c}	6.56±0.01^{a}	6.28±0.03^{b}	0.23±0.02^{b}	0.33±0.02^{c}
	60	9.12±0.02^{b}	10.22±0.02^{b}	0.58±0.02^{a}	0.50±0.01^{b}	6.33±0.01^{a}	6.21±0.02^{b}	0.34±0.01^{a}	0.41±0.01^{b}
	90	9.66±0.03^{a}	11.42±0.04^{a}	0.63±0.01^{a}	0.59±0.01^{a}	6.19±0.04^{a}	6.13±0.05^{b}	0.40±0.01^{a}	0.49±0.02^{a}

Key: LDPE= Low Density Polyethylene; a_w =Water activity; TA= Titratable Acidity; CLEF= Cassava-Leaf Enriched Flour; Means with the same letter along the columns within each packaging materials are not significantly different

Mean scores for the sensory descriptive attributes of maize only and cassava-leaf enriched instant porridge from flours stored in different packaging materials are shown in Table 8.3 (Appendix 5). Irrespective of the packaging material used, the colour brownness, number of specks, rancid intensity, bitterness intensity, and umami taste intensity of the porridge significantly ($p < 0.05$) increased over the storage period. On the contrary, overall aroma, astringent aftertaste, corny flavour, starchy flavour, stickiness, and rough texture intensities of both maize-only and cassava-leaf enriched instant flour significantly ($p < 0.05$) decreased over the storage period. Sweetness intensity significantly decreased in maize-only flour but remained unchanged in cassava-leaf enriched flour over the storage period. Also, porridge made from flour stored in LDPE had higher scores of colour brownness, number of specks, rancid flavour, bitterness, umami flavour, starchy flavour and rough texture in both maize-only and cassava-leaf enriched porridge as compared to the same flours stored in paper bags.

Aroma and sweetness intensities decreased higher in porridge from flour stored in LDPE than in paper bags. There was no significant difference between the two packaging materials for astringency intensity in maize-only flour and corny flavour in cassava-leaf enriched flour over the storage period. Stickiness scores of porridges were higher in flour stored in LDPE for maize-only flour than in paper bags but for cassava-leaf enriched flour scores were higher for flour stored in paper bags than in LDPE over the storage period.

Descriptive sensory attributes coefficients loading matrix to principal components for paper packaged instant flour are shown in Table 8.4. It was found that there were three major components out of the twelve descriptors for both cassava leaf enriched instant flour and control sample along the storage time. Component one had eight, component two had two and component three had one for cassava leaf enriched instant flour, while component one had nine, component two had three and component three had two attributes for the control sample.

Table 8.4. Loading matrix coefficients for attributes on PCA for cassava leaf enriched and maize instant flour packed in paper bags

Attributes	**CLEF**			**Control**		
	Prin1	**Prin2**	**Prin3**	**Prin1**	**Prin2**	**Prin3**
Colour			0.56034		0.75872	
Specks	0.71298			-0.89948		
Overall aroma	-0.62935			0.91728		
Rancid aroma	0.84198			-0.77458		
Sweetness				0.73901		
Bitterness	0.71680				0.61156	
Umami	0.80446			-0.58637		
Astringent	-0.70568				0.69269	0.58278
Corny flavour		-0.77123		0.88900		
Starchy flavour		0.63570		0.68991		-0.42552
Stickiness	-0.66871			0.84474		
Rough texture	-0.64710			0.83044		

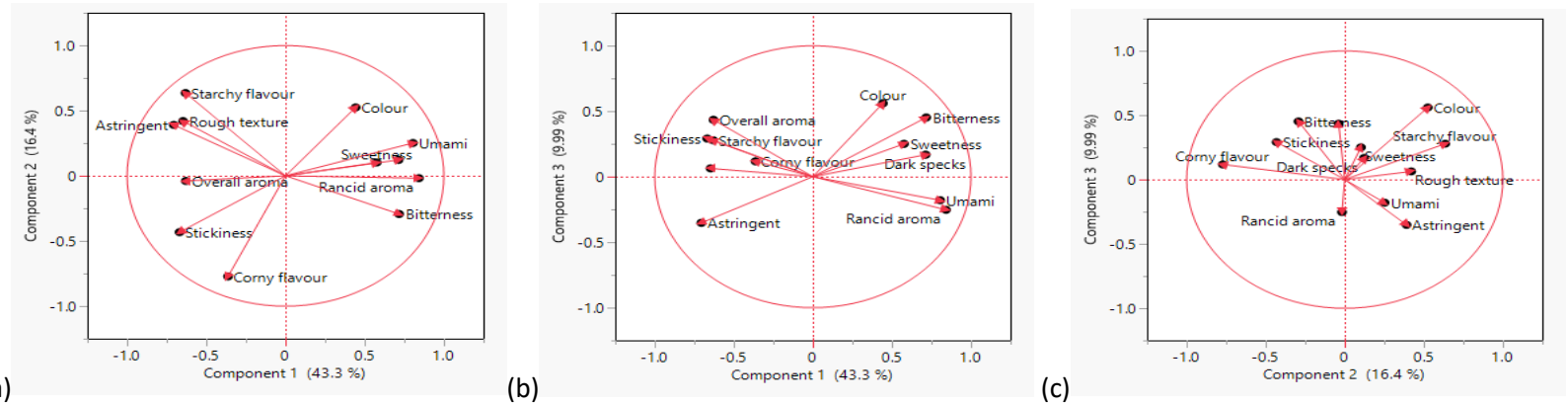
Descriptive sensory attributes coefficients loading matrix to principal components for low-density polyethylene packaged instant flour are shown in Table 8.5. Three major components

were identified out of twelve attributes. Component one had ten, component two had two and component three had only one attribute for cassava leaf enriched and maize only instant flour.

Table 8.5. Loading matrix coefficients for attributes on PCA for cassava leaf enriched and maize instant flour packaged in low-density polyethylene bags

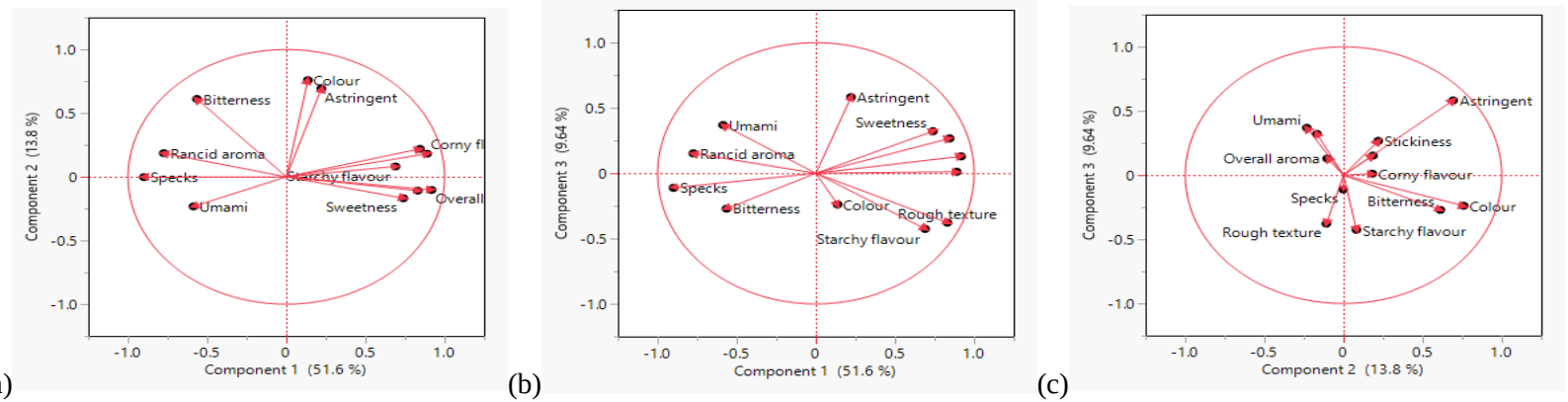
Attributes	**CLEF**			**Control**		
	Prin1	**Prin2**	**Prin3**	**Prin1**	**Prin2**	**Prin3**
Colour	0.76917				0.57831	
Specks	0.92439			-0.92483		
Overall aroma	-0.84623			0.95129		
Rancid aroma	0.91414			-0.91463		
Sweetness	0.43075		0.81012	0.91096		
Bitterness	0.90046			-0.89969		
Umami	0.95357			-0.68716		-0.49918
Astringent	-0.82720				0.86275	
Corny flavour		-0.78533		0.94305		
Starchy flavour		0.74629		0.86552		
Stickiness	-0.74416			0.88232		
Rough texture	-0.65355			0.86728		

A graphical representation of how descriptive attributes are loaded on principal components and the interaction between the components are shown in Figures 8.2 to 8.5. Component one accounted for 43.3% of the loading, component two accounted for 16.4% and component three accounted for 9.99%, giving a total of 69.69% for cassava leaf enriched instant flour packaged with paper bags by principal components (Figure 8.2). However, component one accounted for 51.6%, component two accounted for 13.8% and component three accounted for 9.64% and giving a total of 75.04% for the control sample packaged in paper bags (Figure 8.3). Cassava leaf enriched instant flour packaged with low-density polyethylene bags had also three major principal components, whereby component one accounted for 58.3%, component two accounted for 16.1% and component three accounted for 8.07% and giving a total of 82.47%, while control sample packaged in LDPE, component one accounted for 67.7%, component two 11.6% and component three 7.28%, giving a total of 86.58%.



(a) (b) (c)

Figure 8.2. Pictorial representation of descriptive parameters loading on principal components for paper bags packaged cassava leaf enriched instant flour for 90 days storage (a) PC 1 vs PC 2 (b) PC 1 vs PC 3 (c) PC 2 vs PC 3



(a) (b) (c)

Figure 8.3. Pictorial representation of descriptive parameters loading on principal components for aluminum laminated paper bags packaged control sample for 90 days storage (a) PC 1 vs PC 2 (b) PC 1 vs PC 3 (c) PC 2 vs PC 3

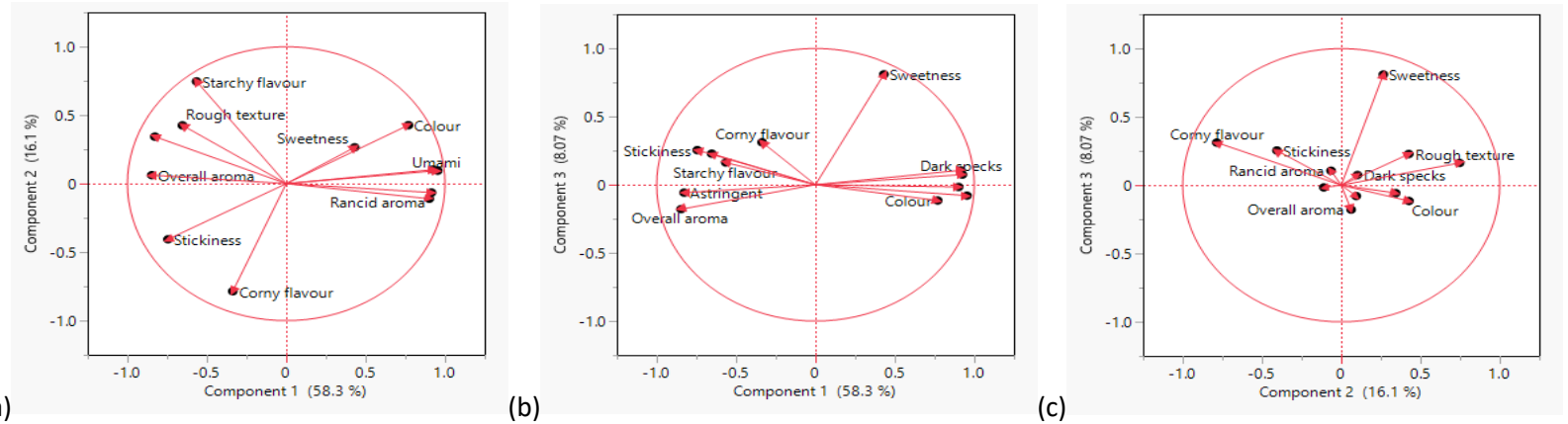


Figure 8.4. Pictorial representation of descriptive parameters loading on principal components for LDPE bags packaged cassava leaf enriched instant flour for 90 days storage (a) PC 1 vs PC 2 (b) PC 1 vs PC 3 (c) PC 2 vs PC 3

