

**OPTIMIZATION OF EXTRUSION COOKING FOR A PROTEIN AND DIETARY
FIBRE RICH INSTANT FLOUR FROM A COMPOSITE BLEND OF RICE (*Oryza
sativa*), SORGHUM [*Sorghum bicolor* (L.) Moench] AND BAMBOO (*Yushania alpina*)
SHOOTS**

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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

JULY, 2021

DECLARATION AND RECOMMENDATION

Declaration

This Thesis is my original work and has neither, wholly or in parts been presented nor concurrently been presented for the conferment of any degree in Egerton University or any other institution.


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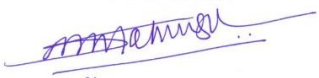
Recommendation

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DEDICATION

This Thesis is dedicated to my family.

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ABSTRACT

Consumer interest in convenience foods is expected to continue growing world-wide due to the ever-busy lifestyle and urbanization. The challenge has been the nutritional shortcomings linked with convenience foods such as higher glycaemic index values since they are extruded from sifted cereals which are very high in starch, but low other nutrients. Thus, the objective of this study was to innovatively develop a high protein and high dietary fibre instant flour from composite blends of rice (*Oryza sativa*), sorghum (*Sorghum bicolor*) and bamboo (*Yushania alpina*) shoots. First stage was to formulate blends from the three ingredients by optimization of protein, fibre and other nutrients using the Mixture Design Analysis (MDA) modelling approach. Second stage was to determine the effect of extruder ingredient variables: feed blending, feed moisture and feed rate on the resultant products physico-chemical and shelf-life properties. Optimization modelling by MDA was by use of Minitab[®] software. Processing employed a factorial design where five blends (100:0:0, 70:30:0, 60:30:10, 55:30:15 and 50:27:23 for rice, sorghum and bamboo shoot flour (BSF) respectively, on dry weight basis), three levels of water addition (15, 20 and 25 kg/h) and two feed rates (1,800 and 2,100 kg/h) were used. A single screw dry extruder with barrel temperature of 250°C and screw speed of 1,480 rpm were used. The dry extrudates were milled to particle size of 0.2 µm to obtain instant flour. Rice was rich in carbohydrates at 77.7%, BSF were richest in protein and fibre at 27.2% and 23.66%, respectively, while sorghum had the highest total phenolic and condensed tannins of 45.51 (mg GAE/kg) and 2.51 (mg CE/g), respectively. Optimization showed that increasing the amounts of BSF and sorghum in rice resulted in significant increase in all nutritional components of resultant blends. Optimum blend was established to have a ratio of 50:27:23 for rice, sorghum and BSF, respectively, with 13.4% protein, 6.2% fibre and 3.9% total minerals. Extrusion increased total phenolics and *in-vitro* protein digestibility by 96.3 and 36.9%, respectively in instant flour as compared to their respective raw blends. Extrusion reduced dietary fibre and condensed tannins by 14.9 and 88.7%, respectively. Increasing BSF in feed blends significantly caused increase in carbohydrates by 0.3-4.0% and fat by 0.01-0.2% while reducing protein loss from 1.4% to 0.03% and increasing fibre loss from 0.03% to 2.4%. Water addition rate caused significant increase of carbohydrates but reduced the loss of fibre and increased loss of fat. Feed rate only significantly affected carbohydrates. Hence, the findings of this study for the first time demonstrate that sorghum and bamboo shoots offer potential for development of novel and nutritious instant flour that can meet diverse consumer needs.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
ASAL	Arid and Semi-Arid Land
BD	Bulk Density
BSF	Bamboo Shoots Flour
CE	Catechin Equivalent
Cfu/g	Colony forming units per gram of the sample
CHO	Carbohydrates
CI	Concentration Index
CML	Carboxyl Methyl Lysine
CT	Condensed Tannins content
DMB	Dry Matter Balance
DOE	Design of Experiment
EPR	Energy to Protein ratio
ER	Expansion Ratio
EV	Energy Value
FA	Fatty Acids
FAO	Food and Agriculture Organization of the United Nations
GAE	Gallic Acid Equivalent
GI	Glycaemic Index
GOK	Government of Kenya
HCN	Hydrogen Cyanide
IDF	Insoluble Dietary Fibre
IVPD	<i>In-Vitro</i> Protein Digestibility
KEFRI	Kenya Forest Research Institute
MAG	MonoAcylGlycerols
MC	Moisture Content
MD	Managing Director
MDA	Mixture Design Analysis
MFC	Mau Forest Complex
MRP	Maillard Reaction Products
NTFP	Non-Timber Forest Products

OHC	Oil Holding Capacity
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PROC GLM	Procedure for General Linear Model
Rpm	Revolutions per minute
RTE	Ready-To-Eat
SAS	Statistical Analysis system
SC	Swelling Capacity
SME	Specific Mechanical Energy
TAG	TriAcylGlycerols
TCC	Total Coliforms Content
TDF	Total Dietary Fibre
TMB	Total Mass Balance
TP	Total Phenolic content
TVC	Total Viable Content
TVP	Texturized Vegetable Products
WAI	Water Absorption Index
WHC	Water Holding Capacity
WHO	World Health Organization
WSI	Water Solubility Index
YM	Yeasts and Moulds
Δ NtCm	Changes on Nutritional components

LIST OF SYMBOLS

\$	United States America dollar
%	Percentage
[A]	Concentration of a substance
[A _{lim}]	The standard acceptable maximum concentration limit
[A _o]	Initial concentration of a substance
°C	Degrees Celsius
μ	Micro
°	Degrees
°K	Degrees kelvins
®	Registered trade mark
a*	Red to Green scale of colour
b*	Yellow-blue scale of colour
C*	Chroma
D	Screw diameter
<i>e</i>	Axial flight width
<i>E_a</i>	Apparent activation energy (J/mol)
h	Barrel channel height
<i>h</i>	Planck's constant (6.63×10^{-34} J.S)
h*	Hue angle
J	Joules
k	Reactions rate constant
K _B	Boltzmann's constant (1.38×10^{-23} J/°K)
<i>k_o</i>	The pre-exponential factor of Arrhenius model
L*	Lightness scale of colour
Ln	Natural logarithm
M _f	Extruder feed rate (Kg/h)
mol	Moles
N	Number of flights
<i>N</i>	Screw speed in revolutions per minute
<i>P</i>	Power rating in kW
Q ₁₀	Temperature coefficient
R	Molar gas constant (8.314 J/K/mol)

R^2	Coefficient of determination
t	Pitch
T	Absolute temperatures in Kelvins
T	Torque of the extruder (N.M)
T_g	Glass transition temperature
T_{ref}	Absolute reference temperature
δ	Clearance
ΔG^*	Change in Gibbs free energy of activation (kJ/mol)
ΔH^*	Change in enthalpy of activation (J/mol)
ΔS^*	Change in entropy of activation (J/mol °K)
ΔX	Change on component X
φ	Helix angle
Φ	Helix angle
ω	Angular velocity (radians/s)
ϵ	Flights thickness

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Any food to be consumed without further heating or processing can be defined as a convenience Ready-To-Eat (RTE) product. Some of the preliminary process performed on foods during processing such as slicing, washing, chopping, and preservation are always not considered though necessary in the unit operations of processes that define convenience foods. Consumer interest in RTE snacks is currently trendy. It is expected to grow rapidly over the next decades, mainly due to their convenience, availability, appearance, taste and texture (Brennan *et al.*, 2013; Omwamba & Mahungu, 2014). It was projected that the worldwide market for convenience RTE foods to rise to USD 630 billion by 2020. The rise in the market of the RTE foods has been alluded to arise from the demand of the customers for protein-rich foods, foods with functional properties and presence of snacks believed to be organic. Hitherto, the most consumed and popular RTE products emanates from potato chips amounting to around 24% of the world's total sales. Next to the potato chips, are the cereal products that are extruded that account for around 23% of the total global sales with a difference of only 1% from the potato chips (Makowska *et al.*, 2018). The RTE foods with high fibre content have attracted attention due to various health benefits associated with them. Though various technologies exist for processing RTE foods such as canning, extensively, cooking and processing through extrusion has provided an opportunity and convenience in the processing of cereal-based convenience RTE foods.

The process of product development recently has gained importance with extrusion processing contributing much to the sector enhancing production of products that are convenient to the consumers. These products include but to mention a few modified starch, pasta, breakfast cereals, baby formulations, snacks (puffed products), and foods with texturized proteins (Altan & Maskan, 2011). The main reported for the adoption of this technology is its extreme versatility (Han & Tran, 2018; Offiah *et al.*, 2018). Extrusion process is versatile as it can be used to produce products with different shapes and textures that provide a product pool for consumer choice and likeability. Versatility in terms of its ease of operation and ability to produce a variety of textures and shapes which appeal to the consumers. In extrusion, there is utilization of high temperatures in combination with short-time and it simultaneously combines pressure, mechanical shear and thermal heating to achieve the desired quality of the extruded product. For achievement of the extruded products by the extrusion process, different

biochemical changes occur on the raw materials such as protein denaturation, starch gelatinization, cooking process and dextrinization leading to formation of dextran during the heating process, and hence formation of the convenience RTE foods. During the process of extrusion, there is sudden decrease in pressure as the high-pressured product exit the die causes the product to puff due to increased number of gas cells in the product resulting to a puffed texture. This texture profile due puffing, essential biochemical reactions, together with the cooked characteristics of the extruded convenience food makes them very appealing to many consumers.

Raw materials from cereals-based and tuber-based crops have been used widely in the extruded products. However, they are very rich in carbohydrates/starch but relatively low in other nutritional components. Therefore, the cereal and tuber-based convenience RTEs are energy intense but low in protein levels (Devi *et al.*, 2013). Originally, whole-grain sources were used as raw materials for many of the cereal-based convenience food. The cereal-based products such as the corn flakes and bran flakes were obtained from the steamed grains by the flaking process. Recently, with the increase and invention of different process and flour separation and refining technologies, compositing technology has been applied in making of the extruded snacks where different flours are used and not a whole grain (Brennan *et al.*, 2013). The use of refined flours in the current world has been catapulted by what the consumers want in reference to the foods the whites consume which are fine-textured and tastier. However, the downside of milling and refining process is that it removes the bran which is rich in essential phytochemicals, minerals and fibre. Therefore, the refined cereal flours are clearer or white compared to then non-refined and they have high amounts of starch, low bioactive components for instance the arabinoxylan and ferulic acid, with less fibre, minerals, and vitamins that are highly concentrated on the outside layer of the cereal grain (Broekaert *et al.*, 2011; McCance & Widdowson, 2014). As a consequence, resultant convenience RTE food products from refined flours exhibit high glycaemic index (GI) values (Brennan *et al.*, 2013).

Hitherto, researchers and product developers have concentrated on enriching the cereal based RTE food products with protein, dietary fibre and other bioactive ingredients. This is achieved by incorporating protein, dietary fibre and bioactive compounds rich food ingredients. For example, RTE food products containing pumpkin (Norfezah *et al.*, 2011), soy and sorghum [*Sorghum bicolor* (L.) Moench] (Omwamba & Mahungu, 2014), tomato processing by-products (Dehghan-Shoar *et al.*, 2011), fruits and vegetable by-products. Some of the fruits that have been used include apples, pears, oranges, peaches, blackcurrants, cherries and

artichokes. Vegetables used include asparagus, onion, carrot, pomace and cauliflower (Stojceska *et al.*, 2008), coconut (Okafor & Ugwu, 2014) and yam (Alves *et al.*, 2002). These ingredients are high in fibre besides being rich in proteins, vitamins and bioactive compounds. The fruits and vegetables were reported to affect positively not only the nutritional composition of the RTE food products, but also the sensory parameters. However, reports on utilization of food ingredients that are high in dietary fibres (18-21% dry weight basis), proteins (17-24% dry weight basis), and bioactive compounds such as bamboo shoots during processing of RTE products using extrusion cooking technologies are scarce. According to Singhal *et al.* (2018), the food potential of bamboo shoot per se remains unexploited despite being reported to be very rich in the macronutrients: protein, carbohydrate, mineral and dietary fibre. Bamboo shoots have been reported to confer health benefits attributed to its components exhibiting prebiotic capability, nutraceutical capacity, and having compounds that can freely scavenge radicals (Chongtham *et al.*, 2011; Nirmala *et al.*, 2007).

Final RTE extruded products and their qualities vary depending on the extrusion parameters. These parameters include the cooking extrusion parameters that entails the feed rate, feed moisture, temperature of the barrel, extruder type, configuration of the screw, speed of the screw, and the composition of the raw materials among many others (Miller & Mulvaney, 2000). Maize, rice (*Oryza sativa*), oats, potato, sorghum, millet, barley, cassava and wheat are commonly used flours as raw materials in extrusion process to provide starch which is a key component that provides structure to convenience foods (Campden, 2001). Among the above cereals, producers have grown much interest in using rice flour as composite flour in convenience foods as a result of its appealing white colour, its tasteless nature, digestibility easiness and hypoallergenicity. On nutritional basis, the protein composition of rice is relatively low (averaging 6 - 8 grams/100grams dry basis) as compared to other cereal based crops, and therefore, need to enhance the protein and other nutritional components of snack products made from it (Dalbhagat *et al.*, 2019; Omwamba & Mahungu, 2014).

Incorporating sorghum in rice presented promising alternative of enriching the rice-based convenience foods (Omwamba & Mahungu, 2014). Sorghum is an important crop which is able to tolerate low rainfall of semi-arid areas but its protein solubility and functionality is low. However, extrusion processing improves its protein solubility and functionality (Llopart *et al.*, 2014). Several studies have shown that incorporating sorghum flour into other cereal flours is the cheapest way of enriching them with nutrients such as vitamins, minerals, fibre and polyphenols (Abah *et al.*, 2020). Based on the health benefits associated with phytochemicals

in the sorghum, and its peculiar nutritional composition, it has higher potential in product development and making of nutritious functional products (Patil *et al.*, 2018). In comparison to rice and maize, in terms of extrusion processing, sorghum has almost similar properties to the latter two cereals (Ratnavathi & Patil, 2013).

Therefore, making of a RTE food product from blends of rice flour, sorghum flour and bamboo shoot flour blends will have nutrient contents high enough to meet daily recommended requirements for proteins, dietary fibre and calories, among other nutrients. However, to determine the appropriate blends, mixture design analysis (MDA) technique was critical during ingredients optimization. The purpose of incorporating bamboo shoots of *Yersinia alpina* species and sorghum (*Sorghum bicolor* L.) into rice (*Oryza sativa*) was to develop a blend that contains at least 10% protein, 5% fibre and 3% minerals on dry weight basis. These ingredient formulation variables can be optimized through the use of Mixture Design Analysis (MDA) to obtain the best blend (Abadou *et al.*, 2020). Thus, one of the aims of this study was to develop a RTE food product that is nutritious and convenient using locally available but, underutilized foods. This was informed by the current global trend on addressing food and nutrition security in emerging world economies. The trend involves enriching locally available and underutilized foods through the processing into convenient value-added food products such as RTE to meet the changing lifestyles. During the value addition processing, extruder ingredient variables vis-à-vis: feed composition, moisture and feed rate, are among the key variables that influence the nutritional properties of a food during extrusion (Camire, 2011).

Mixture Design Analysis (MDA) has been widely used in the optimization of ingredients during formulation. According to Arteaga *et al.* (1993), the mixture design was created to enable the study of the effects of each ingredient in a formulation. The design provides critical information on the importance of the ingredient interaction in a formulation (Montgomery, 2017). Mixture designs differ from other designs such as Taguchi and the response surface methodology which are usually used in the optimization of the process variables. For the MDA process, the responses generated are usually a computation of the amounts of each variable commonly referred to as runs. The MDA optimization has an advantage over other systems used in optimization studies because it only requires few experimental populations, enables understanding of how the variables interact between themselves and lastly, MDA has been proved to be efficient in discovering the experimental conditions that are essential for running experiment (Bezerra *et al.*, 2019). Thus, it is widely used in the formulation and

development of new products, designing the same products, and improvement of products that already exist (Hu, 2017; Montgomery, 2017).