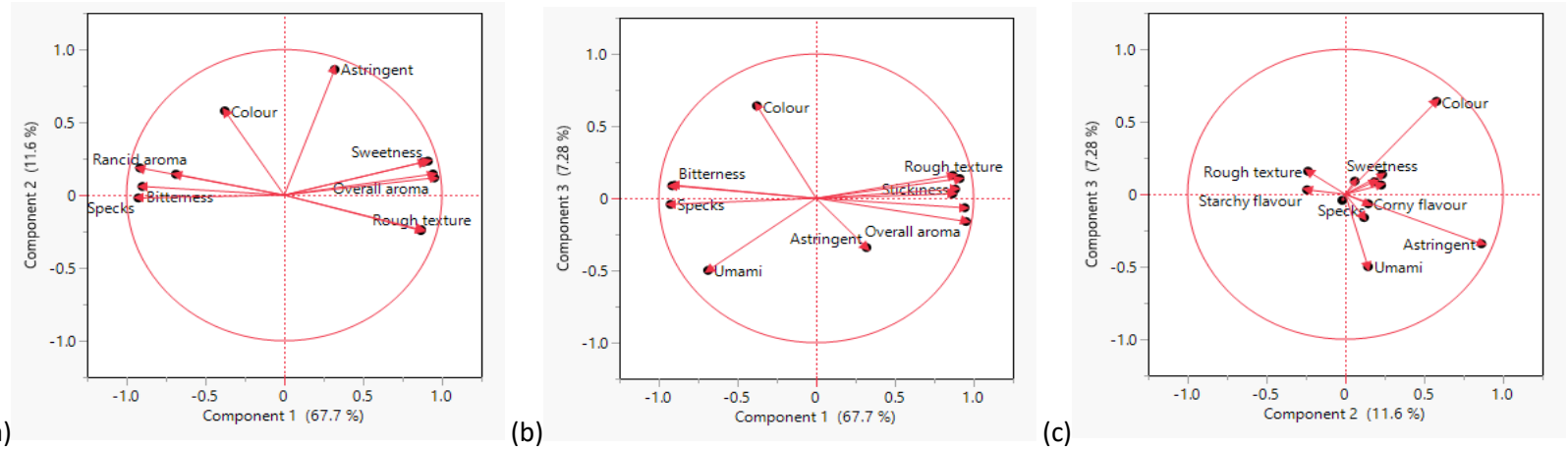


Figure 8.5. Pictorial representation of descriptive parameters loading on principal components for LDPE bags packaged control sample for 90 days storage (a) PC 1 vs PC 2 (b) PC 1 vs PC 3 (c) PC 2 vs PC 3

8.4 Discussion

As indicated in Figure 8.1, microbial and physicochemical properties of instant flour increased significantly ($p < 0.05$) during the storage period except for pH. It was noted that the increase was higher in flour stored in LDPE bags when compared to flour stored in paper bags. At the end of the storage period of 90 days, the increase of moisture, a_w, titratable acidity, TVC and YM were higher in flours that were stored in LDPE bags than in paper bags by 5.2%, 9.8%, 13.6%, 17.8% and 56.3%, respectively. The observed increase in moisture of the instant flour could be attributed to the hygroscopic nature of the products, the storage environment (temperature, relative humidity) and most importantly the nature of the packaging material (Jan *et al.*, 2017). In this case, LDPE could be more permeable than paper bags, which allowed more moisture from the environment to get to the product with time. Consequently, an increased product moisture level caused an increase in the water activity and microbial growth. Based on the observation by Zhang *et al*. (2021) that water activity denotes the potential for shelf stability of the food product, which implies that the stability of flour stored in LDPE bags has a lower stability than when stored in the paper.

Studies have shown that packaging materials with low oxygen transmission rate and low water vapour transmission rate protect the product from oxidation and moisture gain or loss (Jafarzadeh & Jafari, 2020; Sady *et al*., 2021). In this case, the reason that makes flour stored in LDPE has lower stability than in paper bags could be that LDPE had higher oxygen and water transmission rate. The moisture content of the flour is very important regarding its shelf life, lower the flour moisture, the better its storage stability (Liu *et al*., 2019). Moisture is also of great importance for the safe storage of cereals and their products regarding microorganisms, particularly certain species of fungi. At lower moisture fungi will not grow but at about 14% or slightly above, fungal growth takes place leading to physicochemical, nutritional and sensory property losses (Los *et al.*, 2018).

Type of packaging material used in this study significantly ($p < 0.05$) influenced the growth rate of TVC and YM in instant flours as shown in Table 8.1. The cassava-leaf enriched instant flour that was stored in LDPE had 15.2% and 69.5% more TVC and YM count, respectively, after the storage period when compared to the same flour stored in paper bags. Similarly, maize-only instant flour that was stored in LDPE had 20.4% and 44.9% more TVC and YM count, respectively, in comparison to the same flour stored in paper bags after storage. Apart from TVC and YM, the lack of coliforms during the storage period indicates microbial safety, especially the lack of enteric pathogens to the consumers, hence packaging material contributes

towards assuring these safety levels. But the growth of YM could be linked to the loss of quality because this type of micro-organism has been linked to the spoilage of low-water activity foods such as flour. Therefore, the risk of spoilage of instant flours would be higher if stored in LDPE bags than in paper bags.

Besides microbial growth, the packaging materials used in this study also significantly ($p < 0.05$) influenced the physicochemical properties of instant flours as indicated in Table 8.2. The cassava-leaf enriched instant flour that was stored in LDPE had 4.2%, 8.5% and 16.3% higher moisture, water activity and titratable acidity, respectively, after the storage period when compared to the same flour stored in paper bags. Likewise, maize-only instant flour that was stored in LDPE had 6.3%, 12.7% and 7.5% higher moisture, water activity and titratable acidity, respectively, after the storage period when compared to the same flour stored in paper bags. Cassava-leaf enriched instant flour that was stored in LDPE had 2.9% lower pH compared to the same flour stored in paper bags while maize only had 1.3% lower pH compared to the same flour stored in paper bags after the storage period.

Period of storage and the type of the packaging material significantly ($p < 0.05$) influenced the intensity of sensory attributes of instant flours as indicated in Table 8.3. In both packaging types, the appearance of the flours (brown colour and number of specks), taste (bitterness and umami) and rancid aroma increased in intensities. Storage time significantly ($p < 0.05$) reduced the overall aroma, astringent after-taste, corny flavour, starchy flavour, stickiness and rough texture intensities of instant flours stored in both packaging materials. Therefore, the storage period reduces the intensities of desirable attributes such as overall aroma, stickiness and rough texture while increasing the intensities of undesirable attributes to the consumer such as a number of specks, bitterness and rancid off-flavour (Heiniö *et al.*, 2016).

As indicated in Table 8.3, instant flour from only maize stored in LDPE packages had 13.4%, 18.6%, 55%, 74.1% and 10.0% higher intensity scores for colour brownness, number of specks, rancid, bitterness and umami taste, respectively, than same flour stored in paper bags after the storage period. On the contrary, the same instant flour when stored in paper bags recorded 37.9%, 59.3%, 93.3%, 43.0%, 41.7% and 54.6% higher intensity scores for overall aroma, sweetness, corny flavour, starchy flavour, stickiness, rough texture, respectively, than same flour stored in LDPE after the storage period. Cassava leaf enriched instant flour stored in LDPE had 23.4%, 13.3%, 56.7%, 28.8%, 27.4%, 12.5% and 42.9% higher intensity scores for colour, specks, rancid, bitterness, umami, stickiness and rough texture, respectively than same flour stored in paper bags after the storage period. On other hand, cassava leaf enriched instant

flour stored in paper bags had 18.2%, 20.0%, over 100% and 16.7% higher intensity scores for aroma, sweetness, astringent after taste and starch flavour, respectively than the same flour stored in paper bags after the storage period. Generally, paper packaging material after storage had a higher score for desirable attributes such as overall aroma and sweetness while LDPE had a higher score for undesirable attributes such as bitterness and rancid off-flavours. Rancid off-flavours occur when the reaction between oil in flour and oxygen through the packaging material, where LDPE had a higher transmission of oxygen as compared to paper bag (Bolumar *et al*., 2016).

In this study, principal component analysis was performed to compare which sensory attribute determines the shelf life stability of instant flour as indicated in Tables 8.4 and 8.5, and Figure (8.2-8.5). There were three principal components identified for each packaging type and instant flour. Cassava leaf-enriched instant flour stored in paper bags accounted for, a total variation of 69.69% of which component one contributed 43.3%. Principal component one was highly correlated with rancid aroma, umami taste, bitterness, number of specks and astringent (Table 8.4). This indicates that these attributes should be given priority for shelf life testing of cassava leaf-enriched instant flour stored in paper bags. However, cassava leaf-enriched instant flour stored in low-density polyethylene accounted for, a total variation of 82.47% of which component one accounted for 58.3%. Principal component one was highly correlated with umami taste, the number of specks, rancid aroma, bitterness overall aroma, astringent, colour and stickiness (Table 8.5). These attributes determine the change in sensory characteristics of cassava leaf-enriched instant flour stored in low-density polyethylene bags. The higher total variation (86.58%) of attributes during storage was observed for maize-only instant flour stored in low-density polyethylene bags (Figure 8.5). This explains that instant flour developed from maize flour only and packaged with low-density polethylene had the least stability among the others in terms of sensory attributes.

The loss of sensory qualities or the emergence of organoleptic faults that are obvious to the customer, such as off-odors or discoloration, signal the end of the shelf life (Corradini, 2018). Sensory analysis is used to track the gradual deterioration of particular sensory qualities or overall sensory acceptability during storage (Corradini, 2018). The end of the shelf life is also indicated by safety issues stated as upper threshold values for microbiological growth and development or migration of dangerous chemical substances. Due to their potential health effects, they represent necessary acceptable boundaries. The end of shelf life defined in terms of safety limitations typically differs from that determined based on organoleptic

unacceptability, i.e., rejection based only on sensory qualities would represent a longer shelf life. They are typically imposed by regulatory agencies. Most food products' shelf lives are impacted by changes in their sensory properties. In this environment, research on food deterioration mechanisms, as well as the creation and use of new technologies, has become a significant concern for sensory shelf-life estimation of foods (Giménez, 2012).

8.5 Conclusion

It can be concluded from this study, that quality attributes of instant porridge flour from maize enriched with cassava leaves were significantly affected by packaging material and time of storage. During the storage period, both microbial and physicochemical properties of the instant flour significantly increased with time, though that increase was higher in flour stored in LDPE bags as compared to the same flour stored in paper bags. Also, packaging material and time of storage significantly increased intensities of undesirable sensory properties such as rancid aroma, number of specks and bitterness while at the same reducing the intensities of desirable attributes such as overall aroma, stickiness and rough texture.

CHAPTER NINE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

This chapter provides a brief review of some of the methodologies used in this study. Subsequently, the main findings of this research are explained. This comprises the effects of microbial fermentation on nutritional and anti-nutritional contents of maize and cassava leaf flour, extrusion cooking variables on the protein quality, in vitro protein digestibility and functional properties of maize-cassava leaf composite extruded porridge flour. Furthermore, descriptive sensory characteristics and consumer acceptability of extruded maize-cassava leaf composite porridge are explained. The effect of packaging materials and storage durations on physicochemical properties, microbial quality and descriptive sensory characteristics of maize-cassava leaf composite extruded porridge flour is also elaborated. Finally, the overall conclusions and recommendation are indicated.

9.1 Methodology

9.1.1 Raw materials selection

The use of locally available food crops could be ideal to address nutrition-related problems in developing countries. Maize and cassava leaves were used in this study. Maize is an important food crop in Africa. According to FAO (2021), maize is grown on over 40 M ha of land in sub-Saharan Africa (SSA). In vast swathes of sub-Saharan Africa, maize is the staple food with consumption of up to 450 g/person/day (Ekpa *et al.*, 2019). In most parts of Africa, maize is consumed as a porridge in different consistencies; thin or thick. The ratio of flour to water, flour particle size, the method of preparation, the degree of starch gelatinization and enzymatic hydrolysis, and the inclusion of other ingredients determine the consistency of the porridge. For instance, a thin porridge made from maize flour is a common practice to nourish young children in Ethiopia. However, protein energy malnutrition persists in regions where maize is the only option for consumption, partly due to compositional maize characteristics, nutrient loss during processing and consumer preferences (Bamidele & Fasogbon, 2020).

Cassava leaf flour was used in this study due to its high nutritional profile compared to its root counterparts. Cassava is a major staple food in sub-Saharan Africa (SSA), providing an important source of calories and it is a priority for food security for the increasing population (Adiele, 2020). Cassava leaves contain high nutritional values, and thus could be used as a potential ingredient in the production of various products (Saragih *et al.*, 2020).

9.1.2 Analytical methods

The high-performance liquid chromatography (HPLC) technique was used for essential amino acid determination in this study. Pre-column derivatization of amino acids followed by their resolution by reverse-phase high-performance liquid chromatography (RP-HPLC) is the preferred method for quantitative amino acid analysis (Gheshlaghi *et al*., 2008). This is because the derivatization step introduces covalently bound chromophores necessary not only for interactions with the apolar stationary phase for high resolution but also for photometric or fluorometric detection (Gheshlaghi *et al*., 2008). Alkaline hydrolysis with NaOH for tryptophan analysis is a preferred method because acid hydrolysis causes a loss (Ritota & Manzi, 2020).

Utilizing biological or enzymatic techniques, protein digestibility is assessed (Laleg *et al*., 2019). Frequently used techniques for determining *in vitro* protein digestibility include single-enzyme and multi-enzyme approaches (Li *et al*., 2020). A single enzyme assay is used in the pepsin method. Finding the test food's original protein content is necessary for this procedure. Following that, digestion is carried out using pepsin under precise circumstances that mimic the state of the stomach. In the digested test food, the residual protein content as insoluble protein is calculated and expressed as a percentage of the starting protein content. This approach does have some limitations, such as the possibility of underestimating protein digestibility for proteins that are resistant to pepsin due to their primary structure or the acid stability of their tertiary structure (Bessada *et al*., 2019). Due to these reasons, the multi-enzyme method was used in this study. This method requires a short time to determine protein digestibility compared to the single enzyme assay.

9.2 Main findings of this research

In this study, microbial fermentation with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* strains caused a significant reduction in the anti-nutritional contents of maize and cassava leaf flour. Most importantly, the cyanogenic glycoside content in cassava leaf flour was reduced significantly and found within the standard. Fermentation of maize and cassava leaf with these strains improved the nutritional values, especially the protein content by reducing anti-nutrients. Phytase enzyme production during the fermentation process, for instance, could be responsible for the decrease in phytate that is present in maize in the form of myo-inositol hexa-phosphate (IP6) and promotes the breakdown process (Troesch *et al*., 2013). Microorganisms or endogenous phytase can both be created spontaneously (exogenous

phytase). Figure 9.1 illustrates how phytases can dephosphorylate phytate in a step-by-step fashion to a sequence of lower inositol phosphate esters (Myo-inositol penta-phosphate to Myo-inositol mono-phosphate), and eventually to inositol and inorganic phosphorus (Vashishth *et al*., 2017).

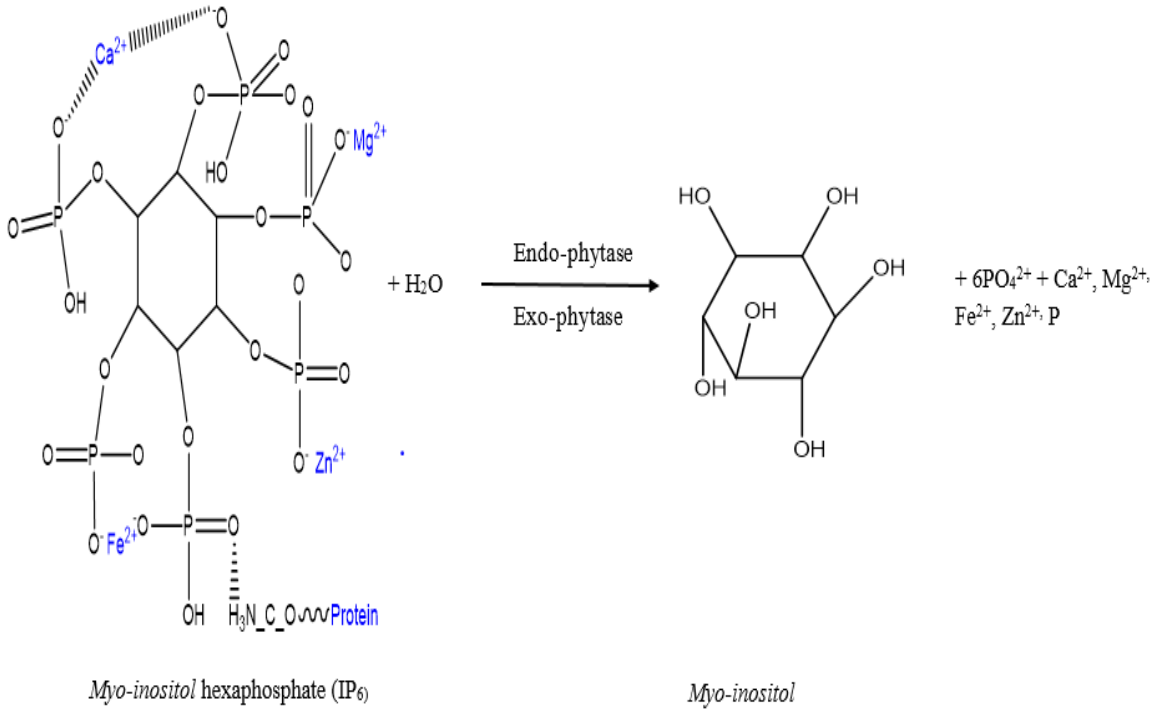


Figure 9.1. Phytate degradation process

In this study, the protein content of fermented cassava leaf had a strong negative correlation with cyanide, oxalate, phytate and tannin contents. Though cassava leaf is known for its high nutritional contents, especially protein, these anti-nutrients affect the bioavailability of proteins if appropriate processing techniques are not applied (Latif *et al*., 2020). The reduction of cyanogenic glycoside could be facilitated by the production of linamarase (exogenous) by the two microorganism strains used during fermentation in addition to the natural linamarase (endogenous) present in cassava leaves as described in Figure 9.2. Linamarase (EC 3.2.1.21; β-D-glucoside glucohydrolase) constitutes a group of β-glucosidase (hydrolase/cellobiase) that catalyses the hydrolysis of glycosidic linkages through the transfer of glycosyl groups and converts into glucose and acetone cyanohydrin which further reduced into acetone and hydrogen cyanide. The released hydrogen cyanide which is an indicator of the presence of linamarin and lotaustralin in cassava is volatile and can be lost during further processing (Feng *et al*., 2003). Yeast and lactic acid bacteria (LAB) are the most common strains that have been frequently associated with the production of linamarase during the fermentation of cassava

(Behera & Ray, 2017). Among the lactic acid bacteria, *Lactobacillus plantarum* is the most predominant strain for producing linamarase enzyme for cyanogenic glycoside hydrolysis (Behera & Ray, 2017).

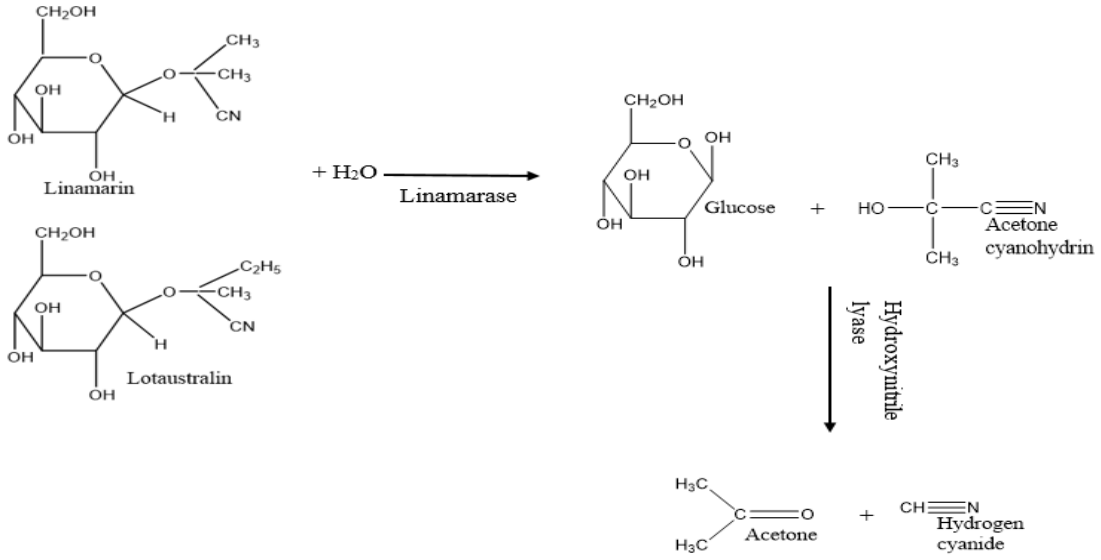


Figure 9.2. Linamarin or lotaustralin degradation through linamarase and hydroxy nitrile lyase

The increasing proportion of cassava leaf flour in the composite significantly increased the protein content of the extruded instant flour. This could be because the high protein content found in cassava leaf in nature or enhanced during fermentation caused the gross protein level in the composite to increase. In addition, the increase in protein could come from the extrusion process as it affects the different bonds and increases the protein availability (Zhang *et al*., 2019) as shown in Figure 9.3. Hence, this product (extruded instant flour) could be used as a means to tackle protein energy malnutrition. Furthermore, this product contains essential amino acids in better quantity which are found low for cereal-based products. The most limiting essential amino acid in cereal-based products is lysine. However, in this study, a considerable amount of lysine was found, which can solve the problem of lysine deficiency.