1.2 Statement of the Problem

Consumer demand for convenience foods is on the rise and is expected to remain that way. Many of these convenience foods are made from refined cereal flours which are low in protein and dietary fibre content. This has caused convenience foods to be linked to health-related challenges such as exhibiting high glycaemic index values that is a predisposing factor for obesity and diabetes type 2. This has called for the improvement of their nutrient composition through enriching with other ingredients. Recent studies have focused on incorporating food materials that are high in protein and dietary fibre into convenience foods during processing. Bamboo shoots are known to have high dietary fibre and protein content while sorghum is rich in minerals among other nutrients. Therefore, the role they play and can play in human diet cannot be understated. However, their utilization in many parts of the developing world, including Kenya, have remained to be as traditional cuisines but not on commercial scale. This is attributed to either lack or minimal processing technologies of bamboo shoots and sorghum that ensures increased accessibility, safety and availability. Especially *Yushania alpina* which is a native bamboo species in Kenya has not been utilized in enrichment of extruded convenience foods. One of such processing technologies that can increase sorghum and bamboo shoot utilization is to make a protein rich convenience food through extrusion cooking process. The aim of this study was therefore to develop an instant flour blend from rice, sorghum and *Yushania alpina* shoots that can be used in making convenience foods. To achieve this, there was need to not only investigate the effect of ingredients formulation and extrusion parameters in making the snack, but also employ mixture design analysis (MDA) in order to optimize these factors.

1.3 Objectives

1.3.1 General Objective

To contribute to improving the nutritional value of rice-based extruded convenience foods through incorporating bamboo shoots and sorghum to optimize protein, dietary fibre and polyphenols.

1.3.2 Specific Objectives

- i. To determine the effect of ingredients optimization on the physico-chemical properties of the composite flour blend containing rice, sorghum and bamboo shoots

- ii. To determine the effect of the extrusion cooking conditions optimization on nutritional properties of the extrudates from composite flour blend containing rice, sorghum and bamboo shoots
- iii. To determine the effect of the extrusion cooking conditions optimization on the microbial load and shelf life of extrudates from composite flour blend containing rice, sorghum and bamboo shoots
- iv. To determine the effect of the extrusion cooking conditions optimization on the physico-chemical properties of extrudates from composite flour blend containing rice, sorghum and bamboo shoot

1.4 Hypotheses

- i. Ingredient's optimization has no significant effect on the physico-chemical properties of the composite flour blend containing rice, sorghum and bamboo shoots
- ii. Optimization of the extrusion cooking processing conditions has no significant effect on the, nutritional properties of the extrudates from composite flour blend containing rice, sorghum and bamboo shoots
- iii. Optimization of the extrusion cooking processing conditions has no significant effect on the microbial load and shelf life of extrudates from composite flour blend containing rice, sorghum and bamboo shoots
- iv. Optimization of the extrusion cooking processing conditions has no significant effect on the physico-chemical properties of extrudates from composite flour blend containing rice, sorghum and bamboo shoots

1.5 Justification

With increasing health consciousness amongst consumers coupled with the rising demand for functional foods, there are great opportunities for sorghum and bamboos utilization in processing and production of different nutritious foods. This will enhance socio-economic growth and related health and nutritional benefits, and development of the respective communities. Their utilization will ensure that the convenience foods provide not only optimal vital nutrients to the consumers but also reduce the risk of low glycaemic index foods. Similar convenience foods made from soy bean are already in the market. Bamboo shoots consumption has been found to confer health benefits to humans beyond providing basic nutrients. According to Nongdam and Tikendra, (2014) and Nirmala *et al.* (2014), bamboo shoots have a very are highly perishable and therefore, for them to last longer and for future consumption and availability, value addition is necessary and, in the process, retaining phytochemicals and

macronutrients. Additionally, more efforts are required in production of novel food products innovatively made using bamboo shoots incorporation apart from their common consumption as vegetables only. Moreover, higher nutritional value in bamboo shoots, micronutrients of about 0.68-1.38% such as bioactive compounds (for instance β -sitosterols, campesterols and stigamasterols phenolic compounds and phytosterols), and minerals and likehave the potential for being used as essential ingredients in coming up with functional foods (Chongtham *et al.*, 2011; Nirmala *et al.*, 2014). The optimization techniques such as MDA will reduce the cost and time required for the formulation and the preparation of the respective RTEs compared to conventional experimentation. Convenience foods can be useful during times of emergencies to mitigate against hunger and malnutrition among the vulnerable populations. This study aimed at making an instant flour with 10% protein and 5% dietary fibre which can be used in making snack bars and instant porridge flour.

1.6 Scope and Limitations of the Study

The research was on on edible shoots from only one species of bamboo, *Yushania alpina*, which is native in Mt. Elgon, Kenya (map is given in Appendix I). *Yushania alpina* from Mt. Elgon was chosen because it is the only place in Kenya where bamboo shoots reported to be consumed (Karanja, 2017). Mt Elgon lies between latitude 0⁰ 48' and 1⁰ 30' North, and longitude 34⁰ 22' and 35⁰ 10' East. It is characterized by undulating landscape with the altitude rising from 1800 m above sea level in the south to about 4300 m to the north, both in Kenya. The mean annual rainfall is 1800 mm with a pattern showing bimodal type of rainfall. The long rains occur between March and June, while the short rains are from September to November. The temperature varies between 14 °C and 24 °C with lower altitude experiencing a higher temperature (Kiptum *et al.*, 2011). The study will focus on optimizing extrusion cooking conditions of only one blend that will have optimum protein and dietary fibre after formulation.

1.7 Definition of Terms

Convenience foods this are food prepared either ready to eat (RTE products) or intermediately prepared (requires minimal final cooking) and the preparation processes (energy input, culinary skills and partial cooking) are done by the processors/distributor and not by the consumer at the home-based kitchen (De Boer *et al.*, 2004).

Design of Experiment this is a section of statistics application which handles research planning, how the research is conducted, analysis of data, and interpretation of tests that are under control to do evaluation of different factors that regulate a variety of parameter (Li & Du, 2006).

Food materials science is an emerging discipline of Food Science, adopted from Materials Engineering field, that deals with exploration of food crop and animal materials' composition and structure with a sole purpose of achieving innovations in ingredients and processes so as to meet quantitative and qualitative food demand occasioned by growing world population.

Glycaemic index is the physiological ability of a carbohydrate food to raise blood sugar after consumption measured on a scale of 0 -100 in comparison to equal amount of reference food (glucose).

Mass balance is the concept applied to the analysis of physical systems by accounting for material entering and leaving a system and mass flows hence resulting in conservation of mass. This concept is derived from the fundamental laws of physics which states that mass can neither be created nor destroyed, that is, mass is conserved.

Mixture design analysis is the special class of designed experiments in which the product under investigation is made up of several ingredients that form mixtures or composite blends. The response is the quality or performance of the product based on some criterion that depends on the relative proportions of the ingredients.

Non-Timber Forest Products (NTFP) have been defined as any product other than timber that is naturally produced in forests and can be harvested for human use without cutting down trees. Foods such as bamboo shoots, nuts, berries, mushrooms and seeds, or non-food items such as oils, perfumes and medicinal plants are classified under this category.

Optimization is the statistical process of designing a system by adjusting set of parameters using mathematical functions without violating constraints with the purpose of minimizing cost and maximizing throughput and/or efficiency.

Thermodynamics is a concept in physical science that deals with energy, its transformations and application in food processing, derived from the phenomena of law of conservation of energy which states that can neither be created nor destroyed.

CHAPTER TWO

LITERATURE REVIEW

2.1 Convenience Foods

Foods that require very minimal handling, for example mild heating/warming for ready-to-eat products or rehydration in hot/cold water for ready-to-cook/ready-to-heat foods are classified as convenience foods (Saxena, 2017). The convenience foods industry, especially snacks sector, is reported to be one leading in terms of fastest growth among the food sectors in global market. The majority of the world's population have made the snack foods to be an integral part of their eating habits. Globally, the convenience foods market was estimated to grow by 3.2 % from \$1.1 trillion in 2011 to \$1.3 trillion in 2016 (Yu *et al.*, 2016). Currently, consumers are health conscious as well as showing a preference for foods that requires less time to cook or already cooked foods such as snacks or ready-to-serve foods (Ghoshal, 2018). Developing countries, including African countries, have also recorded increase in demand for convenience foods and change in traditional eating habits among their populations.

Since social changes are with no doubt altering the pattern of how traditionally foods are prepared, it can therefore be alluded that proper designing of convenience foods can provide that important contribution to nutrition in societies. According to De Boer *et al.* (2004), the three key reasons identified as perceived potential determinants for convenience foods increase among consumers are include the aspect of value for money, value for health and the element time. Therefore, this poses a challenge to food processors and researchers to continually develop cost effective nutritious convenience foods that will meet and exceed this dynamic lifestyle of consumers. Hence, current research approach on convenience foods is focused on fusing food materials science and processing technologies in addressing the above mentioned three key reasons. This Chapter therefore focuses on reviewing information on possibility of fusing extrusion processing technology with food materials science to develop nutritious convenience food using rice, sorghum and bamboo shoots.

2.2 Use of Extrusion Technology in Processing of Convenience Foods

2.2.1 Extrusion Cooking

Food extrusion is basically defined as the process of driving food materials to flow under a variation of processing operations units such as kneading, melting and/or shear, through an extruder die which is predesigned to shape and/or expand the material (Offiah *et al.*, 2018). Extrusion cooking involves the use of high temperature from the steam injected into the barrel

of the extruder or from friction in barrel and the mechanical energy dissipated by the screws which then transforms the biopolymers into a plasticized and flow-able melt hence establishing of intra- and intermolecular chemical bonds. Extrusion cooking or hot extrusion phenomena arise when food materials inside the extruder are heated to temperatures above 100°C (Chen & Rosenthal, 2009). Cooking and high-pressure of 3400-13800 kPa are predesigns of the extruder that ensures thermal energy is conveyed into the raw materials in the barrel before exiting a die (Kazemzadeh, 2012). So as to reduce plasticization temperature and circumvent undesirable thermal degradation effects, usually a plasticizer is added. Plasticizers are defined as the compounds made of molecules with very low weight and possess the ability to penetrate into polymer matrix thereby reducing the glass transition state (T_g) through weakening intermolecular forces causing increase in the volume and mobility. The most known universal plasticiser used in feed and food processing is the moisture/water. However, there very little information is available regarding the efficiency of other approved plasticizer in such systems (Ahmad *et al.*, 2018; Matveev *et al.*, 2000).

Due to the element of versatility, extruders as equipment of processing convenience foods allows the production of a variety of foods of nutritional importance. Versatility is attributed to the inherent blending capability of extruders and simultaneously process sundry ingredients which is key to principle of exploitation applied in the development of novel and functional foods (Ramachandra & Thejaswini, 2015). Hence, versatility of extrusion technology provides a handy opportunity for development convenience foods are very rich in nutrients from a wide range of raw materials and proving a foundation for value addition (Pathak & Kochhar, 2018). So, it is no coincidence that extrusion cooking remains to be the most common technique of manufacturing convenience foods from food plants that are rich in proteins which has recently captured not only the interest but also the attention of food processors and researchers over the recent years (Ajita & Jha, 2017). Besides snack foods and breakfast cereals, extrusion is increasingly being applied in improving the nutritional quality of convenience foods such as increasing the amount of insoluble dietary fibre (IDF), resistant starch, total dietary fibre (TDF), polyphenols and antioxidant compounds (Stojceska *et al.*, 2010).

There are three main stages in the formation of all the convenience foods: First is the formation of a dough. Dough is formed through hydration of starch polymers which causes formation of mass that assumes fluid properties which is vital in shaping of individual pieces. Secondly is then the heating of the formed dough mass which results in water to be superheated and be

released rapidly as vapour leading to the dough mass puffing and lastly, the drying of the product to low moisture levels is done so as to stabilize it by forming a hard brittle structure (Guy, 2001). Starch granules are melted to remove all their crystalline structure during dough formation stage in starch-based raw materials. This is the reason that informs convenience cereal-based foods formulations, where the primary ingredient must either be a grain itself or a grain-derived component so as to provide the functional properties. Extruded products may be processed from flour, whole-grain, or grain fractions (Offiah *et al.*, 2018).

Extrusion cooking has several advantages but the main ones are as follows: 1) Versatility: this is through the ability to alter formulation of ingredients and processing parameters so as to produce/manufacture a limitless number of food products, such as cereal-based, sugar-based and protein-based categories. 2) High productivity attributed to a continuous processing regime which gives a much greater production capability than other processing regimes. 3) Low costs associated with labour, operations and space requirements. 4) High quality products associated with simultaneous combination of high temperatures and short time required in extrusion cooking which helps in retaining heat sensitive components. Lastly, 5) This process not associated with effluents because of low moisture operations (Chen & Rosenthal, 2009).

Besides extrusion cooking, there is also another extrusion technology known as cold extrusion which is worth mentioning. In cold extrusion, also known as forming, there is no addition of external heat to the extruder as the feed is pumped through it (Singh & Heldman, 2009). This then means that these cold/forming extruders operate at comparatively very much lower pressure and without use of heat energy than extrusion cooking. Cold type extruders are used in manufacturing of food include pasta extruders, pretzel formers and any of the many of these types have been designed for sole purpose of forming a dough-like consistency and shaping of ingredients that will later be cooked to a final consistency (Kazemzadeh, 2012). As stated earlier that purpose of this review is to focus in exploring extrusion processing technology in development of convenience foods, cold extrusion will not be emphasized as extrusion cooking.

2.2.2 Extruder

In summary, an extruder basically constitutes of a pump, heat exchanger and a continuous pressure- temperature bioreactor (Berk, 2009). Bioreaction gravitates into transformation of raw ingredients into intermediate and finished products that will have modified characteristics (Gray & Chinnaswamy, 1995; Pathak & Kochhar, 2018). Bioreaction is facilitated by mechanical shear and thermal energy to food ingredients (Sreejith, 2019). Based on mode of

operations, extruders can further be grouped into: Hydraulic ram also known as piston type, roller-type and screw-type. Hydraulic and roller type extruders are purposively excluded from being focused on in this review.

Screw-type extruders exist in two different configurations: single-screw and twin-screw (Muthukumarappan & Karunanithy, 2012; Offiah *et al.*, 2018; Singh and Heldman 2009). As shown in Figure 2.1, screw-type extruders are segmented into four main sections: feed, transition, metering and die.