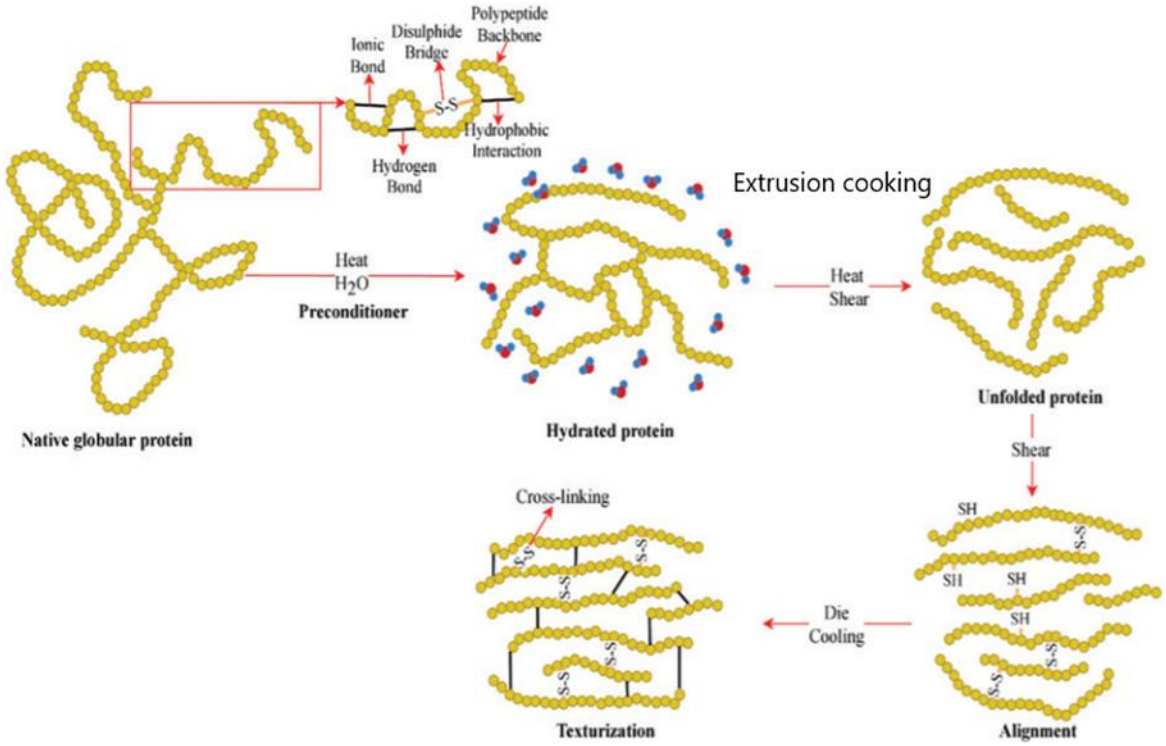


Figure 9.3. Schematic diagram showing the change in protein structure during extrusion cooking; modified from Vatansever *et al*. (2020)

Water absorption and solubility indices are important attributes which determine the functional properties of a given product. Water absorption and solubility indices of the extruded flour are triggered by the gelatinization and melting of molecules during extrusion. The water solubility index is often used as an index for the degradation of molecular components, indicating the number of soluble polysaccharides released from fibre components during extrusion (Tabibloghmany *et al*., 2020) as shown in Figure 9.4. In this study, supplementation of maize flour with cassava leaf flour for the development of extruded instant flour improves its macro and micro mineral contents, such as calcium, potassium, magnesium and iron. The combination of formulation and extrusion temperature significantly influenced both descriptive sensory characteristics and consumer acceptability of instant porridge developed from maize enriched with fermented cassava leaves.

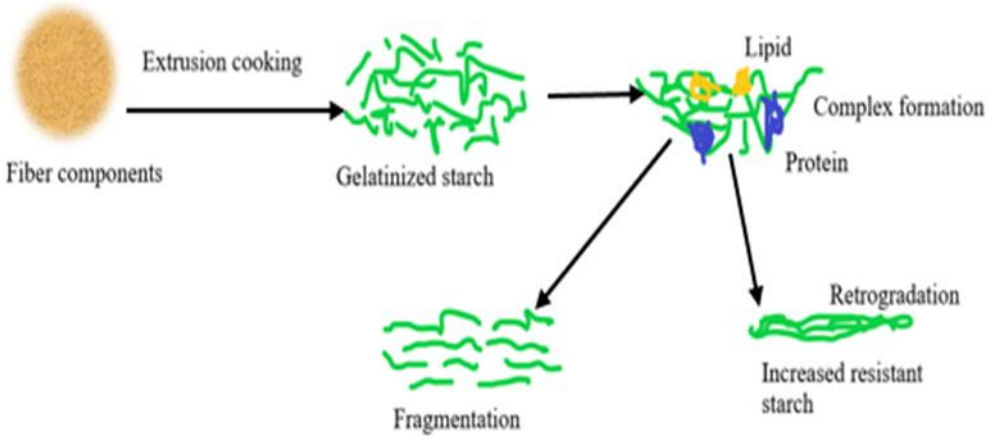


Figure 9.4. Conformational changes of macromolecules during extrusion

Descriptive analysis (DA) provides a way to obtain an objective description of the evaluated product in terms of perceived sensory attributes. Knowing the relationship among the attributes of the product is useful in product development and marketing as well as in customer service (Calviño *et al*., 1996). In this study, the twelve descriptive attributes developed by descriptive panels described the product very well. The principal component analysis showed clearly which attributes should be given priority during the development of maize-cassava leaf instant porridge flour (Figure 9.5).

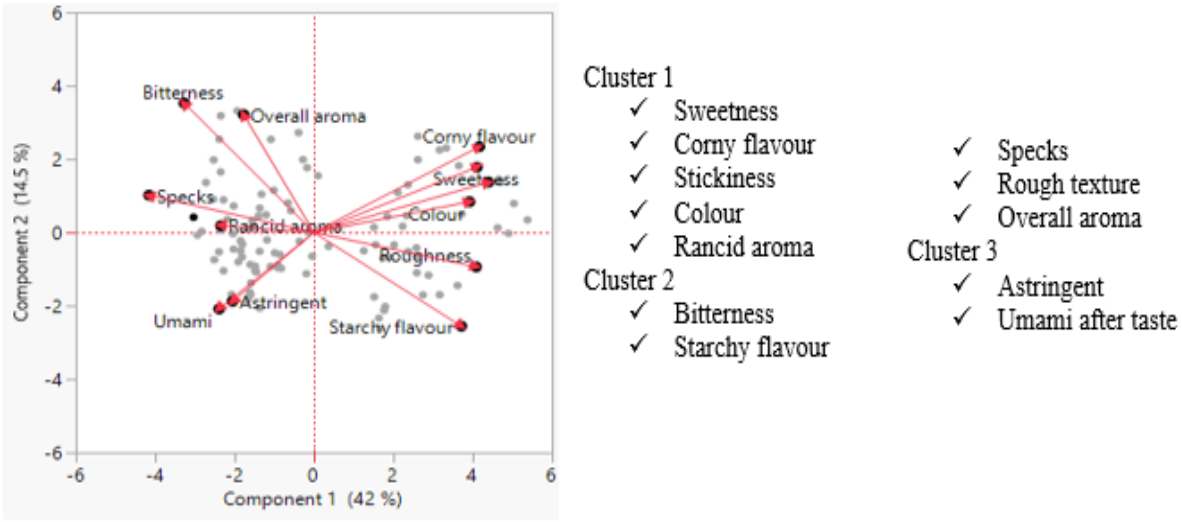


Figure 9.5. Biplot and clusters of descriptive attributes

According to Ethiopian standards, any product shall be free from pathogenic micro-organisms and shall comply with maximum limits for total plate counts of 10^3 cfu/g, yeasts and moulds of 10^3 cfu/g and absent for coliforms. In this study, the maximum total viable counts, and yeasts and moulds were $5.25x10^1$ cfu/g and $3.56x10^1$ cfu/g, respectively for instant flour stored for 90 days, which is within the limits. In addition, coliforms were not detected for each packaged

sample. The overall quality attributes of the developed instant porridge flour were significantly affected by packaging material and time of storage. The intensities of sensory attributes were reduced significantly during the 90 days storage time. This indicates that the sensory attribute's intensity is reduced through time, which can be used as a quality deterioration criterion (Ares *et al*., 2006). Instant flour packed in aluminium paper bags showed better stability compared to low-density polyethylene-packed ones for all attributes tested (Figure 9.6).

Aluminium laminated paper bags Low density polyethylene

Figure 9.6. Types of packaging materials used for this study

9.3 Conclusions

In this study, instant porridge flour was developed using maize and cassava leaf composite flour. The nutritional values of maize flour were improved after the application of microbial fermentation. Fermentation of cassava leaves with *Lactobacillus Plantarum* and *Saccharomyces cerevisiae* assured its safety and improved its nutritional values. The addition of microbial fermented cassava leaf flour with extrusion cooking variables changes the nutritional and functional properties of instant porridge flour.

The protein content which is the most important macro-nutrient was increased as a result of the supplementation of maize with microbial fermented cassava leaf flour. The protein digestibility and essential amino acid profiles which determines the quality of protein were improved as a result of increasing cassava leaf proportion and extrusion cooking variables. In addition, the functional properties were improved, which are important for further processing and determining the acceptability of the product. In this study, the overall optimum extrusion cooking conditions were obtained at an extrusion temperature of 100^{o}C, feed composition of 12% and feed moisture of 14%.

The sensory characteristics of the developed instant porridge were varied due to extrusion cooking variables and cassava leaf flour proportion in the composite flour. The developed instant porridge was accepted positively by the majority of the test panels.

The shelf life properties were assessed by keeping the instant flour for 90 days storage time. During this period of storage, the spoilage indicator micro-organisms tested for the instant flour were below the maximum limits. However, the intensity of sensory attributes was reduced through the storage time. Moreover, in this study, the aluminium laminated paper packages were found appropriate for packaging instant flour developed from composite flour of maize and cassava leaf compared to low-density polyethylene packaging materials.

9.4 Recommendations

Application of microbial fermentation for food ingredients which have toxin compounds such as cassava leaves is a preferred method over spontaneous fermentation which is mostly used so far, because of risks such as the occurrence of pathogenic micro-organisms, chemical contaminants and toxic compounds of microbial origin can be minimized by using known pure strains. Therefore, the use of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* starter cultures in the fermentation of maize and cassava leaf flour is recommended for producing maize and cassava leaf flour with enhanced nutritive value, which could be used to fight malnutrition. Increasing the proportion of fermented cassava leaf flour in the composite flour up to 15% during extrusion cooking for the production of instant flour, increases the protein content and its quality, however, it affects the sensory attributes negatively. Hence, there should be a mechanism to be applied for maintaining the sensory attributes at the desired level. Supplementation of cassava leaf flour with maize flour during extrusion of maize-based products improves the functional properties, *in vitro* protein digestibility and mineral contents. However, further studies are needed to be conducted for *in vitro* starch digestibility and other minerals which are not addressed in this study. Aluminium laminated paper bags can be recommended to be the preferred packaging material for instant porridge flour developed from maize enriched with cassava leaf. However, other types of packaging materials can be investigated to gain insights into how they influence the physicochemical and sensory properties of this product. For inclusive estimation of the end of the shelf life of the developed instant flour, further studies need to be conducted using additional parameters from nutritional and microbial perspectives.