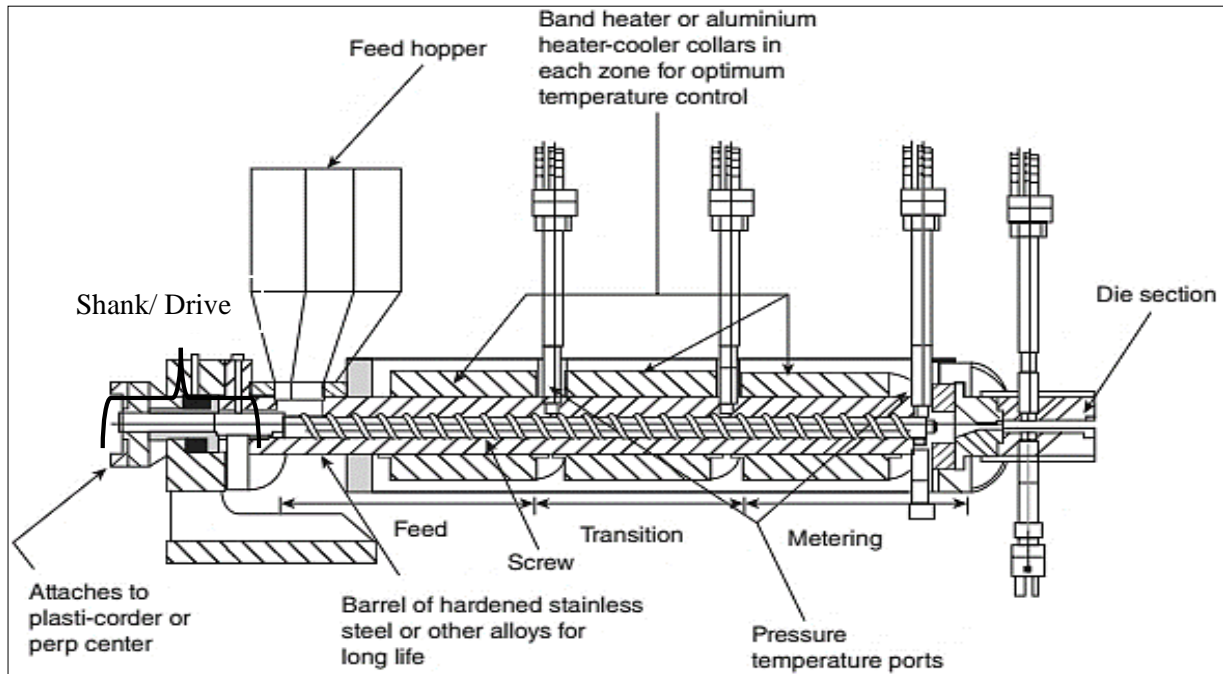


Figure 2. 1: A schematic illustration of an extruder and its principal sections

Source: Muthukumarappan and Karunanithy (2012) with some modifications

Key components of the extruder that influence unit operations are:

1) Screws

The roles of screws are transport materials in the extruder outlet, mix the ingredients, bring about the melting and generate a homogeneous stable supply of polymer melt in barrel (Ajita, 2018; Yacu, 2011). The following are important screw parameters as provided in the illustration of Figure 2.2:

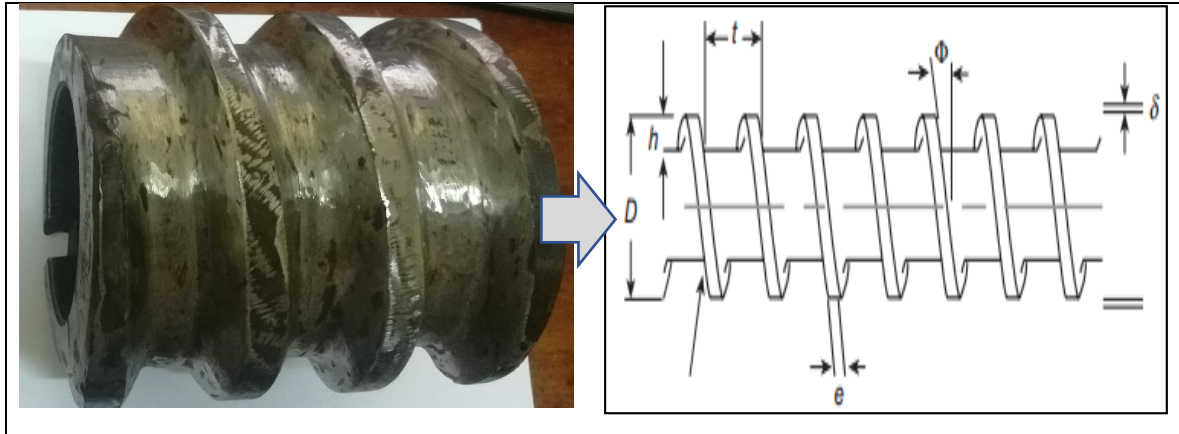


Figure 2. 2 : A photo and schematic illustration of an extruder screw and its parameters

Source: Muthukumarappan and Karunanithy (2012) with some modifications

Where: t = Pitch, which is the distance between consecutive flights; Φ = Helix angle which is the angle between the flight and the line perpendicular to the screw shaft which usually varies between 12 and 15°; h = Channel depth is the distance from the top to the root of the flight
Screw diameter (D)= is the distance between two flights across the shaft; Clearance between flight tips and barrel (δ) is usually 0.5mm and this ensures efficient pumping of the material and Axial flight width (e) of a screw is usually recommended to be 10% of screw diameter.

2) Barrel

Extruder barrel is the cylinder casing, which sometimes is jacketed, that houses the screw(s). Barrel are mechanically designed to be very strong so as to not only endure the pressure developed in it during operation but also to resist tear and wear due to friction created by moving metallic parts (Frame, 1994; Harper & Clark, 1979; Muthukumarappan & Karunanithy, 2012).

3) Die

This are the orifices fitted at the exit end of the barrel. They are the openings through which the extruded product passes through. Dies influence products' physical properties, increased barrel volumetric capacity, residence retention time and energy input (Ajita & Jha, 2017; Fang & Hanna, 2010; Harper & Clark, 1979; Muthukumarappan & Karunanithy; 2012).

4) Feed System

Extruder feed system comprises of the hopper, feed throat, speed auger, feeder weigh belts, steam injection system and water feed system that are designed to ensure that not only loading of the raw materials into the barrel is constant and but also remains uninterrupted throughout operation (Harper & Clark, 1979; Muthukumarappan & Karunanithy, 2012; Ramachandra & Thejaswini, 2015; Sreejith, 2019). Dry extruders are special because they capitalize on internal

friction as their source of heat for cooking. Originally dry type extruders were developed to processing whole soybeans but in recent times they have diversified to process other foods (Riaz, 2019; Riaz, 2001). This type of extruder is of particular interest because it was used for work in Chapters 4, 5 and 6.

5) Shank

The shank, also called the drive system, key function is to facilitate provision of power that causes screws to rotate in the barrel of the extruder. The drive usually consists of a drive mechanism usually an electric/direct current motor, gear reduction, torque transfer and bearings support systems (Harper & Clark, 1979; Muthukumarappan & Karunanithy, 2012).

2.2.3 Extruder Variables

Apart from extruder type, other primary variables can be classified into two broad groups: extruder variables and ingredient variables as illustrated in Figure 2.3.

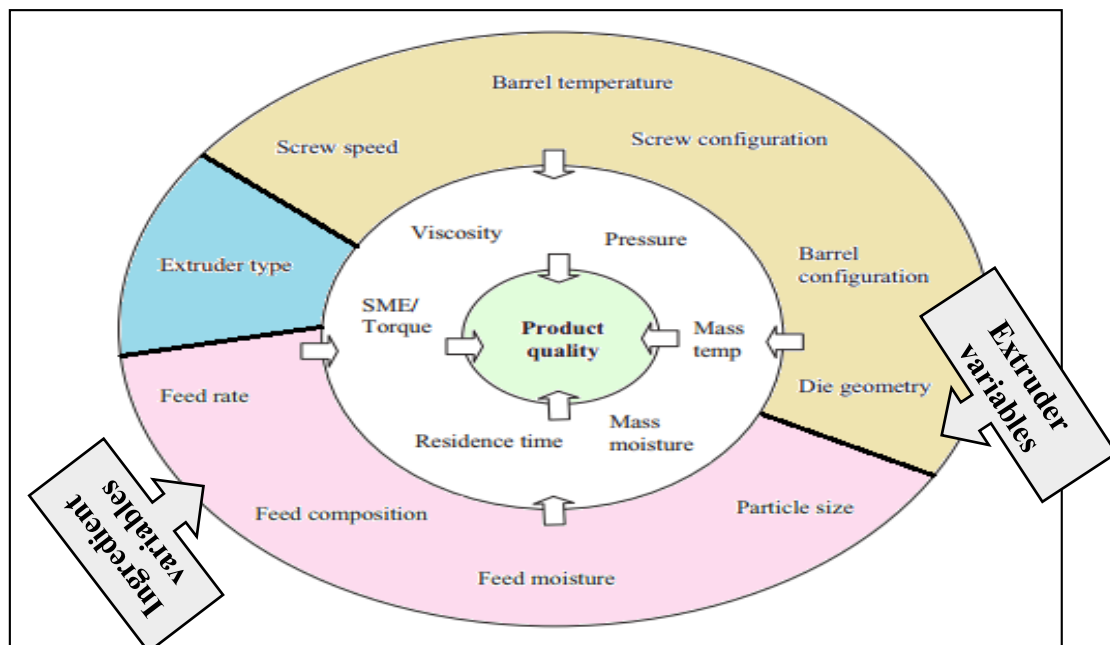


Figure 2.3 : Interrelationships among extruder variables and ingredient variables

Source: Muthukumarappan and Karunanithy (2012) with some modifications

Ingredient variables include feed: 1) composition, 2) moisture, 3) rate and lastly 4) particle size. Extruder variables include screw speed and configuration, barrel temperature and configuration and die geometry (Bhattacharya, 2011; Moscicki & Van Zuilichem, 2011). Both ingredient and extruder variables must be manipulated at varied degrees in the development of new convenience foods. Manipulation of ingredient variables is usually guided by the scientific understanding of food materials.

2.3 Frontier role of material science on processing nutritious convenient foods

2.3.1 Influence of materials science in Food product development

According to Lilliford *et al.* (2008), Food Materials Science can simply be defined as study of the architecture of living biological structures/systems with purpose of deriving food fit for human consumption. Natural biological systems are sourced from plants and animals (Vincent, 2008). On other hand, Food processing is defined as the cumulative operations that together culminate in the conversion of biological systems based on specific and non-specific interactions among and between components into foods respectively (Tolstoguzov, 2008). According to Hermansson (2008), food biopolymers from biological systems are proteins and polysaccharides. Polymer science utilization is diversifying at a growing rate from of synthetic polymer (plastics) industries to in Food Science and Technology (Sanauacos & Maroulis, 2001). Food biopolymers determine the structure of the extruded products which consequently affect quality of the resultant products (Kim *et al.*, 2009).

2.3.2 Relationship between extrusion and material science

Effect of material science and extrusion on the quality attributes of final product are encompassed in the four feed variables: composition, moisture, particle size and rate as illustrated in Figure 2.4. Degree of manipulation of these variables has the potential to give forth infinite number of extruded food products. Feed composition consists of proximate constituents of the specific raw material or blend of raw materials, which all contribute differently towards the quality of the extruded product. Another variable, feed moisture, plays a critical role that determines multiple chemical and biochemical reaction as well as flow behaviour of melt that happen in the barrel of the extruder. Also, particle size of the feed is another variable that plays an important role based on the principle of surface area to volume ratio that determines moisture distribution, heat transfer and viscosity in the barrel. Feed rate determines the mass flow rate within the reaction chamber/barrel and thus the residence time. (Muthukumarappan & Karunanithy, 2012).

Two of the ingredient variables: Feed rate and moisture levels, are usually manipulated during extrusion cooking. Feed rate is usually controlled by the motor attached to the feed hopper while feed moisture can be controlled by either preconditioning or injecting water to the barrel section. Feed particle size is manipulated during milling operation where sieve size and type of the miller determines final diameter particles. Last but very important is the composition of the feed which is manipulated during blending, if more than one ingredient is used. Choice of

ingredient to be used in the blending is dictated by the nutritional and physical properties that are desired to be embodied in the final product (Xu, 2020).

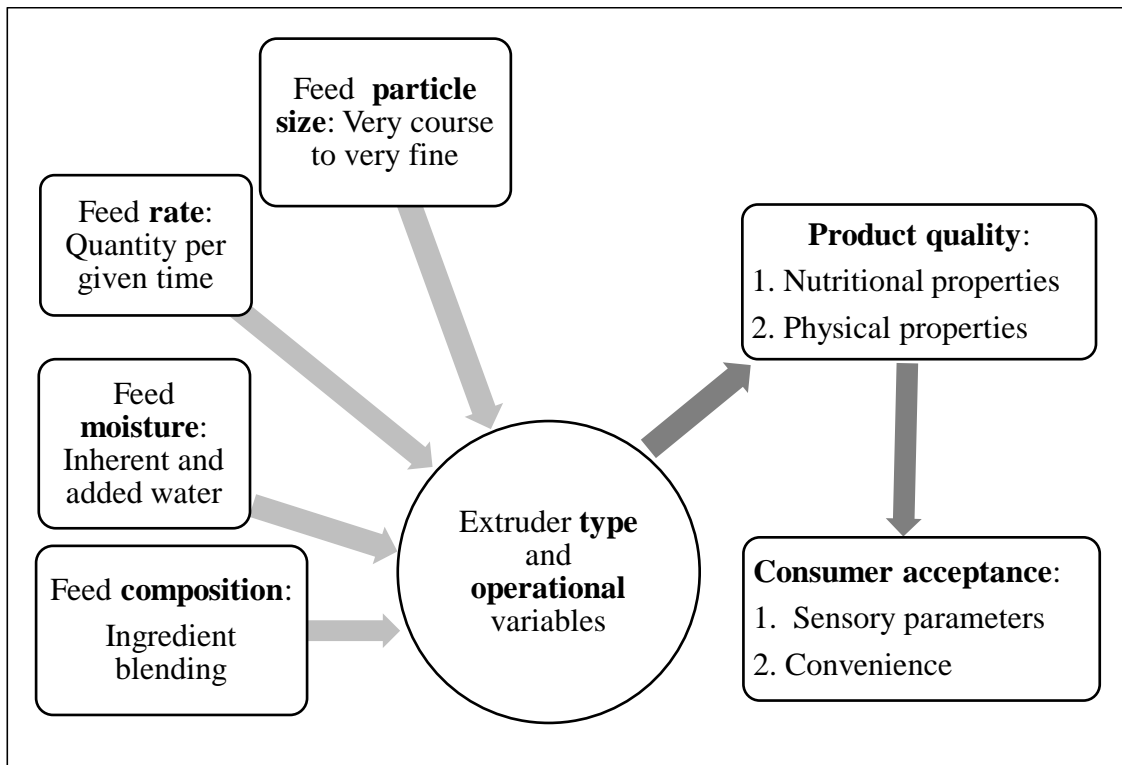


Figure 2. 4: Effect of extruder variables and ingredient variables on product quality

2.4 A case study of materials science and extrusion cooking

2.4.1 Possible utilization of bamboo shoots to enrich rice and sorghum for development of a nutritious convenient food

Some studies have shown that bamboo shoots dietary fibres possess two attributes that are very important: 1) ability to strongly adsorb cholesterol in the human gut during digestion and, 2) potential prebiotic component. Therefore, this is the reason why bamboo shoots are regarded as a bioactive ingredient holds numberless possibilities in functional foods production (Wu *et al.*, 2020).

2.4.2 Rice (*Oryza sativa*)

Rice is the most used base ingredients manufacture of convenience foods. This is because starch granules of rice are the rated to be smallest, doesn't affect the taste of the product and are easiest to digest in comparison to other grain starches. This property of excellent digestibility has made rice flour to find extensive use in the manufacture of infant formulas and functionally as the alternative to wheat for gluten-intolerant individuals. However, rice starch composition exhibits very high variability, for instance normal rice varieties have amylopectin

of 73–85% while waxy rice varieties have 100% amylopectin. A by-product of rice milling is broken rice is a cheaper alternative. The main shortcoming of rice is that during processing, proteins of rice exhibit poor functional properties when compared to other plant proteins (Offiah *et al.*, 2018).

2.4.3 Sorghum [*Sorghum bicolor* (L.) Moench]

Sorghum [*Sorghum bicolor* (L.) Moench] along with wheat, rice, maize and barley are the most common staple foods consumed globally. Starch granules in sorghum are uniquely present in the pericarp. Sorghum grain is a good source of nutrients, phenolic compounds, neutral in flavour and sometimes slightly sweet therefore it makes very adaptable flour for multiple uses. Sorghums can be milled directly into whole-grain flour to produce cookies, cakes, brownies, breads, pizza, pasta, pancakes, waffles, fermented and unfermented flat bread, porridges, malted into alcoholic and non-alcoholic beverages and extruded products (Asif, 2011).

Extrusion processing of crops categorized as underutilized grains and pseudo cereals has provoked a particularly unique interest to the cereal processing research and new products development as avenue of ensuring food and nutrition security. Grains such as sorghum (*Sorghum bicolor* L.), millets (pearl millet, finger millet and others), rye (*Secale cereal* L.) and ancient wheat species (einkorn, emmer, spelt wheat) have been recently classified as underutilized (Sinkovic, 2016). Sorghum utilization in RTE has increased because it is not only gluten free but also rich in nutrients especially high amount of dietary fibre, protein, minerals and phytochemicals. Sorghum is also known to possess functional properties such as anti-oxidant, anti-celiac, anti-carcinogenic, anti-diabetic and cholesterol reducing abilities Extruding sorghum has been reported to increase the bioavailability of catechins (Camire, 2011)

2.4.4 Bamboo shoots

Bamboo shoots are known to be rich in constituents that have functional attributes and they include active peptides, amino acids, polysaccharides, flavonoids, phenolic compounds and phytosterols (Ge *et al.*, 2017). Besides nutritional profile that has attracted attention in fortifications and management of health-related conditions, bamboo fibre is now a common ingredient in blends that that are used to manufacture breakfast cereals, fruit juices, bakery and meat products, sauces, shredded cheeses, cookies, pastas, snacks, frozen desserts, and many other food products (Maroma, 2015; Mustafa *et al.*, 2016). One advantage of bamboo as a plant is that it is a fast-growing crop in different agro-climatic conditions in the world and therefore it possess the potential to meet the increasing demand of edible fibre by the food industry.