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APPENDICES

Appendix 1. List of publications and conference presentation

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ORIGINAL RESEARCH

Food Science & Nutrition Open Access WILEY

Effect of solid state fermentation on proximate composition, antinutritional factors and in vitro protein digestibility of maize flour

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Abstract

Cereals including maize generally have limiting amino acids particularly lysine. In most cases, spontaneous fermentation is used to improve the nutritional profiles of maize-based products. However, in such fermentation, biological risks including the presence of pathogenic microorganisms, chemical contaminants, and toxic compounds of microbial origin such as mycotoxins pose a health risk. The aim of this study was, therefore, to improve the nutritional properties of maize flour by reducing antinutritional factors through microbial fermentation by strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* and their cocultures. A factorial experimental design was used to evaluate the effect of fermentation setups and time on proximate composition, antinutritional factors, and in vitro digestibility of proteins in maize flour. During 48 h of fermentation, protein content was improved by 38%, 55%, 49%, and 48%, whereas in vitro protein digestibility improved by 31%, 40%, 36%, and 34% for natural, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and their coculture-fermented maize flour, respectively. The highest improvement in protein content and its digestibility was observed for *Lactobacillus plantarum* strain-fermented maize flour. Phytate, tannin and trypsin inhibitor activity were reduced significantly ($p < .05$) for natural, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and coculture-fermented maize flour. The highest reduction of phytate (66%), tannin (75%), and trypsin inhibitor (64%) was observed for coculture-fermented maize flour. The two strains and their cocultures were found feasible for fermentation of maize flour to improve its nutritional profiles more than the conventional fermentation process.

KEYWORDS

antinutritional factors, in vitro protein digestibility, *Lactobacillus plantarum*, proximate

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ORIGINAL ARTICLE

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Effect of microbial fermentation on nutritional and antinutritional contents of cassava leaf

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Funding information
EP-Nuffic, Grant/Award Number: NICHE/ETH/179

Abstract

Cassava leaves serves as a source of alternative proteins for people in developing countries who could not easily access the available protein sources. However, its use is limited by the presence of toxic compounds, particularly cyanogenic glycosides. Thus, use of appropriate processing technique is indispensable to reduce the toxic compounds to a safer limit before utilization of cassava leaf. The objective of this study was therefore to evaluate the effect of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* strains, and their co-culture on nutritional contents and antinutritional factors of cassava leaf during fermentation. A 4 × 5 factorial experimental design was used to determine the effect of fermentation setups and time on chemical composition, antinutritional contents, in vitro protein digestibility and mineral contents of cassava leaf. During 48 h of fermentation, a significant change ($p < 0.05$) in moisture, protein, fiber, fat, and ash contents were observed. Protein content was improved by 34.91%, while in vitro digestibility of protein was improved by 28.07% during 48 h of *L. plantarum* fermentation. Cyanide, oxalate, tannin, and phytate contents were decreased significantly ($p < 0.05$) for all fermentation setups. The highest reduction in cyanide (97.17%) and oxalate (86.44%) was achieved under *L. plantarum* fermentation. The highest reduction in tannin (93.25%) and phytate (91.11%) was achieved under co-culture fermentation of cassava leaf. A significant ($p < 0.05$) reduction of mineral contents except iron was observed during 48 h of fermentation. A significant ($p < 0.0001$) strong negative correlation was found between protein with cyanide (−0.8164), oxalate (−0.7991), phytate (−0.7851), and tannin (−0.6906). In vitro digestibility of protein also showed a strong significant ($p < 0.0001$) negative correlation with phytate (−0.9628), oxalate (−0.9407), cyanide (−0.9305), and tannin (−0.8493). Application of *L. plantarum* and *S. cerevisiae* in cassava leaf fermentation showed significant improvement of nutritional qualities by reducing the antinutritional factors and toxic compounds. Fermentation of cassava leaf using these strains ascertain utilization of cassava leaf for human consumption to tackle protein energy malnutrition.

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Full Length Research

Functional properties, *in vitro* protein digestibility and mineral contents of extruded flour developed from maize-cassava leaf composites

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ABSTRACT: Application of extrusion cooking technology in product development is getting a high priority due to its effectiveness in quality improvement. Response surface methodology has been used extensively to optimize extrusion variables. The objective of this study was to evaluate the functional properties, *in vitro* protein digestibility and mineral contents of maize-cassava leaf composite extruded instant porridge flour using response surface methodology. Box-Benkhen design was used for formulation and optimization of the process variables. Extrusion variables; extrusion temperature (80°C – 120°C), feed composition (cassava leaf flour proportion) (5% -15%) and feed moisture (14% - 18%) were used as input variables whereas, water absorption index (WAI), water solubility index (WSI), *in vitro* protein digestibility (IVPD), and minerals were used as responses. Results obtained showed that, WAI and WSI increased significantly ($p<0.05$) as a result of increase in extrusion temperature and feed composition. WSI showed a significant ($p<0.05$) positive correlation with IVPD and mineral contents. IVPD was significantly ($p<0.05$) improved by extrusion temperature and feed composition. Calcium, potassium, magnesium and iron contents were significantly ($p<0.001$) increased as a result of increase in feed composition. Supplementation of cassava leaf flour during extrusion of maize based products improved its functional properties, IVPD and mineral contents, hence suitable for formulation of nutritious extruded food products.

Keywords: Cassava leaf, extrusion cooking, instant porridge flour, optimization, response surface methodology.

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Optimization of Extrusion Cooking Variables for Production of Protein Enriched Maize-Cassava Leaf Composite Instant Porridge Flour

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Abstract

Protein energy malnutrition is one of the major public health problems in the developing countries. The objective of this study was to develop protein enriched instant porridge flour from maize and cassava leaf composites. Response Surface Methodology (RSM) in Box-Behnken design was used for formulation and optimization of the process variables. A co-rotating twin screw extruder was used with the combination of the three variables; extrusion temperature (80-120°C), cassava leaf flour proportion (5-15%) and feed moisture (14-18%). Results obtained showed that, the protein and lysine contents were found in the ranges of 11.45 to 20.1% and 1.98 to 6.2 g/100g of protein, respectively. There was a significant ($p < 0.001$) increase in the protein and essential amino acid contents of the extruded flour due to supplementation with cassava leaf flour. The optimum extrusion variables that could give optimum proximate composition and essential amino acid profiles were; extrusion temperature (118°C), feed composition (8%) and feed moisture (14%) with a composite desirability of 99.8 percent. The optimum value of protein after optimization was 16%. Therefore, 100 grams serving of maize-cassava leaf extruded instant porridge can provide 47% of the recommended daily allowance of protein (34 g/day) for children up to 12 years old with enhanced protein quality. Therefore, extrusion cooking can be used effectively for production of maize-cassava leaf composite instant porridge flours that have enhanced nutritional quality.

Keywords

Cassava Leaf, Composites, Extrusion Cooking, Instant Porridge, Maize, Optimization

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OF RECOGNITION

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Endeavor Research Private Limited and
Advisory Board Members of NutriFood-2022 applaud

Zemenu K. Terefe

Hawassa University, Ethiopia

For his phenomenal and worthy Oral presentation on

Effect of solid state fermentation on proximate composition, anti-nutritional factors and in vitro protein digestibility of maize flour

at

Webinar on Food Science and Nutrition
held on March 28, 2022

Chyer Kim,
Agricultural Research Station,
Virginia State University, USA

Appendix 2. Letters for necessary procedures

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OFFICE OF THE DIRECTOR GRADUATE SCHOOL

Ref: KD16/18504/17 Date: 21st August, 2019

Hawassa University
Ethical Review Board
ETHIOPIA

Dear Sir/Madam,

RE: MR. ZEMENU KERIE TEREFE REG. NO. KD16/18504/17

This is to introduce and confirm to you that the above named student is in the Department of Dairy & Food Science, Faculty of Agriculture, Egerton University.

He is a bona-fide registered PhD student in this University. His research topic is **"Development of Protein Enriched Maize – Cassava Leaf Composite Extruded Instant Porridge Flour."**

He is at the stage of collecting field data.

Your kind assistance to him will be highly appreciated.

Yours faithfully,

DIRECTOR, BOARD OF POST GRADUATE STUDIES
21 AUG 2019
EGERTON UNIVERSITY, P.O. Box 536-20115 EGERTON

Prof. Nzula Kitaka
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

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HAWASSA UNIVERSITY
COLLEGE OF MEDICINE AND
HEALTH SCIENCES
Institutional Review Board

Meeting No: 01/2012

Ref. No: IRB/269/12
Date: 05/11/2019

Name of Researcher(s): ***Zemenu Kerie Terefe, Mary Omwamba, Abdul K.Faraj, John M. Nduko***

Topic of Proposal: ***Development of protein enriched maize-cassava leaf composite extruded instant porridge flour***

Dear researcher(s),

The Institutional Review Board (IRB) at the College of Medicine and Health Sciences of Hawassa University has reviewed the aforementioned research protocol with special emphasis on the following points:

1. Are all principles considered?
   - 1.1. Respect for persons: Yes ☑ No ☐
   - 1.2. Beneficence: Yes ☑ No ☐
   - 1.3. Justice: Yes ☑ No ☐
2. Are the objectives of the study ethically achievable? Yes ☑ No ☐
3. Are the proposed research methods ethically sound? Yes ☑ No ☐

Based on the aforementioned ethical assessment, the IRB has:

A. Approved the proposal for implementation ☑ -Approval period from **Nov 5/ 2019** to **Nov 4/ 2020**
B. Conditionally Approved ☐ -Element Approved: **Protocol Version No. 1**
C. Not Approved ☐ -Follow up report expected in **6 months**

Obligation of the PI:

1. Should comply with the standard international and national scientific and ethical guidelines
2. All amendment and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 3 days of the event
4. End of study, including manuscript should be reported to the IRB

Yours faithfully,

Dawit Jember
Institutional Review Board Chairperson.

+046 820 92 90 Fax: + 046 2208755 1560 Hawassa

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Hawassa University
School of Nutrition, Food Sceince and Technology
Academic Center of Excellence in Human Nutrition

ቁጥር ሀ/ሥ/ም/ሣ/ቴ/1225/2012
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Appendix 3. Consumer sensory evaluation sheet

Please write the numbers in the appropriate boxes to show your agreement.

Please rinse your mouth between tastes. Date: _____Time: _____

Sex: Female ☐ Male ☐ Age: _____

Table 1.Sensory evaluation ballot for acceptance test

S. No.	Product codes	Sensory Attributes					
		Appearance	**Taste**	**Aroma**	**Flavour**	**Texture**	**Overall acceptability**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
Use these numbers to show your degree of liking in the appropriate boxes: 7= like extremely, 6= like very much, 5= like, 4= neither like nor dislike, 3= dislike, 2= dislike very much, 1= Dislike extremely							

Comments: _____

_____

Thank you for your valuable contribution!

Appendix 4. Quantitative descriptive analysis (QDA) ballot

Instructions: Please rinse your mouth between tastes. Date: _____Time:_____

Sex: Female ☐ Male ☐ Age: _____

Code: _____

1. Appearance
A. Colour

Light dark brown

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0 1 2 3 4 5 6 7 8 9 10

B. Specks

No specks many specks

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0 1 2 3 4 5 6 7 8 9 10

2. Aroma
A. Overall aroma

Not intense very intense

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0 1 2 3 4 5 6 7 8 9 10

B. Rancid aroma

Not rancid rancid aroma

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0 1 2 3 4 5 6 7 8 9 10

3. Taste
A. Sweet

Not intense very intense

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0 1 2 3 4 5 6 7 8 9 10

B. Bitter

Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

4. Aftertaste

A. Umami

Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

B. Astringent

C. Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

5. Flavour

A. Corny flavour

Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

B. Starchy flavour

Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

6. Texture

A. Stickiness

Not intense very intense

0 1 2 3 4 5 6 7 8 9 10

B. Rough texture

Smooth very intense

0 1 2 3 4 5 6 7 8 9 10

Appendix 5. ANOVA table for some parameters from respective objectives

1. ANOVA table for objective 1 part one (3.1)

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
Ft	4	LP LpSc N SC
Fhrs	5	0 12 24 36 48

Number of Observations Read	60
Number of Observations Used	60

Dependent Variable: Moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	87.00211833	12.42887405	70.97	<.0001
Error	52	9.10658000	0.17512654		
Corrected Total	59	96.10869833			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ft	3	4.02964500	1.34321500	7.67	0.0002
Fhrs	4	82.97247333	20.74311833	118.45	<.0001

Dependent Variable: Protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	142.4744067	20.3534867	118.10	<.0001
Error	52	8.9618333	0.1723429		
Corrected Total	59	151.4362400			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ft	3	3.3387333	1.1129111	6.46	0.0008
Fhrs	4	139.1356733	34.7839183	201.83	<.0001

Dependent Variable: Fibre

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	56.00019167	8.00002738	96.18	<.0001
Error	52	4.32542667	0.08318128		
Corrected Total	59	60.32561833			

Source	DF	Anova SS	Mean Square	F Value	Pr > F

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Ft	3	3.97523167	1.32507722	15.93	<.0001
Fhrs	4	52.02496000	13.00624000	156.36	<.0001

Dependent Variable: Fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	27.78345833	3.96906548	70.25	<.0001
Error	52	2.93782667	0.05649667		
Corrected Total	59	30.72128500			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ft	3	1.36936500	0.45645500	8.08	0.0002
Fhrs	4	26.41409333	6.60352333	116.88	<.0001

Dependent Variable: Ash

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	16.17121667	2.31017381	35.04	<.0001
Error	52	3.42787667	0.06592071		
Corrected Total	59	19.59909333			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ft	3	1.65124000	0.55041333	8.35	0.0001
Fhrs	4	14.51997667	3.62999417	55.07	<.0001

Dependent Variable: CHO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	179.3774233	25.6253462	52.96	<.0001
Error	52	25.1622100	0.4838887		
Corrected Total	59	204.5396333			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ft	3	15.8939400	5.2979800	10.95	<.0001
Fhrs	4	163.4834833	40.8708708	84.46	<.0001

2. ANOVA table for objective 1 part two (3.2)

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
Fermentation setups	4	Lp Mixed N Sc

Class Level Information		
Class	**Levels**	**Values**
Fermentation time	5	0 12 24 36 48

Number of Observations Read	60
Number of Observations Used	60

Dependent Variable: HCN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	167544.3375	23934.9054	237.44	<.0001
Error	52	5241.8462	100.8047		
Corrected Total	59	172786.1837			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Fermentation setups	3	9161.6040	3053.8680	30.29	<.0001
Fermentation time	4	158382.7335	39595.6834	392.80	<.0001

Dependent Variable: Oxalate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	52099.80054	7442.82865	712.87	<.0001
Error	52	542.91496	10.44067		
Corrected Total	59	52642.71550			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Fermentation setups	3	435.99802	145.33267	13.92	<.0001
Fermentation time	4	51663.80252	12915.95063	1237.08	<.0001

The ANOVA Procedure

Dependent Variable: Tannin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	25838.77478	3691.25354	191.40	<.0001
Error	52	1002.84208	19.28542		
Corrected Total	59	26841.61686			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Fermentation setups	3	1173.85643	391.28548	20.29	<.0001
Fermentation time	4	24664.91834	6166.22959	319.74	<.0001

Dependent Variable: Phytate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	15195.07178	2170.72454	356.48	<.0001
Error	52	316.64671	6.08936		
Corrected Total	59	15511.71849			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Fermentation setups	3	445.69625	148.56542	24.40	<.0001
Fermentation time	4	14749.37553	3687.34388	605.54	<.0001

3. ANOVA table for objective 2 part one (4.1)

Design expert output

A. Protein

Source	SS	df	MS	F-value	P-value	
Model	32.75	9	3.64	63.54	0.0001	significant
A-Extrusion temperature	5.95	1	5.95	103.90	0.0002	
B-Feed composition	25.81	1	25.81	450.64	< 0.0001	
C-Feed moisture	0.2211	1	0.2211	3.86	0.1066	
AB	0.0000	1	0.0000	0.0004	0.9841	
AC	0.0072	1	0.0072	0.1261	0.7370	
BC	0.0576	1	0.0576	1.01	0.3620	
A^2	0.3567	1	0.3567	6.23	0.0548	
B^2	0.0420	1	0.0420	0.7334	0.4309	
C^2	0.2528	1	0.2528	4.41	0.0897	
Residual	0.2864	5	0.0573			
Lack of Fit	0.2499	3	0.0833	4.57	0.1848	not significant
Pure Error	0.0365	2	0.0182			
Cor Total	33.04	14				

B. Lysine

Source	SS	df	MS	F-value	P-value	
Model	5.99	9	0.6654	435.38	< 0.0001	significant
A-Extrusion temperature	2.17	1	2.17	1422.21	< 0.0001	
B-Feed composition	2.93	1	2.93	1915.94	< 0.0001	
C-Feed moisture	0.0528	1	0.0528	34.56	0.0020	
AB	0.5112	1	0.5112	334.50	< 0.0001	
AC	0.1600	1	0.1600	104.69	0.0002	
BC	0.0552	1	0.0552	36.13	0.0018	
A^2	0.0057	1	0.0057	3.71	0.1122	
B^2	0.0707	1	0.0707	46.23	0.0010	
C^2	0.0231	1	0.0231	15.14	0.0115	
Residual	0.0076	5	0.0015			
Lack of Fit	0.0068	3	0.0023	5.21	0.1652	not significant
Pure Error	0.0009	2	0.0004			

Cor Total	6.00	14				

4. Anova table for objective 2 part two (4.2)

A. Water absorption index

Source	**SS**	**df**	**MS**	**F-value**	**P-value**	
Model	6.49	9	0.7212	17.10	0.0030	significant
A-Extrusion temperature	3.24	1	3.24	76.79	0.0003	
B-Feed composition	0.7812	1	0.7812	18.52	0.0077	
C-Feed moisture	0.5778	1	0.5778	13.70	0.0140	
AB	0.0016	1	0.0016	0.0379	0.8532	
AC	0.3540	1	0.3540	8.39	0.0339	
BC	0.0100	1	0.0100	0.2371	0.6469	
A^2	0.1351	1	0.1351	3.20	0.1336	
B^2	0.0636	1	0.0636	1.51	0.2741	
C^2	1.28	1	1.28	30.35	0.0027	
Residual	0.2109	5	0.0422			
Lack of Fit	0.1003	3	0.0334	0.6044	0.6721	not significant
Pure Error	0.1106	2	0.0553			
Cor Total	6.70	14				

B. Water solubility index

Source	**SS**	**df**	**MS**	**F-value**	**P-value**	
Model	121.47	9	13.50	20.07	0.0021	significant
A-Extrusion temperature	24.43	1	24.43	36.33	0.0018	
B-Feed composition	77.50	1	77.50	115.25	0.0001	
C-Feed moisture	3.64	1	3.64	5.42	0.0674	
AB	5.81	1	5.81	8.64	0.0323	
AC	1.37	1	1.37	2.04	0.2130	
BC	4.80	1	4.80	7.13	0.0443	
A^2	0.7728	1	0.7728	1.15	0.3327	
B^2	2.56	1	2.56	3.81	0.1086	
C^2	0.4082	1	0.4082	0.6070	0.4712	
Residual	3.36	5	0.6725			
Lack of Fit	1.90	3	0.6339	0.8680	0.5746	not significant
Pure Error	1.46	2	0.7303			
Cor Total	124.83	14				

C. In vitro protein digestibility

Source	**SS**	**df**	**MS**	**F-value**	**P-value**	
Model	72.86	9	8.10	13.60	0.0051	significant

A-Extrusion temperature	6.37	1	6.37	10.71	0.0221
B-Feed composition	48.86	1	48.86	82.09	0.0003
C-Feed moisture	1.24	1	1.24	2.08	0.2084
AB	8.58	1	8.58	14.43	0.0127
AC	2.86	1	2.86	4.80	0.0800
BC	0.0870	1	0.0870	0.1462	0.7179
A^2	1.80	1	1.80	3.02	0.1426
B^2	1.42	1	1.42	2.39	0.1829
C^2	1.39	1	1.39	2.34	0.1864
Residual	2.98	5	0.5951		
Lack of Fit	0.8948	3	0.2983	0.2867	0.8351 not significant
Pure Error	2.08	2	1.04		
Cor Total	75.84	14			

Table 5.4. Effect of extrusion variables on proximate composition of maize-cassava leaf composite extruded instant flour

Runs	Independent variables			Response variables						
	ET (^{o}C)	FC (%)	FM (%)	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	CHO (%)	Calories (kcal/100g)
1	+1(120)	0(10)	+1(18)	8.0 ± 0.03^{g}	16.43 ± 0.06^{f}	2.06 ± 0.04^{e}	2.29 ± 0.02^{d}	4.68 ± 0.04^{c}	68.83 ± 0.05^{g}	359.58 ± 0.30^{f}
2	0(100)	+1(15)	-1(14)	8.22 ± 0.05^{f}	18.11 ± 0.06^{d}	2.2 ± 0.03^{d}	3.06 ± 0.07^{c}	4.6 ± 0.04^{d}	66.87 ± 0.06^{i}	359.72 ± 0.18^{f}
3	0(100)	0(10)	0(16)	8.2 ± 0.11^{ef}	17.17 ± 0.03^{e}	2.23 ± 0.05^{d}	2.97 ± 0.05^{c}	4.66 ± 0.07^{c}	67.74 ± 0.09^{h}	359.71 ± 0.79^{f}
4	-1(80)	+1(15)	0(16)	9.45 ± 0.01^{a}	20.1 ± 0.11^{a}	2.21 ± 0.04^{d}	3.32 ± 0.07^{a}	4.28 ± 0.03^{e}	63.96 ± 0.07^{l}	356.13 ± 0.25^{g}
5	0(100)	0(10)	0(16)	8.5 ± 0.01^{de}	17.04 ± 0.02^{e}	2.29 ± 0.04^{d}	2.74 ± 0.01^{c}	4.4 ± 0.03^{e}	67.77 ± 0.01^{h}	359.85 ± 0.35^{f}
6	0(100)	-1(5)	+1(18)	7.0 ± 0.02^{i}	15.26 ± 0.05^{h}	2.5 ± 0.05^{c}	1.74 ± 0.08^{f}	3.95 ± 0.07^{f}	71.29 ± 0.07^{d}	368.7 ± 0.30^{d}
7	+1(120)	-1(5)	0(16)	5.45 ± 0.0^{1m}	14.65 ± 0.03^{j}	2.5 ± 0.02^{c}	1.24 ± 0.04^{g}	3.18 ± 0.05^{h}	74.22 ± 0.02^{b}	377.98 ± 0.31^{b}
8	-1(80)	0(10)	-1(14)	8.9 ± 0.02^{b}	18.1 ± 0.06^{d}	2.3 ± 0.05^{c}	3.07 ± 0.06^{b}	4.5 ± 0.02^{d}	66.2 ± 0.10^{j}	357.9 ± 0.27^{f}
9	0(100)	+1(15)	+1(18)	9.0 ± 0.02^{b}	18.8 ± 0.04^{b}	2.2 ± 0.09^{d}	3.19 ± 0.03^{b}	5.07 ± 0.07^{a}	64.93 ± 0.08^{k}	354.72 ± 0.77^{g}
10	+1(120)	+1(15)	0(16)	8.5 ± 0.01^{d}	18.54 ± 0.09^{c}	2.20 ± 0.03^{d}	2.35 ± 0.03^{d}	4.85 ± 0.02^{b}	66.41 ± 0.05^{j}	355.1 ± 0.13^{g}
11	-1(80)	0(10)	+1(18)	8.64 ± 0.06^{c}	18.23 ± 0.06^{d}	2.27 ± 0.03^{d}	3.23 ± 0.05^{b}	4.77 ± 0.04^{bc}	66.09 ± 0.10^{j}	357.71 ± 0.24^{f}
12	+1(120)	0(10)	-1(14)	6.06 ± 0.02^{k}	16.13 ± 0.03^{g}	2.22 ± 0.05^{d}	2.39 ± 0.02^{d}	4.7 ± 0.04^{c}	70.89 ± 0.07^{e}	368.06 ± 0.18^{d}
13	0(100)	0(10)	0(16)	8.35 ± 0.04^{e}	17.31 ± 0.03^{e}	2.32 ± 0.09^{d}	2.7 ± 0.09^{c}	4.37 ± 0.02^{e}	67.65 ± 0.10^{h}	360.72 ± 0.52^{ef}
14	0(100)	-1(5)	-1(14)	5.5 ± 0.03^{l}	15.05 ± 0.08^{i}	2.7 ± 0.03^{b}	1.85 ± 0.09^{e}	3.75 ± 0.05^{g}	73.0 ± 0.12^{c}	376.5 ± 0.15^{c}
15	-1(80)	-1(5)	0(16)	7.28 ± 0.03^{h}	16.22 ± 0.04^{g}	2.25 ± 0.09^{a}	2.05 ± 0.04^{f}	3.8 ± 0.01^{g}	70.34 ± 0.13^{f}	367.48 ± 0.37^{d}
C	100	0	16	6.39 ± 0.05^{j}	11.45 ± 0.03^{k}	3.25 ± 0.06^{a}	1.67 ± 0.06^{f}	2.11 ± 0.08^{i}	76.8 ± 0.16^{a}	382.25 ± 0.22^{a}