Bamboo shoots dietary fibre component has shown prospects that it can play a role of providing many other benefits associated with human health beyond nutrition hence meeting different requirements that are expected to be inherent in functional foods (Song *et al.*, 2018). According to Nirmala *et al.* (2014), contemporary foods that been labelled as functional contain dietary constituents that have been proven to provide health benefits beyond basic nutrition. This is the trait that predestines bamboo shoot as a candidate food that is well endowed with various nutrients, as shown in Table 2.1. For the purposes of utilization of bamboo shoots as an ingredient in the formulation of functional foods, two different forms have been exploited: powder and paste.

Table 2. 1: Nutrient composition of major constituents of bamboo shoots on wet basis

Component	Content (%)	Reference(s)
Moisture Content	88.00-92.00	Mustafa <i>et al.</i> (2016) and Nirmala <i>et al.</i> (2008)
Crude Proteins	2.00-4.00	Nirmala <i>et al.</i> (2008)
Crude Fat	0.30-4.00	Mustafa <i>et al.</i> (2016) and Singhal <i>et al.</i> (2013)
Total minerals	0.60-3.80	Nirmala <i>et al.</i> (2008)
Dietary Fiber	2.10-4.50	Mustafa <i>et al.</i> (2016) and Nirmala <i>et al.</i> (2008)
Carbohydrates	2.00-9.40	Nirmala <i>et al.</i> (2008) and Singhal <i>et al.</i> (2013)

According to Oke *at al.* (2012) bamboo shoots are classified under sprouting vegetables and contains characteristics of vegetables. Starches and dietary fibres such as cellulose, hemicellulose, pectic substances and lignin are the major polysaccharides found in vegetables such as bamboo shoots. Cell walls of young vegetables are primarily made up of cellulose. But as these vegetables age, they become higher in hemicellulose and lignin because which defines their structure. Unfortunately, processing of aged vegetables does not affect their consistency because of hemicellulose and lignin toughness and fibrous nature. Parenchyma cells predominate the structural feature of these vegetables because they are usually located in important regions that metabolism and other functional roles. For instance, parenchymal cells located in the tissues of the vegetables' leaf are organized in such a manner that photosynthesis occurs efficiently, while in cells of tissues located in roots are full of granules of starch. Also, the elasticity and permeability of parenchyma cells directly affects the texture of vegetables while the lipid constituent that is mainly found in the cuticles usually influences their characteristic flavour. Among the vegetables that have been scientifically shown to contribute towards curbing malnutrition and lowering the prevalence of some lifestyle diseases such as hyperlipidemia and diabetes mellitus, bamboo shoots remain the favourites (Wang *et al.*, 2020).

A report by the government of Kenya fingered out that one of the long- term forestry practices to be explored in rehabilitating the dilapidated Mau Forest complex (MFC) is sustainable bamboo production and processing (Government of Kenya, 2009). According to Karanja (2017), there are both indigenous and exotic bamboo species in Kenya. The indigenous variety *Yushania alpina* is native and found in protected areas where felling is prohibited by the government since 1986. Due to the government ban on felling of the *Yushania* species, the Kenya Forest Research Institute (KEFRI) took the initiative and introduced into the country over 20 species exotic species mainly from Asian countries. However, only fifteen of the exotic species have been successfully cultivated in various Kenyan agro-ecological zones. The successful species are *Bambusa bambos*, *Dendrocalamus strictus*, *Dendrocalamus giganteus*, *Dendrocalamus hamiltonii*, *Dendrocalamus asper*, *Dendrocalamus membranacea*, *Bambusa tulda*, *Bambusa vulgaris var. striata*, and *Thyrsostachys siamensis*. Consumption of the shoots of *Alpina* has been reported in Cherengany' and Mt. Elgon regions with an average annual consumption of 1,200 kg in each of the market centres and towns. The major counties with highest bamboo cover in Kenya are Nakuru (8,565 ha), Mt Elgon (10,250 ha), Nyeri (25,133), Kiambu (5,723 ha), Nyandarua (9,060) and Narok (4,207 ha). Globally, bamboo forest has been estimated to covers an area of about 31.5 million ha which is approximately 1% of the total forest cover. Contrary to the reported continuous decline in total global forest area for the last over the last 30 years, bamboo forest area has shown a gradual increase of about 3% per annum. Two Asiatic countries, that is India and China, have the largest total bamboo forest areas of 9.57 million ha and 6.01 million ha, respectively (Wang *et al.*, 2020).

1) Processing of Bamboo Shoot Based Food Products

Freshly harvested bamboo shoots can be processed in very many different methods before consumption. But canning, drying and fermentation are the most used processing methods though roasting, boiling, blanching and pickling have also been explored (Wang *et al.*, 2020). According to Singhal *et al.* (2013), bamboo shoots have been integrated in many recipes such as *halwa*, chutneys, *pulao*, curries, *bhaji* etc. Bamboo shoot flour/powder, juices, beers and crisps have been produced in different regions of the world. Bamboo shoots powder has also been successfully incorporated in cookies, biscuits, nuggets and crackers (Choudhury *et al.*, 2015; Mustafa *et al.*, 2016; Nirmala *et al.*, 2014). It has been reported that bamboo shoots have been included in other food products such as frozen dough, meat, dairy, beverages, spices and condiments and (Zhang *et al.*, 2017). But currently, studies on extrusion cooking of bamboo shoot powder are focussing on producing foods with modified dietary fibre (Ge *et al.*, 2017;

Song *et al.*, 2018). Modified dietary fibres offer a wide spectrum of functional properties that provides potentiality of numerous applications in novel food product development (Elleuch *et al.*, 2011).

Pre-processing unit operations employed during of handling of harvested bamboo shoots culms usually has been linked to the quality features of the final processed products. Drying is one of the effective methods that reduces water activity, inactivate enzymes, reduces hydrogen cyanide (HCN) and inhibit microbial growth contributing to preservation. Upon complete dehydration, the bamboo shoots lose moisture to below 10 g/ 100 g. So as to achieve dehydration, several methods such as use of solar, hot air, microwaving or combination of these techniques can be applied. In ancient years, open air solar drying was the only method which resulted in dried bamboo shoots having unpleasant dark appearance due to the enzymatic and non-enzymatic browning reactions. In modern times, the freshly harvested bamboo shoots are first blanched either in hot water or dilute acid solution before drying which gives a yellowish appearance (Wang *et al.*, 2020).

2) Hazards Associated with Bamboo Shoots

Besides all benefits associated with bamboo shoots, it contains Taxiphyllin (4-hydroxy-(R)-mandelonitrile- β -Dglucopyranoside) which is a cyanogenic glycoside. Taxiphyllin is an unstable compound which breaks down to form two other compounds: cyanohydrins and sugar. Cyanohydrins then rapidly decompose to form hydrocyanic acid (HCN) and a hydrocarbon which could be an aldehyde or a ketone. This hydrocarbon is the one responsible for the characteristic bitterness. HCN concentration levels vary between parts of bamboo shoots and within the same parts of different portions. Bamboo shoots naturally contain very high levels of HCN which could be as much as 1000 mg/kg. However, HCN is removed by upon processing with such methods as involve use of heat or fermentation (Moller & Seigler, 1999; Singhal *et al.*, 2013).

2.4.5 Application of Design of Experiment (DOE) in Optimizing Blends

Design of experiments (DOE) are approaches that systematically borrows statistical methods and apply them to the experimental process (Bezerra *et al.*, 2020). The systematic approach is the one that guides the experimental investigation process of all the variables as designed in a given experiment and have been hypothesized to influence product quality. During the experimental hypotheses' formulations, only variables that have direct role that can improve or enhance intended product's manufacturability, reliability, quality and field performance are identified. Considering that by nature resources are limited, it is very important to only identify

variables that will provide the most of essential information from each experiment to be performed. It is with no doubt that well designed experiments always produce significantly more information from very few experimental runs in comparison to haphazardly or unplanned experiments. The designing of these experiments often happens in four phases: 1) planning, 2) screening (characterization), 3) optimization and 4) verification.

Mixture design analysis (MDA), is a special type of designed experiments where variables, also called factors, are the ingredients or components to make a mixture or blend while the response variable becomes a function of the proportions/amounts of each ingredient. These amounts of each ingredient are characteristically measured by weight, volume and mole ratio (Myers & Montgomery, 1995). Therefore, it can be concluded that MDA is a designed experiment where the proportions/amounts of the components are more important than their magnitude (Mathews, 2005). So as to achieve the goal of developing a nutritious convenience food product from blend of rice, sorghum and bamboo, MDA comes in handy. It helps to cut down the cost and time required to come up with blends.

According to Bezerra *et al.* (2019), the following are types of MDA: 1) Lattice simplex design: This design is generally considered to be the correspondent of factorial experimental designs. This is because experimental points created by the process variables are taken at the ends of the experimental domain. In the case of more than two experimental levels, these experimental points with some consistence are spaced along with the coordinates that represent the variables. Typically, this mixture design consists of a (x, b) coordinates given that x components fit a polynomial regression model on degree of freedom b , where $b-1$ experimental points can be taken equally spaced between levels 0 and 1. 2) Centroid-simplex design: This design was developed to address the inadequacies associated with simplex lattice mentioned above. This is achieved by reducing the number of coefficients of a polynomial regression model and as a consequence reducing the number of experimental points. The results of reducing experimental points leads to economic use of time required to the experiment and quantities of reagents. A polynomial regression function called special cubic model is used for the ternary mixtures. Cubic models are designed in such a way that they contain only terms that represent experimental points related to the presence of components in equal amounts within the demarcated experimental space used. In this, at least one central point is always included in the design so as the model could evaluate possible synergistic and/or antagonistic relationships that appear that could take place during the MDA.

The coordinate systems employed in MDA assume a triangular configuration which enables easier visualization the relationships between the ingredients in a spatial arrangement within the mixture. The ingredients making up the mixture are restricted by one another in such a manner that the components must always add up to the total amount or whole, usually 1.0 or 100%. Applications of mixture experiments are in useful in the formulation of blends from different foods, where the amount of a given nutritional component of interest in the final blend will be depend on contribution of each ingredient for that special component and also the interaction effect between the ingredients (Myers & Montgomery, 1995; Tola & Ramaswamy, 2012). Several computer softwares such MINITAB[®], Design-Expert[®] and MATLAB[®] usually used to perform MDA. Therefore, this explains the choice of MDA by use of MINITAB[®] software to enable optimization of nutrients enrichment process in rice by use of sorghum and bamboo shoots.

2.4.6 Expected Extruded Product

Most convenience foods are processed from starch-based foods which include cereal and tuber-based crops. These foods are usually high amounts in starch, but low in other nutritional constituents. This is the reason that makes many convenience foods to be regarded as energy dense, thus the name empty calorie foods. These convenience foods manifest high glycaemic index (GI) values. Demand for convenience food products which not only satisfy them from hunger but also must be low in fat, rich in dietary fibre, vitamins and minerals is now the growing trend among consumers. Such a healthier convenience could be developed from foods that naturally contain high proportions of essential nutrients (Brennan *et al.*, 2013). Therefore, the ambitious expectation of the rice, sorghum and bamboo shoots convenience food product is to meet not only to meet the nutritional requirement for the consumers but also to possess acceptable sensory attributes.

The term Glycaemic index (GI) is usually used to refer to the physiological ability of a food to elevate blood glucose after ingesting measured on a scale of 0 -100 (Jenkins *et al.*, 2002). It is known as incremental area under the blood glucose response curve elicited by 50 grams portion of available carbohydrate of a test food expressed as percentage of the response after a 50 g portion of a reference food (glucose) taken by the same subject. GI is classified in three categories based on the rate of starch absorption: GI above 70 is high, between 70 and 55 is intermediate and below 50 is low (Matthan *et al.*, 2017). Low GI foods were found to play a role in reducing risks of Cardio Vascular Diseases, diabetes mellitus and certain cancers (Clar *et al.*, 2017).

2.4.7 Mass and Energy Balance

It is anticipated that varying the feed conditions will consequently affect mass and energy properties during extrusion cooking. Mass, m , and energy, E , are properties governed by principles of conservation where they cannot be created and also, they cannot be destroyed in any process. But these properties have a unique ability, in that, they can be converted to each other according to the established engineering formulas (Cengel & Boles, 2007; Dincer & Rosen, 2012). Mass and volume flow rates are related as shown in equation 2.1:

$$\dot{m} = \rho \dot{v} = \frac{\dot{v}}{v} \dots \text{Equation 2. 1}$$

Where: \dot{m} is the mass flow rate, ρ is the density, v is the specific volume and \dot{v} is the volumetric flow rate of a substance in a container.

According to Berk (2009), the principle of mass conservation when applied in the control of volume is essentially expressed as: The net mass transfer to or from a control volume during a time interval Δt is equal to the net change (increase or decrease) in the total mass within the control volume during Δt . The lack of equilibrium within any given system usually results in what is called transport phenomena that includes fluid flow, heat and mass transfer and electric current. In principle, transport phenomena always obey a universal law, which is characteristically similar to the Ohm's law, expressed as: The rate of transport (i.e., the quantity transported per unit time) is proportional to the driving force and inversely proportional to the resistance of the medium to the transport. Models that govern the conservation of mass, energy and momentum are fundamentally regarded as mathematically analogous (Özilgen, 2011).

Fourier's equation (shown in equation 2.2) essentially used in modelling of heat transfer through solid foods which occurs through conduction while Fick's law of diffusion (shown in equation 2.3) is used in describe mass transfer (Özilgen, 2011; Wang & Sun, 2012): These two equations are stated as follows

$$\frac{dQ}{Adt} = -k \frac{\partial T}{\partial z} \dots \text{Equation 2. 2}$$

$$\frac{dm_B}{Adt} = J_B = -D_B \frac{\partial C_B}{\partial z} \dots \text{Equation 2. 3}$$

Where Q =heat transferred, J; T =temperature, K; t =time, s; k =thermal conductivity of the medium, J.s⁻¹.m²; z =distance in the direction of the transport, m; m =mass of substance B transferred, mol; C_B concentration of substance B, mol.m³; D_B diffusivity (coefficient of diffusion) of molecular species B through the medium, m².s⁻¹. The minus sign before the gradients serves to indicate that heat flows in the direction of decreasing temperatures and mass travels in the direction of decreasing concentration.

According to Wang and Sun (2012), thermal processing of foods involves transfer of heat between the source and the centre zone inside the food through an interface which could be the food surface or container wall which can be steady state or unsteady state. As pointed out earlier, heat and mass transfer follow the same pattern which is described by either Fourier's law or Fick's' law. This is because mass transfer in itself assumes two mechanisms:1) molecular diffusion and 2) convective mass transfer. Molecular diffusion mechanism is produced on condition that there exists a concentration gradient of a given component between two different points of the system.

2.4.8 Mass Flow properties

The flow characteristics of a food depends on Newtonian and the non-Newtonian phenomena of that food material and also its time-independent and time-dependent behaviours (Doublier & Lefebvre, 1989). Solute molecules a solution, make random Brownian motions whose intensity tends to exhibit inverse proportionality between liquid/solid systems with diffusion medium due to the molecular matrix getting more rigid (Özilgen, 2011). According to Gray & Chinnaswamy (1995), that net flow inside an extruder is complex and it is a combination of three forms of flow: 1) drag, 2) pressure and 3) leakage as shown in Figure 2.5

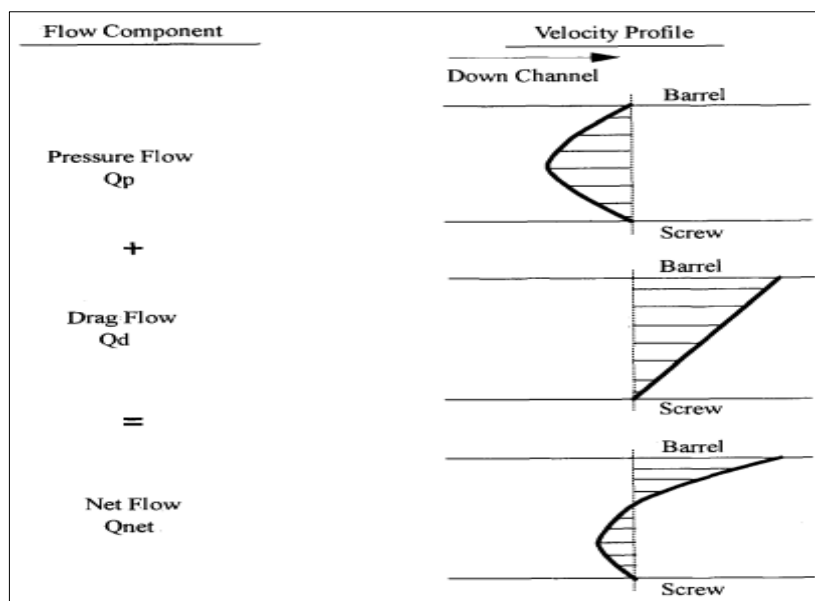


Figure 2. 5: Flow mechanisms of materials within an extruder

Source: Gray and Chinnaswamy (1995)

The drag type of flow is due to velocity of the extruder screw in relation to the barrel. This is because of the velocity of the melt being conveyed down the barrel by means of the screws which is zero at the screw surface but as it moves away from the barrel wall it increases

gradually. As a consequence, drag flow is what is responsible for the forward movement of materials through the barrel of the extruder. On other hand, pressure flow occurs due to the difference in pressure between the extruders' feed zone and the metering zone thereby creating what is known as a pressure gradient between the two points. This means that this type of flow moves in the direction of from the die to the feeding section Pressure is controlled by the size of the die opening and the screw configuration. Lastly there is also leakage flow, which is similar to the pressure flow in terms of being driven by pressure gradient and the same direction flow. It is called leakage flow because it is the gap between the barrel and the screw, also known as the clearance. This type of flow can also happen in the case where screws do not fully intermesh, especially in twin screw extruders.

Therefore, net flow mechanism of a material within an extruder is shown in equation 2.4:

$$Q_{net} = Q_d + Q_p + Q_l \dots \text{Equation 2. 4}$$

Where Q_{net} = Net flow; Q_d = Drag flow; Q_p = Pressure flow and Q_l = Leakage flow.

Properties of food biopolymers directly influence transport properties of foods in the extruder. Examples of food biopolymers that influences the transport characteristics of materials within an extruder are the structural proteins such as elastin, keratin and collagen, storage proteins such as glutenin, globulin, albumins and prolamins, structural polysaccharides such as cellulose, hemicelluloses, pectins, and gums, storage polysaccharides such as starch and lignin. Besides mass transport properties, food biopolymers also influence phase transitions within the extruder barrel. Examples of phase transitions that take place in foods include freezing, glass transitions, gelatinization and crystallization (Sanauacos & Maroulis, 2001).

2.5 Influence of Extrusion on Food Properties

2.5.1 Physical and Rheological Properties

To accomplish extrusion cooking of food, factors such as moisture level of the feed, barometric conditions, thermal properties and shear properties are manipulated to modify the microstructure of the material being processed. As a result, extrusion eventually improves the tastiness, shelf-life, and hydration stability. Also, the solubility, ability to swell, hydration properties, viscosity, the holding capacity of water, lateral expansion, colour, oil holding capacity and texture are improved (Ge *et al.*, 2017). Extrusion influences the rheological properties, for example Young's and elastic modulus, fracture stress, yield stress and strain, Poisson's ratio, coefficient of friction and fracture toughness (Dobraszczyk *et al.*, 1987). Quality assurance of extruded foodstuffs employs use of rheological measurements as a tool

that ensures that variations in raw materials do not have any effect the consistency of the final products. Hence a clear understanding of rheology modelling is very important to a food manufacturer in the bioprocess design, economics and consumer preferences control (Özilgen, 2011). However, among the main food components, starch composition: amylose-amylopectin ratio plays a key in determining the attributes of the resultant product. The amylose component of starch provides weightlessness, springiness, surface and texture uniformities, but a gluey surface. But on other hand, amylopectin causes an extruded product to have a hard texture as well as puffing less. Hence, it has been recommended that an amylose proportion of 5 to 20% in starch causes the extruded snack products to have satisfactory crispness and desirable (Gray & Chinnaswamy, 1995).

The Drag and pressure are the two features act together to dictate the material flow within a screw extruder. Drag flow is caused by screw rotation in the extruder barrel and it is directional proportional to the speed of rotation resulting in a forward viscous flow. On contrary, pressure flow which is caused by the built-up of a higher pressure at the die section of the extruder and it flows in a reverse direction to drag flow. The main factors that affect the overall net flow within the extruder are the rheological properties of food material and the operation conditions such as temperature, pressure, the diameter of the die aperture and screw speed. Overall productivity of the extruder and the optimum operating pressure are main guiding principles in the choosing of the die. (Chen & Rosenthal, 2009). Another rheological property that plays an important role in flow in an extruder is viscosity. In nature most food substances can be described have a thixotropic viscosity because they tend to thin on shearing. This can be translated to mean that as the shear on a given food substance increase, viscosity will proportionally decrease (Gray & Chinnaswamy, 1995). Viscoelastic food materials exhibit both viscous and liquid properties thus displaying elastic recovery from deformation when stress is removed (Gan & Lam, 2014). The optimization of food material processing technology affects the specific mode of viscoelastic foods material degeneration mechanism (Lu *et al.*, 2015). A summary of rheological and viscoelastic properties of foods are given in Figure 2.6.

Study by Brent Jr *et al.* (1997) proved that the storage modulus of extruded melts reduced by about two orders in magnitude from the glassy to rubbery regimes which is attributed to the reduction in physical crosslinks and/or entanglements of molecules. When compared to other flour melts in the extruder, oat melted at above glass transition temperatures due to the higher levels of protein and lipid. Rheological models that are used to study deformation behaviour of foods includes the integral models as well as differential viscoelastic models that derivatives

of dilute solution and concentrated dispersion theories respectively. Prediction of the rheological deformation behaviour of food materials over a wide range of strain rates requires use of suitable constitutive modelling of food polymeric. The food rheology deformation behaviour directly affects the food quality attributes such as sensory qualities and stability (Kokini & Dhanasekharan, 2009).

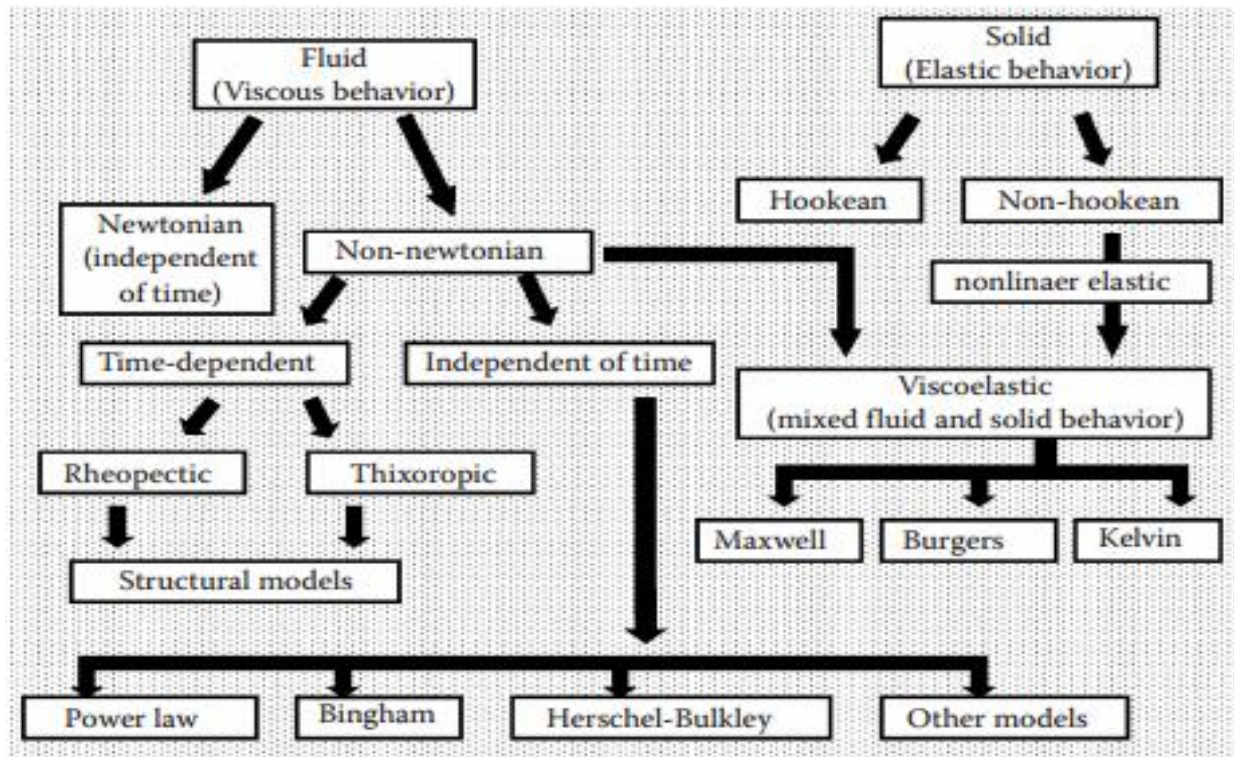


Figure 2. 6: Steffe's classification of rheological and viscoelastic behaviour of fluids and solids

Source: Özilgen (2011)

The assumption of taking an extruder to a rheometer where the feed material exhibits dynamism due to heat and shear pressure helps in understanding the materials behaviour within the extrusion system. The dynamic nature of food materials exists in three different categories: thermoplastic, thermosetting and non-functional. Food materials such as starches and sugars that when heated to a melting point always solidify on cooling belong to a group called thermoplastic raw materials. Some food materials such as proteins that which once they are heated, they undergo glass transition phase and become cured where molecules acquire the tendency to be very strongly held together to an extent that when they cool and reheated, they will fail to follow the same original pathway and therefore not flow are categorised as thermosetting raw materials. The last category is the non-functional food materials such as

minerals, fibres, and cellulose which under temperatures presented in the extruder not only fail to undergo glass transition phase but also do not participate in the building of the matrix of the resultant product. These non-functional food materials when heated to a high enough thermal energy, they will bypass the amorphous phase and decompose. (Kazemzadeh, 2012)

According to Soria and Villamiel (2012) and Tamanna and Mahmood (2015), in addition to the cooking step, extrusion also involves toasting operations that cause non-enzymatic browning to cereal-based food products due to caramelization and Maillard reactions. Both reactions results depend on degree of heating, composite formulation, level of moisture, and hydrogen ion potential. Foods that contain high protein content as well as high reducing sugars content at moderate moisture level tend to undergo Maillard reactions on heating in comparison to caramelization reactions that depend directly on heat and other conditions to cause degradation of carbohydrates. These two non-enzymatic reactions also depend on other chemical reactions that have the probability of taking place during extrusion processing. The cumulative non-enzymatic reactions that happen in extrusion of cereal-based foods greatly contribute to their typical organoleptic characteristics. To control and optimize extrusion conditions during manufacture of cereal-based foods, it is important to evaluate the extent of non-enzymatic browning by use of suitable indicators. Types of indicators used in evaluating non-enzymatic browning include furosine, available lysine, Hydroxymethylfurfural (HMF) furfural products and calorimetric properties. Colour formation has always been used to understand the rate of non-enzymatic browning in foods without taking into account individual kinetic mechanisms because they are extremely complex for cereal products.

2.5.2 Nutritional and Chemical Properties

The amount of nutrients retained after during extrusion is controlled by a number of factors. (Camire, 2011). Combining pressure, temperature and shear exposure effects in an extruder gives rise to several simple and complex chemical reactions and modifications to the nutritional profile food and by extension causing changes to its functional properties. Gelatinization, solubilization and dextrinization/ complex formation of starches, protein denaturation, polymerization/ cross-linking and texturization, partial or complete deactivation of enzymes, non-enzymatic browning, denaturation of vitamins and inactivation of anti-nutritional factors are the biochemical reactions and modification (Offiah *et al.*, 2018; Stojceska *et al.*, 2009).

Denaturation is one of the most important and predominant chemical change among other several that occur on protein during extrusion cooking because it exposes enzyme accessible sites and destroying enzyme inhibitors. This chemical change subsequently improves

properties such as the protein digestibility of that given food product. This is because during denaturation disulfide bond linkages are broken causing formation of smaller units from the dissociation of high molecular weight proteins. Consequently, re-alignment of protein molecules through cross-linking and restructuring leads to extruded food product developing a chewy texturized product. Irrespective of the type of raw food material, blending ratio and extrusion conditions, the Maillard reactions between protein and sugars that takes place eventually leads to the decrease the protein quality, (Steel *et al.*, 2012). Protein-rich plant materials such as soybeans, groundnuts and wheat make products imitate the texture and the appearance of meat during extrusion in a process known as texturization. Texturized vegetable protein products are (TVPs) with the example common of meat extenders in comminuted meat products such as meat patties, sauces and burgers and as meat analogues or imitation meat are gaining popularity among consumers worldwide (Zhang *et al.*, 2018).

Physico-chemical reactions involving gelatinization, hydrolysis, and non-enzymatic browning are very critical in determining final extruded product nutritional and sensory quality (Camire, 2011). Starch gelatinization in itself does not increase or decrease the total starch content of a particular food under extrusion but may cause some degradation that will influence the digestibility of the product. Therefore, starch digestibility of an extruded food product largely depends on the degree of gelatinization that took place during processing. Chemically, gelatinization cleaves the intermolecular hydrogen bonds in starch structure which increases water absorption and breakage of starch granules, consequently causing increased viscosity of the product. This is also the reason why extrusion cooking has been fronted as mechanism of reducing resistant starch in foods. A phenomenon called vaporization which is the rapid expansion of starchy materials that gives extrudates a porous structure upon the exiting the extruder die also takes place during extrusion. Lipids in a food material not only function as plasticizers and lubricants in the extruder barrel by reducing friction but also quality attributes of the extruded foods (Camire, 2011).

Another modification on the physicochemical properties of the extruded products is from lipids forming amylose-lipid complexes during the process of extrusion cooking which also affects the extent of gelatinization of starch. These complexes formations depend on the nature of starch and lipids present in the blend's formulation. (Offiah *et al.*, 2018; Steel *et al.* 2012). Extruders being bioreactors promote starch enzymatic conversion as well as initiate liquefaction and saccharification in a single pass. This is the reason why extruders have been used extensively to chemically modify starches (Cui, 2005).

Another very important chemical reaction that is affected by extrusion cooking is depolymerization of β -glucan. Extrusion causes a partial depolymerization of β -glucans thus improving their physiological activity by increasing availability and that polymer molecular size, although it might be reduced (milling, stirring, pumping), is still effective in plasma cholesterol, glucose, and insulin response improvements (Lazaridou *et al.*, 2007). This is another reason why extrusion cooking is considered to be one of the processing methods that improves the nutritional properties of a food. The depolymerization of β -glucans a chemical reaction could be applied on foods such as sorghum to improve on their functionality.

2.5.3 Microbial properties

Since extrusion cooking occurs at high temperatures and pressure, common spoilage and pathogenic microorganisms are expected not to survive these conditions. Also, these extrusion cooking conditions and drying, which is a unit operation that happens after extrusion, creates unfavourable intrinsic factors to microbial development in the food matrix. These intrinsic factors include low moisture content which exists in bound form and low water activity. As a result, little water in the food matrix could be available for microbial activity. When microbial activity in a food is curtailed causes prolonged shelf life. However, post process handling of extruded products may cause microbial contamination. Levels and type/profile of microbial contaminants not only do they compromise the shelf life of the product but also the safety of consumers. Some microorganisms are known to thrive under extreme conditions, such as low water activity, and thus their presence in extruded foods pose risk of spoilage and pathogenicity. Therefore, it is recommended that hygiene prevails while extruding convenience foods so that they may meet stipulated microbial standards (Cook & Johnson, 2011; Yang *et al.*, 2017).

The rates metabolism and population growth of spoilage microorganisms in foods depend heavily on temperature. Keeping foods at temperatures at zero or below freezing cause increase in shelf life while temperatures over the range of 0–40 degrees Celsius exponentially reduce shelf life. A phenomenon called of Q_{10} used to be quantified in terms the effect of temperature change on microbial activity. Q_{10} is the factor by which growth rate increases with every 10°C increase in temperature (Aisami *et al.*, 2017). This demands that extruded food products be appropriately packaged and stored at temperature which will not accelerate microbial and chemical mechanisms of spoilage (Lopez-Rubio *et al.*, 2004).