Values are mean ± standard deviation, values with different superscripts in column are significant at $p < 0.05$ET= Extrusion temperature, FC= Feed composition, FM= Feed moisture, CHO= Carbohydrate, C= control (maize flour).

Table 5.5. Effect of extrusion variables on essential amino acid contents of maize-cassava leaf composite extruded instant flour (g/100g protein)

Runs	Independent variables			Response variables								
	ET (°C)	FC (%)	FM (%)	Lysine	Isoleusine	Leusine	Valine	Methionine	Threonine	Histidine	Phenylalanine	Tryptophan
1	+1(120)	0(10)	+1(18)	4.52±0.04^{e}	3.34±0.05^{g}	6.85±0.08^{cd}	3.39±0.06^{f}	2.58±0.02^{fg}	2.43±0.02^{efg}	2.27±0.04^{ef}	7.49±0.02^{ef}	1.92±0.01^{cdef}
2	0(100)	+1(15)	-1(14)	5.55 ± 0.06^{b}	4.6 ± 0.03^{c}	7.35 ± 0.09^{ab}	4.2 ± 0.02^{b}	3.0 ± 0.07^{abc}	2.75 ± 0.03^{ab}	2.96 ± 0.02^{a}	7.89 ± 0.01^{a}	2.04 ± 0.01^{bc}
3	0(100)	0(10)	0(16)	4.8 ± 0.09^{d}	3.91 ± 0.04^{e}	7.01 ± 0.05^{c}	3.7 ± 0.09^{d}	2.97 ± 0.04^{abc}	2.57 ± 0.04^{cd}	2.41 ± 0.04^{d}	6.62 ± 0.03^{i}	1.81 ± 0.05^{f}
4	-1(80)	+1(15)	0(16)	6.2 ± 0.02^{a}	5.21 ± 0.04^{a}	7.51 ± 0.03^{a}	4.55 ± 0.05^{a}	3.11 ± 0.04^{a}	2.77 ± 0.02^{a}	3.04 ± 0.05^{a}	7.76 ± 0.05^{b}	2.11 ± 0.05^{b}
5	0(100)	0(10)	0(16)	4.81 ± 0.03^{d}	4.14 ± 0.07^{d}	6.85 ± 0.04^{cd}	3.64 ± 0.02^{d}	2.98 ± 0.03^{abc}	2.51 ± 0.04^{de}	2.38 ± 0.01^{de}	7.55 ± 0.01^{de}	2.01 ± 0.03^{bcd}
6	0(100)	-1(5)	+1(18)	4.2 ± 0.04^{f}	2.0 ± 0.04^{j}	5.38 ± 0.01^{f}	3.56 ± 0.07^{de}	2.51 ± 0.04^{g}	2.3 ± 0.09^{h}	2.22 ± 0.02^{fg}	6.78 ± 0.08^{h}	1.88 ± 0.02^{def}
7	+1(120)	-1(5)	0(16)	3.95 ± 0.09^{h}	1.98 ± 0.07^{j}	5.5 ± 0.04^{f}	3.89 ± 0.03^{c}	2.61 ± 0.05^{fg}	2.31 ± 0.03^{gh}	2.15 ± 0.08^{g}	7.63 ± 0.03^{cd}	2.06 ± 0.01^{b}
8	-1(80)	0(10)	-1(14)	5.75 ± 0.01^{b}	4.96 ± 0.03^{b}	7.44 ± 0.06^{ab}	3.92 ± 0.04^{c}	2.91 ± 0.08^{bcd}	2.51 ± 0.04^{de}	2.8 ± 0.02^{b}	7.71 ± 0.03^{bc}	2.04 ± 0.06^{bc}
9	0(100)	+1(15)	+1(18)	5.2 ± 0.10^{c}	3.98 ± 0.09^{e}	7.28 ± 0.05^{b}	4.21 ± 0.03^{b}	2.8 ± 0.02^{de}	2.64 ± 0.02^{bc}	2.41 ± 0.03^{d}	7.02 ± 0.03^{g}	1.98 ± 0.07^{bcde}
10	+1(120)	+1(15)	0(16)	4.42 ± 0.08^{ef}	3.45 ± 0.02^{g}	6.9 ± 0.03^{cd}	3.63 ± 0.03^{d}	2.99 ± 0.04^{abc}	2.7 ± 0.03^{ab}	2.24 ± 0.02^{fg}	7.41 ± 0.08^{f}	2.01 ± 0.04^{bcd}
11	-1(80)	0(10)	+1(18)	5.14 ± 0.05^{c}	3.97 ± 0.05^{e}	6.53 ± 0.06^{e}	3.57 ± 0.02^{de}	2.69 ± 0.05^{ef}	2.46 ± 0.02^{de}	2.61 ± 0.05^{c}	7.58 ± 0.03^{de}	2.02 ± 0.03^{bc}
12	+1(120)	0(10)	-1(14)	4.33 ± 0.04^{ef}	3.69 ± 0.04^{f}	6.48 ± 0.03^{e}	3.48 ± 0.04^{ef}	2.65 ± 0.05^{efg}	2.45 ± 0.04^{def}	2.37 ± 0.01^{de}	6.2 ± 0.01^{j}	1.98 ± 0.05^{bcde}
13	0(100)	0(10)	0(16)	4.84 ± 0.15^{d}	3.98 ± 0.04^{e}	6.83 ± 0.06^{d}	3.66 ± 0.03^{d}	3.05 ± 0.04^{ab}	2.5 ± 0.05^{de}	2.43 ± 0.04^{d}	7.61 ± 0.05^{cde}	2.08 ± 0.07^{b}
14	0(100)	-1(5)	-1(14)	4.08 ± 0.03^{gh}	2.53 ± 0.01^{h}	5.47 ± 0.08^{f}	3.98 ± 0.04^{c}	2.6 ± 0.07^{fg}	2.33 ± 0.06^{fgh}	2.18 ± 0.04^{fg}	7.9 ± 0.06^{a}	2.3 ± 0.03^{a}
15	-1(80)	-1(5)	0(16)	4.3 ± 0.05^{efg}	2.42 ± 0.03^{h}	5.53 ± 0.05^{f}	3.56 ± 0.06^{de}	2.85 ± 0.08^{cd}	2.39 ± 0.02^{efgh}	2.19 ± 0.01^{fg}	6.56 ± 0.02^{i}	1.84 ± 0.04^{ef}
C	100	0	16	1.98 ± 0.14^{i}	2.23 ± 0.04^{i}	6.89 ± 0.04^{cd}	4.20 ± 0.07^{b}	1.54 ± 0.04^{h}	2.15 ± 0.07^{i}	1.56 ± 0.06^{h}	4.26 ± 0.04^{k}	1.23 ± 0.09^{g}

Values are mean ± standard deviation, and values with different superscripts in the column are significant at $p < 0.05$. ET= Extrusion temperature; FC= Feed composition; FM= Feed moisture; C= control (maize flour).

Table 7.2. Mean scores of the sensory descriptive attributes of extruded maize instant porridge enriched with cassava leaves at different rates and moisture