Water activity of the food describes the energetics of water in a food system, and hence its availability to act as a solvent and participate in chemical or biochemical reactions. Pathogenic

bacteria cannot grow below a water activity of 0.85–0.86, whereas yeast and moulds are more tolerant to a smaller water activity of 0.8 (Chen, 2010). According to Yousef and Balasubramaniam (2013), bound portion of water in a food matrix adheres to food components by physical or chemical binding mechanisms. There are many sites that bind water; these include hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and salt ions. On other hand, unbound water is usually present within the pores or the interstitial tissue of the food. The amount and form of water influence microbial behaviour, as well as the physicochemical and biological reactions in food. The presence of microbial contaminants in a food having favourable a_w , in combination with other factors such as low acidity, can lead to quality deterioration or compromise the safety of foods. An example are fungi which are not only spoilage microbes but some also produce potent toxic and carcinogenic agents. Fungi are responsible for the various degrees of visible deterioration and decomposition of foods which then results in spoilage. Their growth is easily identifiable through rot spots, scabs, slime, cottony mycelium, or coloured sporulating moulds on foods. Moulds may produce abnormal volatile odours as a consequence of fermentative, lipolytic, and proteolytic reactions caused by enzymatic activities on carbohydrates, fats, and proteins constituents of the foods (Marriot, 2006).

2.5.4 Shelf-life Properties

Extrusion cooking together with drying leads to extruded products have extended period for which they will still be acceptable to the consumer. This is because of low moisture content and water activity which raise glass transition temperature hence curtailing all forms of food spoilage that can occur: physical, chemical and microbiological spoilage (Singh & Anderson, 2004). According Haouet *et al.* (2018), two stability testing methods can be used to estimate shelf-life: real-time stability test and accelerated stability test. Real-time stability testing methods involves giving a food product recommended storage conditions and thereafter monitoring the food until it fails the stipulated specification. On the contrary, accelerated stability tests involves storing the said food product at elevated stress conditions such as temperature, humidity, and pH and the monitoring the degradation of the food. This degradation can be predicted using known regression models that interrelate between the acceleration factor and the degradation rate. The most common acceleration factor in shelf-life analysis is temperature because it establishes the regression relationships using the Arrhenius equation. Arrhenius equation is favoured in these studies because it is a formula that brings

out the temperature dependence of reaction rates and energy of activation. The degradation rate depends on the activation energy for the chemical reaction and it is product specific.

According to Mizrahi (2004), accelerated shelf-life testing (ASLT) refer to any method that is capable of evaluating product stability, based on data obtained in a significantly shorter period than the actual shelf-life of the product. ASLT is applicable to any deterioration process that has a valid kinetic model. That process may be chemical, physical, biochemical or microbial. One of the simplest methods used for accelerating the shelf-life testing in foods is what is known as the 'initial rate approach'. It is more applicable in cases where the deterioration conditions of the food can be monitored by an extremely accurate and sensitive known analytical methods. To predict the actual shelf-life for the given food product, one needs only to understand or to evaluate by modelling how the deterioration process behaves as a function of time. The second method of ASLT is use of kinetic model approach which happens to be the most used technique.

The basic procedures for ASLT involves the following steps: 1) Selection of the most desirable kinetically active factors that speed up the deterioration process in a given food. 2) This is followed by running a kinetic study on how the deterioration process at predetermined levels of the accelerating factors affects the rate of deterioration. 3) Then there is the evaluation of the parameters of the kinetic model, accompanied with extrapolating the data to normal storage conditions for that particular food. 4) The extrapolated data or the kinetic model obtained from the study could then be used to predict shelf-life at actual storage condition.

When applying the kinetic model approach, the first question that has to be considered is whether to use a single or multiple factors for accelerating the deterioration reaction. The 'no model' approach is a third method used for the accelerated shelf-life testing method that assumes that a valid kinetic model exists but does not require experiments to evaluate it. This approach may apply only to cases where the kinetically active factor is changing during storage in a monotonically and continuous way. The combination approaches model is the fourth method to accelerated shelf-life testing has the same advantages as using multiple accelerating factors.

2.6 Negative Health Effects associated with Extrusion Cooking

Extrusion cooking involves high temperatures, which triggers a series of chemical reactions between amino acids and reducing sugars which leads to the formation of Maillard reaction products (MRPs). Both beneficial and toxic MRPs can be produced. There is an overall

reduction of protein quality in extruded products, since some amino acids such as lysine are dissipated in the reaction process. Studies have reported that MRPs such as high carboxymethyl lysine (CML) promote diabetes and cardiovascular diseases. Besides MRPs formation, there is also formation of acrylamide which has been classified as a group 2 A carcinogenic compound. The main amino acid contributing to the acrylamide formation is asparagine, especially in the presence of reducing sugars, such as glucose, whereas cysteine, glutamine, arginine and aspartic acid produce only trace quantities of acrylamide (Omwamba & Mahungu, 2014; Singh *et al.*, 2007; Tamanna & Mahmood, 2015).

Acrylamide, a compound which has been classified to be potentially carcinogenic, is believed to be formed in products during extrusion cooking. Since asparagine is a major amino acid in cereals, the possible formation of acrylamide in cereal-based foods should be considered. Variable amounts of acrylamide have been found in cereal-based foods extruded products such as breakfast cereals. The presence of acrylamide, together with that of other undesirable Maillard compounds, such as furosine, HMF, carboxymethyllysine, has been related to formulation and processing conditions of model systems (Soria & Villamiel, 2012).

Despite its expediency in convenience foods development, extrusion cooking has some disadvantages associated with it. Some of these disadvantages include a high initial financial cost associated with investing in this technology and the technical challenges that involve careful selection of extrusion process parameters such as moisture content, feed particle size, feed rate, screw speed, temperature, screw configuration and die shape to avoid reactive and harmful substance formation. As noted above, the bulk of the disadvantages are purely on technical know-how that can be overcome through short training courses. Thus, on comparison, the advantages of extrusion food processing far outweigh the disadvantages (Egal & Oldewage-Theron, 2019).

2.7 Research Gap

Consumption of convenience food products is on the rise globally and is projected that this trend will remain so for some years to come. However, many of these convenience foods have been associated with health problems such as higher glycaemic index values because they are mainly prepared from sifted cereals. These sifted cereals are very high in starch but low in protein, dietary fibres and minerals. Still, due to the health consciousness of consumers, there is a trendy call for nutritious foods. Therefore, this study focuses on contributing towards solving this problem by incorporating bamboo shoots that are high in protein and dietary fibre into the rice-sorghum convenience foods to improve on their nutritional quality. As a result,

there will be increased utilization of bamboo shoots, contributing to food security and nutrition in Kenya and globally. The research gap has been summarized in Figure 2.7.

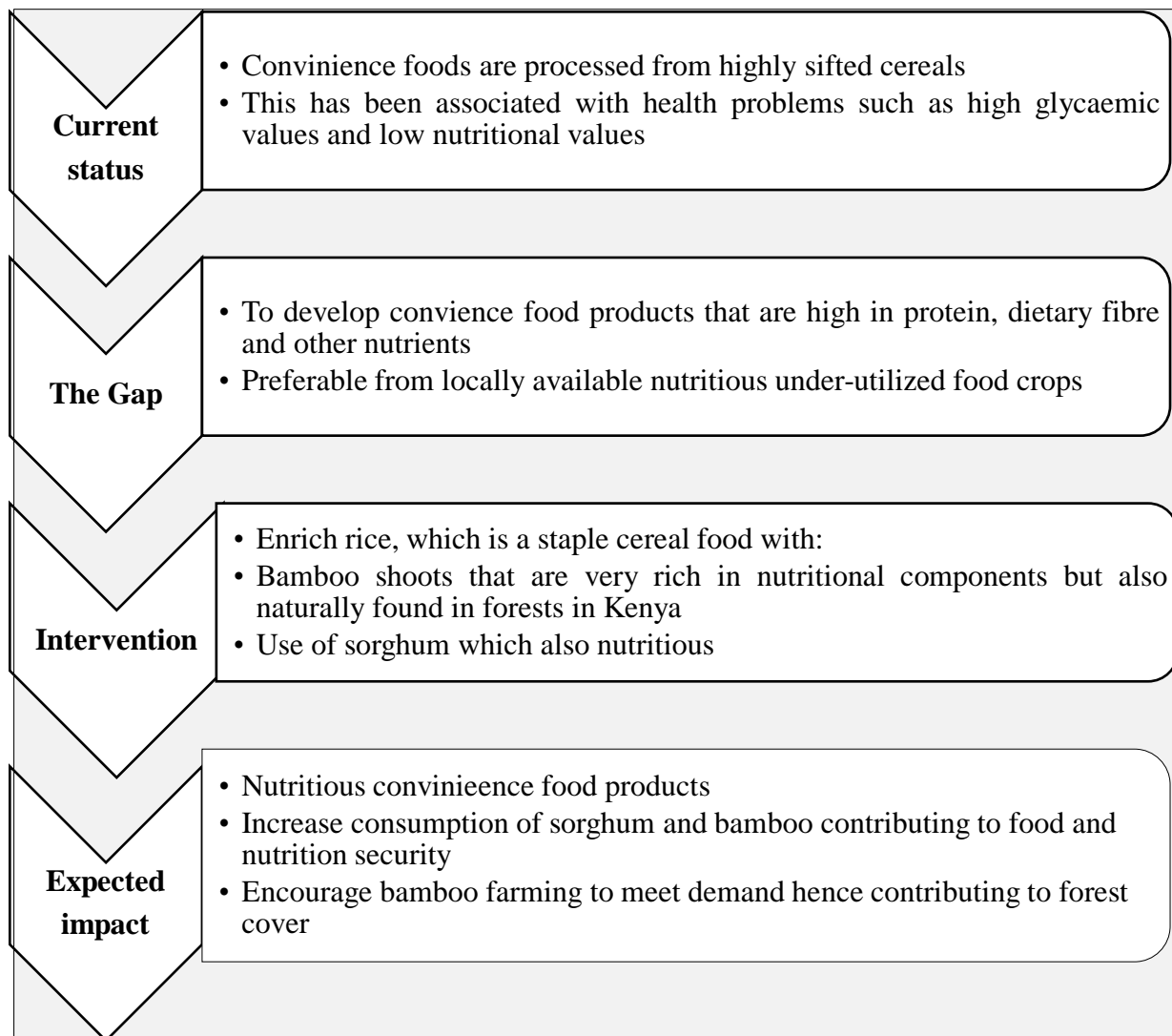


Figure 2. 7: Summary of research gap

2.8 Conceptual Framework

The conceptual framework for the study is shown in Figure 2.8.

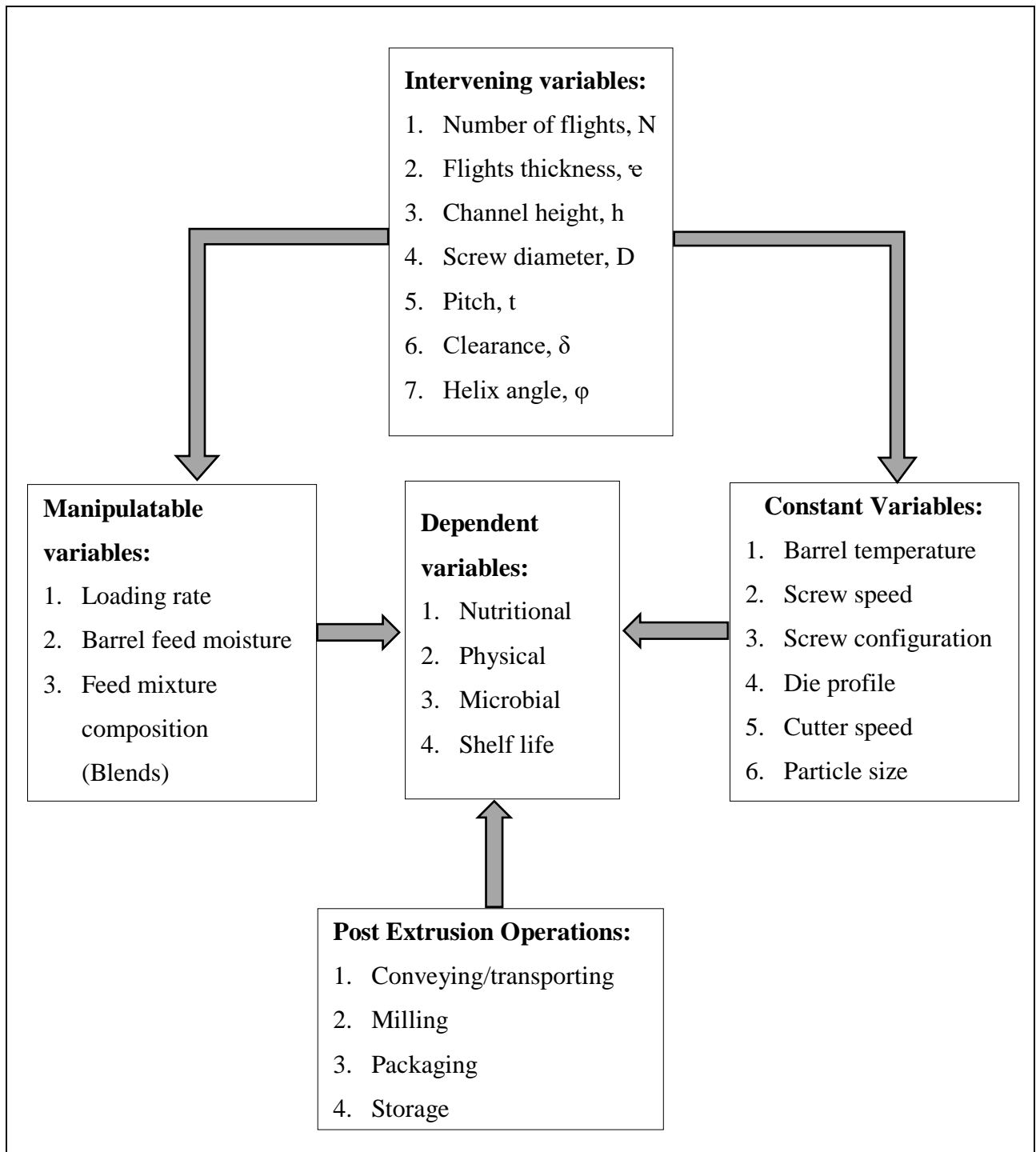


Figure 2. 8: *Conceptual framework for the study*

CHAPTER THREE

OPTIMIZATION OF PROTEIN CONTENT AND DIETARY FIBRE IN A COMPOSITE FLOUR BLEND CONTAINING RICE, SORGHUM AND BAMBOO SHOOTS

Abstract

Initiatives on tackling food insecurity among global emerging economies are being focused on enriching native staple foods with locally available nutritious underutilized crops. The objective of this study was to optimize protein content and dietary fibre in rice (*Oryza sativa*) flour using Sorghum (*Sorghum bicolor* L.) and Bamboo shoots (*Yushania alpina*). An extreme vertices design of mixture approach with 11 runs was employed in the study using MINITAB® software. The 11 blends from 11 generated runs and individual ingredient samples were analysed for nutrients composition. Energy value and energy-to-protein ratio for the samples was calculated. Bamboo shoots flour (BSF) had highest content for all proximate components except total carbohydrates on dry weight basis. Rice had highest content of total carbohydrates at 77.71% and energy to protein ratio of 53.72 kcal/g. Sorghum had highest mean total phenolic and condensed tannins of 45.512 (mg GAE/kg) and 2.512 (mg CE/g) while rice had the least with 0.042 (mg GAE/kg) and 0.102 (mg CE/g), respectively. Fresh bamboo shoots had highest level content of HCN at 117.81 mg/kg. The dried ingredients had a mean HCN content of 2.313, 1.584 and 0.066 mg/kg for dried BSF, sorghum and rice, respectively. Increasing the quantity of BSF and sorghum flour in the blends consequentially increased the protein content, crude fibre and total minerals. The optimum blend was established to be 50:27:23 for rice, sorghum and BSF, respectively. This blend had 13.4% protein, 6.2%, crude fibre and 3.9% total minerals. Regression analysis showed that apart from dry matter, all other constituents were significantly predictable during optimization with $R^2 > 0.7530$. Cluster analysis showed that the nutritional components analysed were in four main clusters. Cluster 1: Dry matter and protein digestibility, cluster 2: Carbohydrates, energy value and energy ratio, cluster 3: Protein, crude fibre and total minerals while cluster 4: Crude fat only. These findings of the optimum composite ratio and other blends can contribute in addressing the food insecurity for low-income countries.

Key words: Optimization, Protein, Crude fibre, Bamboo shoots, Mixture analysis

3.1 Introduction

Cereal crops such as corn, wheat, rice, barley and oat are global staple foods which play key role as major constituent in diets. These cereal and cereal based food products are very rich in

carbohydrates and therefore, significantly contribute to human nutrition. However, cereal and cereal based food products are low in many vital nutrients such as proteins and minerals (Desai, 2019). Some processing techniques associated with cereals involve removal of the germ and bran thus refined cereal products such as polished white rice and refined wheat flour (Bach-Knudsen *et al.*, 2017). Consumption of these refined cereal-based food products has been associated with high glycaemic values which is a predisposing factor to type 2 diabetes and obesity (Dreher, 2018; Palmacci *et al.*, 2019). It has also been reported that communities that depend primarily on cereal-based foods, especially from emerging economies, suffer from health problems related to protein-energy malnutrition and micro-nutrient deficiencies (Ayenigbara, 2013; Barrett & Bevis, 2015; WHO, 2002). According to Hossain *et al.*, (2019) it is estimated over 3 billion worldwide consume rice as a staple food. A joint report by FAO, IFAD, UNICEF, WFP and WHO (2019), Africa, Asia and Oceania regions have recorded multiple burden of malnutrition such as obesity, stunted growth among children, wasting and other diet related non-communicable diseases. Thus, there is a growing trend that calls for the enrichment of staple cereal and cereal based food products with proteins, crude fibre and minerals.

The current trends in development of nutrient dense cereal food products involve integrating plant-based ingredients that are rich in the nutrients of interest. The philosophy of utilizing plant-based ingredients is their assumed affordability and availability as a source of proteins, crude fibre, minerals and phytochemicals as opposed to animal sources. The most commonly used plants for blending in order to improve on specific nutrients in deficit cereal-based foods include soybeans, legume seeds, fruit pomace, gums and ‘pseudo cereals’ such as sorghum, millet, buckwheat, quinoa and *tef* (Aghamirzaei *et al.*, 2013; Montemurro *et al.*, 2019). Recent reports indicate that food blending innovations are utilizing underutilized food crops as fortificants in nutrient deficit cereal-based foods. However, bamboo shoots which have been found to contain high levels of quality proteins, remain underutilized (Karanja, 2017) in Kenya. The bamboo shoots are mainly consumed in Asia. Recent reports indicate that there is a significant and growing demand for the bamboo shoots-based foods around the world (Das, 2019). Reports on the influence of bamboo shoots incorporation on physical, sensory and technological properties of cereal-based food products are scanty (Memariani *et al.*, 2020). Thus, there is need to evaluate both the sensory and technological impact of incorporating bamboo shoots in cereal-based foods. Such information will be critical in the innovative

development of new and improved nutrients and texture food products that address the dynamic consumer demands especially nutrition and palatability of the foods.

Rice is one of the cereal food crops that have widely been used in processing of ready-to-eat snacks, breakfast cereals, porridge flour blends and noodles. This is because it possesses a characteristic blunt taste (Omwamba & Mahungu, 2014). Many studies have focussed on improving the nutritional value of rice-based products by incorporating soya beans, sorghum, millet, edible insects, and peanut (Indriani *et al.*, 2019; Li *et al.*, 2019; Liu *et al.*, 2018). Sorghum is a drought tolerant crop and therefore better placed in fighting food insecurity as well as protein-energy malnutrition through fortification (Serrem, 2011). According to Bhattacharya (2011), diets consumed by a majority of the world's population are based mainly on cereals and contain a small amount of pulses, vegetables, milk, and other animal foods. Consequently, protein-calorie malnutrition, anaemia, and diseases due to vitamin deficiencies are widely prevalent. Protein rich and protective foods such as milk, eggs, meat, and fish are not available in adequate quantities and are sometimes beyond the reach of low-income groups. Nutritionally balanced foods are necessary for maintaining good health in the case of normal humans, but nutritious foods enriched with protective nutrients are essential to improve the health status of vulnerable segments of the population. Sorghum starches are the most commonly used because of their low cost and easy availability. However, very scanty information is available on bamboo shoot being used to enrich rice flour and consequently rice based products. Studies have shown that bamboo is very rich in quality proteins, dietary fibres, minerals and phytochemicals (Choudhury *et al.* 2015; Chongtham *et al.*, 2011). Therefore, it is proposed that preparing a composite flour composed of rice, bamboo shoots and sorghum will improve both the nutritional value and physical properties of the respective food products prepared using the composite flour

The purpose of this study was therefore, to develop a nutrient dense composite flour through an optimization process by standardizing the levels of bamboo shoot flour, sorghum flour and rice flour in the optimal composite flour. An experimental mixture design approach was employed in order to optimize mixtures of flours. The effect of this optimization process on nutritional properties of the composites was analysed. It is hypothesized that use of sorghum and bamboo shoot flour will improve the intensity of nutrients of interest including phytochemicals of the flour blends when rice is the base ingredient.

3.2 Materials and Method

3.2.1 Materials

Edible shoots of *Yushania alpina* (Alpine bamboo) were harvested from Mt. Elgon National Reserve, Kenya (shown in Appendix I). This bamboo species was selected because it is a vast indigenous plant that grows wildly in Kenya and the other East African highland areas. The shoots were harvested at 4-6 weeks after the onset of April-May rainfall. Harvesting was purposively done at 4-6 weeks because beyond this stage shoots become 'woody' due to lignification. The husks were removed, the soft edible portions chopped into small pieces, and immediately dried by fire from wood (as shown in Appendix II) (Karanja, 2017). Fresh bamboo shoots were cut into small pieces, placed in Ziploc bags and immediately transported to the Egerton University food chemistry laboratory in a cool box for cyanide analysis. Rice and red sorghum were procured from the local agricultural market. All the ingredients: Rice, Sorghum and bamboo shoots were milled using a hammer mill and the flour passed through a sieve with average pore size of 800 μm . Rice, sorghum and bamboo shoot flour were then stored in sealed polyethylene pouches.

3.2.2 Experimental Design

This study was carried out in two main stages: Pre-trial and trial. During pre-trial, ingredients to be used were first analyzed for their proximate composition. The protein, crude fibre and total minerals were applied in mixture design to empirically set up, first proportional limits for each ingredient (constrain the ingredients) using Minitab[®] (Version 17.6.1, Minitab, USA). Secondly, the ingredient combination that lay outside the feasible region of optimization was screened out. The trial stage used the constrained mixture design obtained from pre-trial to evaluate the effect of flour proportions (Rice, sorghum and bamboo shoot) on the properties of composite flour. Extreme vertices method of mixture design with constraints that comprised of Rice 0.5- 0.7, Sorghum 0.1-0.3 and Bamboo Shoot Flour 0.0-0.3 on proportional basis so as to have a final mixture total of 1.0 (100%) was employed (Zahiri & Eskandari-Naddaf 2018). This method is used in mixture experiments when constraints are applied on factors (ingredients). Hence, these factor constraints reduce the spatial volume of the factors and the factor level from 0 to 100% or 0.0 to 1.0 on proportional basis. This model generated 11 runs as shown in Table 3.1, where each run formed a composite (Appendix III). Each composite was analyzed in triplicates for proximate and protein digestibility. The composition of the 11 composites were used to empirically determine the optimum blend using the response optimizer. The proximate composition of the optimum blend was also analysed.

Table 3. 1 : Mixture Design extreme vertices model

Std Order	Optimization Order		Ingredient Ratios			Mixture Constraints		
	Run Order	Pt Type	Rice	Sorghum	Bamboo	Totals	Lower	Upper
Sample Name								
Comp 1	2	1	0.70	0.10	0.20	1.0	0.50	0.70
Comp 2	8	1	0.70	0.30	0.00		0.10	0.30
Comp 3	4	1	0.50	0.20	0.30		0.00	0.30
Comp 4	5	0	0.60	0.20	0.20			
Comp 5	6	-1	0.65	0.15	0.20			
Comp 6	7	-1	0.65	0.25	0.10			
Comp 7	9	1	0.60	0.10	0.30			
Comp 8	10	-1	0.55	0.25	0.20			
Comp 9	1	-1	0.55	0.20	0.25			
Comp10	11	-1	0.60	0.15	0.25			
Comp11	3	1	0.50	0.30	0.20			

Key: Std Order=Standard order; Pt Type = Point type; Comp = Composite

3.2.3 Proximate Analysis

1) Moisture and Dry Matter Content

Moisture content of the samples was determined using oven method (AOAC, 2000) Method 950.46. About 2.0 g of each sample was accurately weighed and transferred into a dry aluminium dish. The sample was then dried in a dry air oven at 105 °C/24 hours, and then cooled in a desiccator before weighing. The percent moisture and percent dry matter were calculated using equation 3.1 and 3.2, respectively.

$$\text{Moisture (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100 \text{ Equation 3. 1}$$

$$\text{Dry Matter (\%)} = \frac{\text{Final weight of the sample after drying}}{\text{Initial weight of the sample before drying}} \times 100 \text{ Equation 3. 2}$$

2) Total Minerals content

Total minerals content of the samples was determined using (AOAC, 2000) method 920.39. About 5.0 g of each sample was accurately weighed and placed into dry crucibles. These samples were then ashed in a muffle furnace at 550⁰C/6 hours. The ashed samples were cooled

in a desiccator to room temperature and weighed. Total minerals content was calculated as shown:

$$\text{Total minerals (\%)} = \frac{(\text{Weight of crucible+ash}) - (\text{Weight of crucible})}{\text{Original weight of the sample}} \times 100 \dots \text{Equation 3. 3}$$

3) Crude Fat

Crude fat was determined by the Soxhlet method (AOAC, 2000) Method 920.39. Approximately 5 g ground dry sample were weighed accurately into an extraction thimble and covered with cotton wool. The thimble was placed into the soxhlet extractor and the fat extracted into a tared flask for 8 h using petroleum ether. The solvent was then evaporated in a rotary evaporator and the residue dried in an air oven at 105 °C for 1 h before weighing. Crude fat content on dry weight basis was calculated using equation 3.3.

$$\text{Crude fat (\%)} = \frac{\text{Weight of ried flask with sample} - \text{weight of dried empty flask}}{\text{Weight of the dry sample}} \times 100 \dots \text{Equation 3. 4}$$

4) Crude Protein

Crude protein was determined by the Kjeldahl method (AOAC, 1995) Method 978.04. About 1 g of the sample was weighed into a test tube and digested with 10 ml concentrated H₂SO₄ in presence of a catalyst in a digestion block until the colour turned blue. The digest was then cooled to room temperature before diluting to 100 ml with distilled water and carefully swirled to mix the contents. The test tube was transferred to a distillation unit where 10 ml of 40% NaOH was gradually added to the sample. Distillation continued for about 10 minutes and ammonia produced in the reaction was collected as ammonium hydroxide in a conical flask containing 20 ml of 1% boric acid solution with a drop of methyl red indicator. About 60 ml of the distillate were collected and titrated against 0.1 N HCl until the colour changed to pinkish-orange. A blank was also prepared alongside the samples.

The protein content was then calculated by multiplying the percent nitrogen content by a factor 6.25.

$$\text{Nitrogen (\%)} = N \text{ HCl} \times \frac{\text{Corrected acid volume}}{\text{Weight of sample}} \times \frac{14 \text{ g N}}{\text{Mol}} \times 100 \dots \text{Equation 3. 5}$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25 \dots \text{Equation 3. 6}$$

Where: Corrected acid volume = (mL acid for sample – mL acid for blank), N HCl is Normality of HCl, 14 is the atomic weight of nitrogen and 6.25 is the conversion factor.

5) *In-Vitro* Protein Digestibility

The *In-Vitro* Protein Digestibility was determined using the method 971.09 (AOAC, 2005). Exactly 200 mg of the sample were weighed and suspended in 15 ml of 0.1N HCl with 1.5 mg pepsin (Sigma-Aldrich, USA) in 100 ml conical flask followed by incubation at 37 °C for 3

hours. The mixture was then neutralized with sodium hydroxide. This was followed by a treatment with 4 mg pancreatin in 0.2 M phosphate buffer containing sodium azide and incubated at 37 °C for 24 hrs. Trichloroacetic acid was then added to stop the reactions. The reaction mixture was centrifuged at 5000 g for 5 minutes. Five millilitres of aliquots were obtained and analysed digestibility using equation 3.6.

$$\text{Protein digestibility (\%)} = \frac{\text{Crude protein in supernatant}}{\text{Crude protein in sample}} \times 100 \dots \text{Equation 3. 7}$$

6) Crude Fibre

Crude fibre was determined according to Method 984.04 AOAC (2000). Approximately 2.0 g ground dry sample was accurately weighed into a graduated 600 mL beaker. About 100 mL boiling distilled water and 2.04 M sulfuric acid solution were added. The volume of the mixture was made up to 200 mL with boiling distilled water and maintained at this volume whilst boiling for 30 minutes on a hot plate. The mixture was then filtered using a funnel lightly packed with glass wool. The residue was washed three times with boiling distilled water. The residue and the glass wool were then transferred quantitatively back to the beaker and about 100 mL of boiling distilled water and 25 mL of 1.78 M potassium hydroxide solution added. The volume was made up to 200 mL with boiling distilled water and this volume maintained whilst boiling on a hot plate for 30 minutes. The mixture was filtered again using glass wool and was washed three times with boiling distilled water. The residue was further washed three times with small amounts of ethanol. The residue and glass wool were transferred quantitatively to a porcelain dish and dried in an air oven at 105 °C for 2 hours. The sample was cooled and weighed in the porcelain dish before igniting at 600 °C in a muffle furnace for 2 hours. The sample was cooled in the dish and weighed. The crude fibre content was calculated and expressed as a percentage of the sample on dry weight basis (dwb).

7) Total Carbohydrates

Total carbohydrate content was obtained from the difference between 100% and the percent sum of the values for moisture content, fat, protein, crude fibre and total minerals.

3.2.4. Determination of Total Phenolic Content

The total phenolic content of the samples was determined according to the method described by Li *et al.* (2007). Absorbance at 760 nm was determined using UV-VIS Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan). Sample blank were included in which distilled water replaced the sample. The standard curve (as shown in Appendix IV) was prepared using

Gallic acid as standard. The total phenol content was expressed in mg of Gallic acid equivalents (mg GAE/kg).

3.2.5 Determination of Condensed Tannin Content

The condensed tannin content of the samples was determined by use of the Vanillin-HCl method as described by Price *et al.* (1983). Absorbance at 500nm was measured in UV-Vis Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) with Catechin being used as the standard. Calibration curve is shown in Appendix IV.

3.2.6 Determination of Hydrogen Cyanide

Hydrogen cyanide (HCN) was determined using the picrate acid paper method described by Singh *et al.* (2014). Absorbance was measured at 510 nm using UV-VIS Spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan) against a blank (picrate paper incubated without sample). Total cyanide was obtained by multiplying the absorbance value by 396 which is a gradient factor observed in the normal picrate method (Bradbury, 2009).

3.2.7 Energy value (EV) and Energy-to-protein ratio (EPR)

The energy value/content of the samples was determined by multiplying the values obtained for crude protein, total carbohydrates, crude fat and crude fibre by 4.00, 4.00, 9.00 and 2 Kcal/g respectively, and adding up the results (FAO/GOK, 2018). Energy-to-protein was obtained by dividing energy value of the sample by its crude protein content.

3.2.9 Data Analysis

A statistical package, MINITAB[®], was used to generate experimental points (runs), randomize the runs, perform analysis of variance, fit the second order polynomial models and create graphical representations. Desirability index was used as a measure of assessing on how well the model obtained explains and predicts the variability observed on dependable variables. *Post-hoc* analysis of the experimental means employed use of Scheffe test to determine if the effect of factors was significant at $P < 0.05$. Pearson's correlation K-means cluster analysis for the dependent variables was carried out to classify them into groups of similarities. Data output has been reported as tabulated mean values \pm standard error of the mean and graphical charts.

3.3 Results

3.3.1 Nutrients Composition of the Ingredients

The proximate composition determined for the rice, sorghum and bamboo shoots are shown in Table 3.2. Bamboo shoots flour had significantly higher content than rice and sorghum for all components evaluated except total carbohydrates. Rice had significantly higher content of total

carbohydrates at 77.71% followed by sorghum at 65.76% and bamboo shoots had the least at 19.27%. Also, rice had significantly higher energy to protein ratio of 53.72 kcal/g of protein as compared to bamboo shoots and sorghum. Bamboo shoots had the lowest energy value of 288.92 kcal/100 g.