FC (%)	FM (%)	ET (ºC)	Colour	Specks	Aroma	Rancid	Sweetness	Bitterness	Umami	Astringent	Corny flavour	Starchy flavour	Stickiness	Roughness
0	16	100	8.71±0.32^{a}	1.71±0.39^{e}	8.07±0.40^{a}	0.64±0.21^{a}	7.57±0.30^{a}	1.71±0.32^{c}	2.43±0.25^{abcd}	3.50±0.50^{a}	6.36±0.43^{a}	5.64±0.71^{a}	4.71±0.43^{a}	5.71±0.71^{a}
5	14	100	6.64±0.52^{abcd}	1.86±0.34^{de}	4.64±0.46^{cd}	0.71±0.18^{a}	3.79±0.29^{bc}	2.07±0.37^{bc}	1.93±0.28^{abcd}	2.43±0.49^{a}	3.43±0.38^{bcd}	3.79±0.52^{abc}	3.64±0.24^{ab}	3.21±0.32^{bcde}
5	16	80	6.50±0.61^{abcd}	1.71±0.29^{e}	4.36±0.30^{d}	0.57±0.13^{a}	3.50±0.42^{bcd}	2.14±0.47^{bc}	1.57±0.28^{cd}	2.21±0.36^{a}	3.00±0.36^{bcde}	4.36±0.73^{ab}	4.14±0.28^{a}	3.57±0.32^{bcd}
5	16	120	7.57±0.34^{ab}	2.21±0.24^{cde}	5.14±0.36^{bcd}	1.21±0.26^{a}	4.29±0.47^{b}	1.79±0.34^{c}	1.71±0.24^{bcd}	2.21±0.29^{a}	4.50±0.49^{ab}	3.71±0.74^{abc}	3.79±0.45^{ab}	3.93±0.34^{bc}
5	18	100	6.79±0.65^{abc}	1.57±0.28^{e}	4.57±0.34^{cd}	1.21±0.29^{a}	3.93±0.30^{bc}	2.43±0.37^{abc}	1.50±0.19^{d}	2.57±0.35^{a}	4.29±0.46^{bc}	4.29±0.72^{ab}	3.79±0.32^{ab}	4.00±0.22^{b}
10	14	80	6.00±0.31^{bcde}	3.64±0.26^{bc}	6.29±0.52^{abcd}	1.21±0.24^{a}	2.71±0.42^{cde}	3.86±0.34^{abc}	2.36±0.34^{abcd}	3.57±0.44^{a}	1.93±0.32^{de}	2.07±0.41^{bc}	2.71±0.18^{bc}	2.43±0.17^{cdef}
10	14	120	5.57±0.37^{bcde}	4.36±0.42^{ab}	6.93±0.54^{ab}	1.07±0.17^{a}	3.00±0.19^{bcde}	3.21±0.45^{abc}	2.86±0.47^{abcd}	3.86±0.56^{a}	1.57±0.23^{de}	1.79±0.34^{bc}	1.93±0.30^{c}	1.71±0.10^{ef}
10	16	100	5.57±0.53^{bcde}	3.29±0.26^{bcd}	6.07±0.35^{abcd}	1.29±0.18^{a}	2.57±0.23^{cde}	3.64±0.39^{abc}	2.50±0.22^{abcd}	2.71±0.31^{a}	2.64±0.42^{bcde}	2.64±0.34^{bc}	1.93±0.32^{c}	2.64±0.28^{bcdef}
10	18	80	5.00±0.36^{cde}	3.64±0.40^{bc}	6.36±0.39^{abcd}	1.29±0.24^{a}	2.14±0.24^{de}	4.07±0.58^{ab}	2.36±0.30^{abcd}	3.50±0.63^{a}	1.64±0.18^{de}	1.93±0.46^{bc}	1.79±0.15^{c}	1.86±0.09^{ef}
10	18	120	5.57±0.63^{bcde}	3.71±0.38^{abc}	6.57±0.23^{abc}	1.29±0.10^{a}	2.93±0.32^{bcde}	3.57±0.41^{abc}	3.00±0.24^{ab}	3.07±0.30^{a}	2.64±0.64^{bcde}	2.64±0.45^{bc}	2.43±0.17^{bc}	3.00±0.24^{bcdef}
15	14	100	4.43±0.55^{de}	4.14±0.21^{ab}	7.09±0.25^{ab}	1.50±0.15^{a}	2.21±0.29^{de}	4.50±0.45^{a}	3.29±0.21^{a}	3.79±0.29^{a}	2.29±0.53^{cde}	2.07±0.37^{bc}	2.00±0.22^{c}	3.00±0.41^{bcdef}
15	16	80	4.07±0.34^{e}	3.64±0.30^{bc}	7.21±0.50^{a}	1.57±0.28^{a}	1.86±0.21^{e}	4.57±0.52^{a}	2.93±0.23^{abc}	3.50±0.33^{a}	1.79±0.51^{de}	2.64±0.66^{bc}	2.00±0.27^{c}	2.57±0.13^{bcdef}
15	16	120	5.14±0.48^{cde}	5.21±0.34^{a}	8.00±0.50^{a}	1.50±0.24^{a}	2.57±0.28^{cde}	3.86±0.66^{abc}	2.86±0.30^{abcd}	3.71±0.59^{a}	1.29±0.32^{e}	1.50±0.33^{c}	1.71±0.24^{c}	1.57±0.28^{f}
15	18	100	5.43±0.44^{bcde}	4.21±0.29^{ab}	7.36±0.46^{a}	1.36±0.32^{a}	2.14±0.34^{de}	4.43±0.57^{a}	2.64±0.21^{abcd}	3.86±0.26^{a}	1.36±0.32^{e}	1.50±0.42^{c}	2.43±0.32^{bc}	2.36±0.21^{cdef}

Values are mean ± standard deviation (n=7). Means with the same letter in the column are not significantly different at $p < 0.05$. Key: FC= Feed Composition; FM= Feed Moisture; ET= Extrusion Temperature. The scale used for descriptive sensory profiling was 0 to 10, where 0 represents the lowest intensity and 10 represents the highest.

Table 8.3. Mean scores of the descriptive sensory attributes of instant porridge from flour stored in different packaging materials

Pkg.	Sample	ST	Colour	Specks	Aroma	Rancid	Sweetness	Bitterness	Umami	Astringent	Corny flavour	Starchy flavour	Stickiness	Rough texture
LDPE	Control	0	8.60±0.43^{a}	1.50±0.35^{c}	7.60±0.37^{a}	0.80±0.25^{c}	7.50±0.89^{a}	1.50±0.35^{b}	2.70±0.25^{b}	3.40±0.71^{a}	6.40±0.58^{a}	5.50±0.65^{a}	4.80±0.54^{a}	6.20±0.86^{a}
		30	8.50±0.32^{a}	3.30±0.25^{b}	4.40±0.33^{b}	1.70±0.12^{b}	4.30±0.34	2.90±0.43^{b}	3.30±0.34^{ab}	2.40±0.29^{a}	2.90±0.29^{b}	3.50±0.35^{b}	2.30±0.34^{b}	2.60±0.19^{b}
		60	9.30±0.25^{a}	4.70±0.25^{a}	3.60±0.24^{bc}	2.80±0.20^{a}	3.80±0.41^{b}	4.40±0.33^{a}	4.20±0.44^{a}	2.80±0.34^{a}	1.90±0.29^{b}	2.40±0.29^{b}	1.70±0.25^{b}	2.10±0.29^{b}
		90	9.30±0.25^{a}	5.10±0.19^{a}	2.90±0.19^{c}	3.10±0.19^{a}	2.70±0.34^{b}	4.70±0.25^{a}	4.40±0.33^{a}	2.60±0.19^{a}	1.50±0.16^{b}	2.00±0.16^{b}	1.20±0.34^{b}	1.10±0.29^{b}
	CLEF	0	5.10±0.70^{b}	5.00±0.45^{b}	8.00±0.71^{a}	1.10±0.29^{c}	2.50±0.32^{a}	4.90±0.56^{c}	2.90±0.43^{c}	4.30±0.64^{a}	1.50±0.42^{a}	1.50±0.35^{a}	1.90±0.29^{a}	1.80±034^{a}
		30	7.30±0.25^{a}	7.30±0.37^{a}	5.90±0.19^{b}	3.50±0.27^{b}	2.60±0.19^{a}	6.60±0.33^{b}	6.20±0.37^{b}	2.00±0.22^{b}	1.40±0.19^{a}	0.90±0.19^{a}	0.80±0.12^{b}	1.20±0.12^{ab}
		60	7.50±0.47^{a}	8.30±0.25^{a}	4.80±0.44^{b}	3.80±0.25^{ab}	3.04±0.23^{a}	8.00±0.22^{ab}	7.30±0.37^{ab}	2.20±0.37^{b}	0.90±0.24^{a}	0.80±0.20^{a}	0.60±0.10^{b}	0.70±0.12^{b}
		90	7.90±0.43^{a}	8.50±0.35^{a}	4.40±0.33^{b}	4.70±0.25^{a}	2.90±0.19^{a}	8.50±0.35^{a}	7.90±0.43^{a}	1.10±0.29^{b}	0.90±0.19^{a}	0.60±0.10^{a}	0.90±0.19^{b}	1.00±0.16^{ab}
Paper	Control	0	8.60±0.43^{a}	1.50±0.35^{c}	7.60±0.37^{a}	0.80±0.25^{c}	7.50±0.35^{a}	1.50±0.35^{a}	2.70±0.25^{b}	3.40±0.71^{a}	6.40±0.58^{a}	5.50±0.65^{a}	4.80±0.54^{a}	6.20±0.86^{a}
		30	8.60±0.40^{a}	2.80±0.25^{b}	6.50±0.35^{a}	1.10±0.19^{bc}	6.30±0.72^{ab}	1.40±0.19^{a}	3.00±0.22^{ab}	2.60±0.24^{a}	4.20±0.34^{b}	4.00±0.22^{ab}	2.90±0.19^{b}	2.80±0.25^{b}
		60	8.70±0.25^{a}	3.80±0.37^{ab}	4.90±0.43^{b}	1.90±0.19^{ab}	5.20±0.37^{bc}	2.70±0.25^{a}	3.30±0.25^{ab}	3.40±0.19^{a}	3.10±0.43^{b}	4.30±0.25^{ab}	2.40±0.37^{b}	2.40±0.37^{b}
		90	8.20±0.20^{a}	4.30±0.25^{a}	4.00±0.22^{b}	2.00±0.22^{a}	4.30±0.41^{c}	2.70±0.46^{a}	4.00±0.47^{a}	2.60±0.29^{a}	2.90±0.19^{b}	2.86±0.21^{b}	1.70±0.30^{b}	1.70±0.12^{b}
	CLEF	0	5.10±0.70^{ab}	5.00±0.45^{b}	8.00±0.71^{a}	1.10±0.29^{b}	2.50±0.32^{a}	4.90±0.56^{b}	2.90±0.43^{c}	4.30±0.64^{a}	1.50±0.42^{a}	1.50±0.35^{a}	1.90±0.29^{a}	1.80±0.34^{a}
		30	4.60±0.33^{b}	6.90±0.43^{a}	7.40±0.53^{ab}	2.30±0.41^{ab}	2.90±0.29^{a}	5.30±0.25^{ab}	3.90±0.19^{bc}	3.10±0.19^{ab}	1.80±0.20^{a}	0.90±0.19^{a}	1.00±0.22^{ab}	1.30±0.25^{ab}
		60	6.50±0.35^{a}	7.00±0.50^{a}	6.80±0.58^{ab}	2.70±0.34^{a}	3.20±0.37^{a}	6.50±0.27^{a}	5.20±0.37^{ab}	2.70±0.25^{ab}	0.90±0.19^{a}	0.90±0.19^{a}	1.00±0.22^{ab}	1.00±0.16^{ab}
		90	6.40±0.33^{ab}	7.50±0.35^{a}	5.20±0.37^{b}	3.00±0.22^{a}	3.48±0.27^{a}	6.60±0.40^{a}	6.20±0.44^{a}	2.50±0.35^{b}	0.90±0.19^{a}	0.70±0.12^{a}	0.80±0.20^{b}	0.70±0.20^{b}

Key: Pkg= Type of package material; CLEF= Cassava-Leaf Enriched Flour; ST= Storage Time; Means (n = 7) with the same letter in the column within each treatment are not significantly different at $p < 0.05$.

5. Anova table for objective 3

The ANOVA Procedure
Dependent Variable: Apperance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	185.8571429	4.4251701	5.64	<.0001
Error	377	295.7714286	0.7845396		
Corrected Total	419	481.6285714			

Dependent Variable: Taste

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	203.0952381	4.8356009	6.24	<.0001
Error	377	292.2952381	0.7753189		
Corrected Total	419	495.3904762			

Dependent Variable: Aroma

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	224.8571429	5.3537415	5.33	<.0001
Error	377	378.7047619	1.0045219		
Corrected Total	419	603.5619048			

Dependent Variable: Flavour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	273.0809524	6.5019274	6.83	<.0001
Error	377	358.6595238	0.9513515		
Corrected Total	419	631.7404762			

Dependent Variable: Texture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	226.9809524	5.4043084	5.60	<.0001
Error	377	363.5809524	0.9644057		
Corrected Total	419	590.5619048			

Dependent Variable: Overall acceptability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	257.5952381	6.1332200	7.08	<.0001
Error	377	326.4880952	0.8660162		
Corrected Total	419	584.0833333			

Appendix 6. Some pictures during experimentation