Table 3. 2: Proximate composition for rice, sorghum and bamboo shoots used in the optimization process

Ingredient	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Total minerals (%)	Crude Fibre (%)	Total CHOs (%)	PD (%)	EV (Kcal/100g)	EPR (Kcal/g of Protein)
Bamboo	89.89±0.37 ^a	27.31±0.17 ^a	6.14±0.40 ^a	16.27±0.04 ^a	23.66±0.14 ^a	19.27±2.74 ^c	63.58±1.06 ^b	288.92±7.37 ^b	10.58±0.30 ^c
Rice	86.35±0.18 ^b	6.47±0.32 ^c	0.85±0.13 ^c	0.29±0.02 ^c	1.34±0.28 ^c	77.71±0.59 ^a	85.61±0.83 ^a	345.79±0.75 ^a	53.72±2.51 ^a
Sorghum	89.49±0.03 ^a	9.65±0.01 ^b	3.61±0.03 ^b	1.82±0.05 ^b	8.65±0.18 ^b	65.76±0.22 ^b	63.90±0.61 ^b	351.43±0.27 ^a	36.43±0.01 ^b

Key: Std= Standard; CHOs= Carbohydrates; PD= Protein Digestibility; EV= Energy Value; EPR= Energy to protein ratio;

Means with the same letter along the column are not significantly different at P<0.05

3.3.2 Optimized Composite

Proportions of ingredients required to make a flour blend with optimum protein, crude fibre and total minerals are shown in Figure 3.1. Blending rice, sorghum and bamboo shoots at a ratio of 50:27:23 respectively gives 13.39% protein, 6.16% crude fibre and 3.98% total mineral contents with a composite desirability of 0.993.

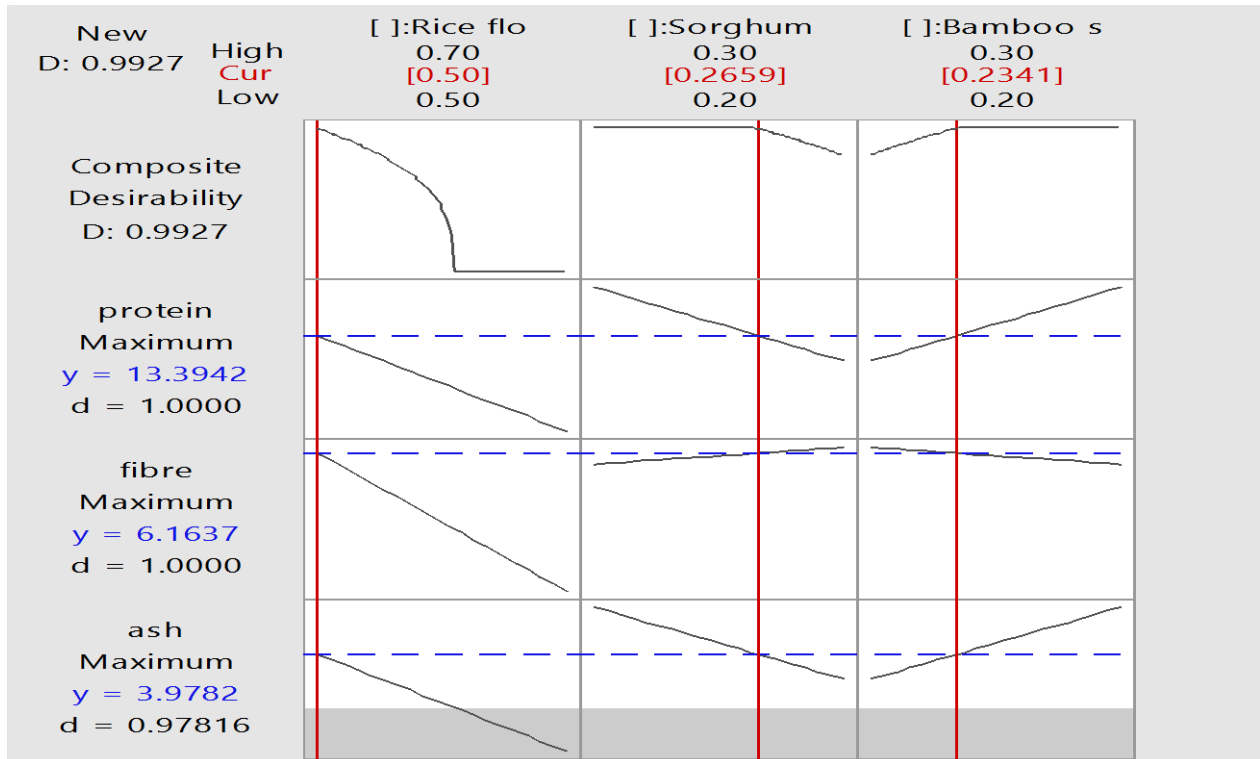


Figure 3. 1 : Optimization plots indicating best possible blend proportions for rice, sorghum and bamboo shoots that gives optimum protein, crude fibre and total minerals

3.3.3 Proximate Composition of Individual Runs used in the Optimization

Nutritional composition of composite blends used in the optimization process is shown in Table 3.3. Composite 2 which was 70:30 for rice to sorghum, respectively had the highest carbohydrates content of 73%, while the energy value of 346 kcal/100 g and energy to protein ratio of 52.28 kcal/ g of protein were observed. Composite 3 that was 50:20:30 for rice, sorghum and bamboo shoots respectively, had the highest content of protein of 13.6%, while the crude fibre content of 10.94% and total minerals of 5.37% were observed. This blend exhibited the lowest content of carbohydrates of 57%, while the energy value of 322.49 kcal/100 g and energy to protein ratio of 23.71 kcal/ g of protein were observed.

Table 3. 3: Proximate composition of the different blend composites used during optimization

Sample Name	Dry Matter (%)	Total minerals (%)	Crude Protein (%)	Crude Fibre (%)	CHO (%)	PD (%)	Crude Fat (%)	EV (Kcal/100 g)	EPR (Kcal/g of Protein)
Comp 1	89.74±0.20 ^a	3.67±0.06 ^c	10.70±0.18 ^d	8.01±0.18 ^d	65.73±0.17 ^b	65.65±0.19 ^a	1.63±0.08 ^b	336.44±0.88 ^c	31.48±0.60 ^c
Comp 2	87.94±0.23 ^b	0.76±0.01 ^e	6.60±0.20 ^f	5.66±0.01 ^e	73.11±0.06 ^a	60.08±0.58 ^c	1.82±0.01 ^a	346.49±0.91 ^a	52.58±1.38 ^a
Comp 3	89.16±0.33 ^{ab}	5.37±0.01 ^a	13.60±0.08 ^a	10.94±0.05 ^a	57.42±0.23 ^d	61.49±0.31 ^{bc}	1.84±0.02 ^a	322.49±1.13 ^{de}	23.71±0.06 ^e
Comp 4	89.49±0.07 ^a	3.79±0.01 ^c	11.37±0.01 ^c	8.86±0.02 ^{cd}	63.76±0.09 ^{bc}	64.35±0.19 ^{ab}	1.70±0.01 ^b	333.59±0.26 ^c	29.33±0.04 ^d
Comp 5	89.10±0.13 ^{ab}	2.99±0.37 ^c	10.21±0.51 ^{de}	7.76±0.25 ^d	66.45±0.95 ^b	64.85±0.41 ^a	1.69±0.05 ^b	337.38±1.70 ^c	33.22±1.73 ^c
Comp 6	88.69±0.08 ^{ab}	1.89±0.19 ^d	8.57±0.31 ^e	6.81±0.23 ^{de}	69.69±0.66 ^{ab}	62.41±0.37 ^b	1.74±0.02 ^{ab}	342.27±0.81 ^{ab}	40.06±1.50 ^b
Comp 7	89.36±0.24 ^a	5.32±0.04 ^a	13.37±0.02 ^{ab}	9.15±0.35 ^b	59.73±0.05 ^d	62.23±0.26 ^b	1.78±0.11 ^a	326.76±0.21 ^d	24.43±0.06 ^e
Comp 8	89.70±0.18 ^a	3.40±0.23 ^c	10.78±0.39 ^{cd}	8.20±0.62 ^d	65.64±1.50 ^b	60.04±0.17 ^{cd}	1.69±0.08 ^b	337.27±2.50 ^c	31.39±1.31 ^c
Comp 9	88.81±0.11 ^{ab}	4.52±0.03 ^{ab}	12.47±0.05 ^b	9.74±0.10 ^{ab}	60.94±0.29 ^{cd}	61.69±0.02 ^b	1.80±0.02 ^a	329.31±1.10 ^d	26.41±0.16 ^e
Comp 10	87.24±0.03 ^b	4.48±0.03 ^b	10.42±0.22 ^{de}	9.03±0.23 ^{bc}	61.72±0.02 ^c	62.01±0.10 ^b	1.59±0.03 ^b	320.93±0.39 ^e	30.82±0.60 ^{cd}
Comp 11	88.55±0.20 ^{ab}	3.91±0.03 ^{bc}	11.71±0.41 ^{bc}	9.01±0.11 ^c	61.83±0.09 ^c	58.04±0.29 ^d	2.08±0.06 ^a	330.94±0.62 ^{cd}	28.34±0.93 ^{de}

Key: Std = Standard; CHOs= Carbohydrates; PD= Protein Digestibility; EV= Energy Value; EPR= Energy to protein ratio; Means with the same letter along the column are not significantly different at p<0.05.

Graphical representation of the sweet spot which shows region of optimum blend and contribution of each ingredient to protein, crude fibre and total minerals in the composite blends during optimization is shown in Figure 3.2. It was found that increasing proportions of bamboo shoot in the composite blend immensely increased protein content and total minerals. Both bamboo shoots and sorghum contributed in the increase of crude fibre.

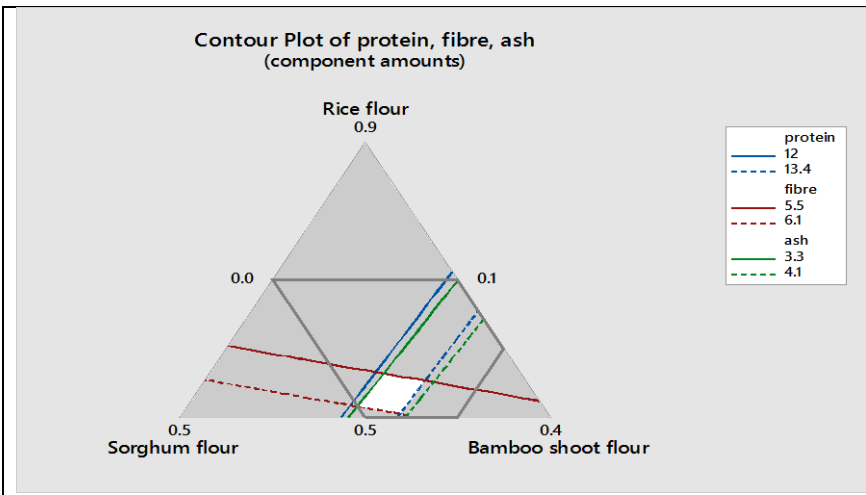


Figure 3.2 a: Sweet spot region indicating where optimum blend lies

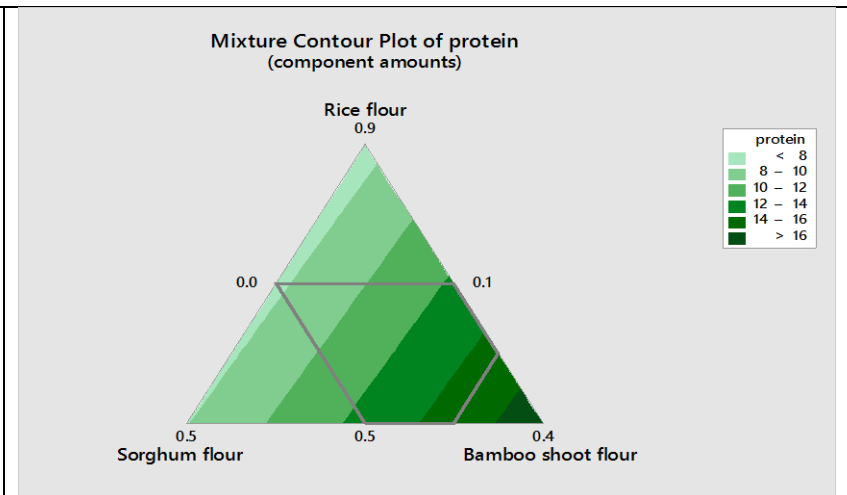


Figure 3.2 b: Contribution of each ingredient used in the blending to protein content

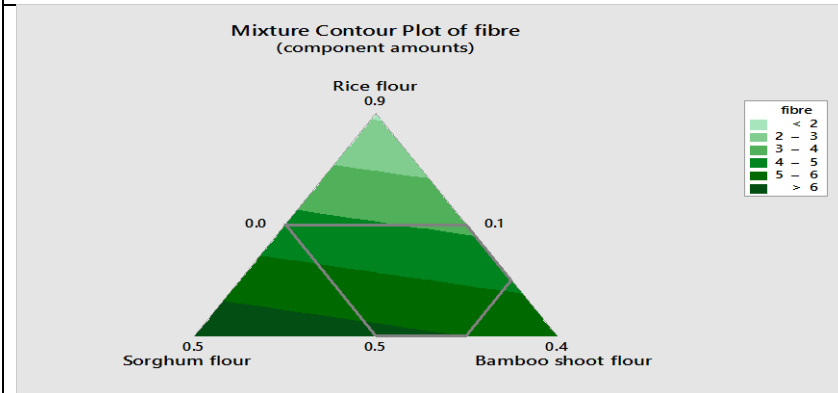


Figure 3.2 c: Contribution of each ingredient used in the blending to crude fibre content

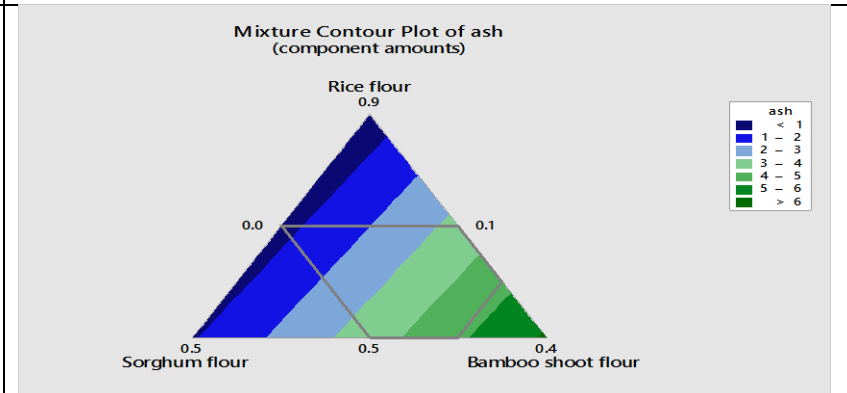


Figure 3.2 d: Contribution of each ingredient used in the blending to total minerals content

Figure 3.2 : Overlaid contour plots of the effect of mixture components

3.3.4 Effect of Optimization on Mixture Components

Regression coefficients for the proximate composition due to contribution mixture components are shown in Table 3.4. It was observed that apart from dry matter, all other constituents are significantly predictable when making a flour blend from rice, sorghum and bamboo shoots. Total minerals content had a higher predictability with $R^2=0.9553$ while crude fibre had the least with $R^2=0.7530$.

Table 3. 4 : Predicted regression model equations

Parameter	Predicted regression model ^{***}	R ²
Crude protein	$Y=10.36X_1+38.33X_2+46.77X_3$	0.8981
Total minerals	$Y=1.62X_1+16.01X_2+27.60X_3$	0.9553
Crude fibre	$Y=12.2X_1-8.75X_2+51.10X_3-78.52X_1X_3+54.39X_2X_3$	0.9028
Carbohydrates	$Y=67.83X_1+29.97X_2-43.53X_3$	0.9353
Crude fat	$Y=2.31X_1+13.33X_2+4.71X_3-18.04X_1X_2-14.50X_1X_3$	0.7530

Key: X_1 , X_2 and X_3 were the mixture components; Rice, Sorghum and Bamboo shoot flours respectively; R^2 = Coefficient of determination; ^{***} backward elimination regression procedure was used and non-significant terms at $p<0.05$ were removed from the equations.

Cluster groups of dependent variables based on Pearson's correlation coefficients are shown in Figure 3.3. It was observed from the dendrogram that the nutritional components analyzed are in four main clusters. Cluster 1 had dry matter and protein digestibility, cluster 2 had carbohydrates, energy value and energy ratio, cluster 3 had protein, crude fibre and total minerals while cluster 4 had crude fat only.

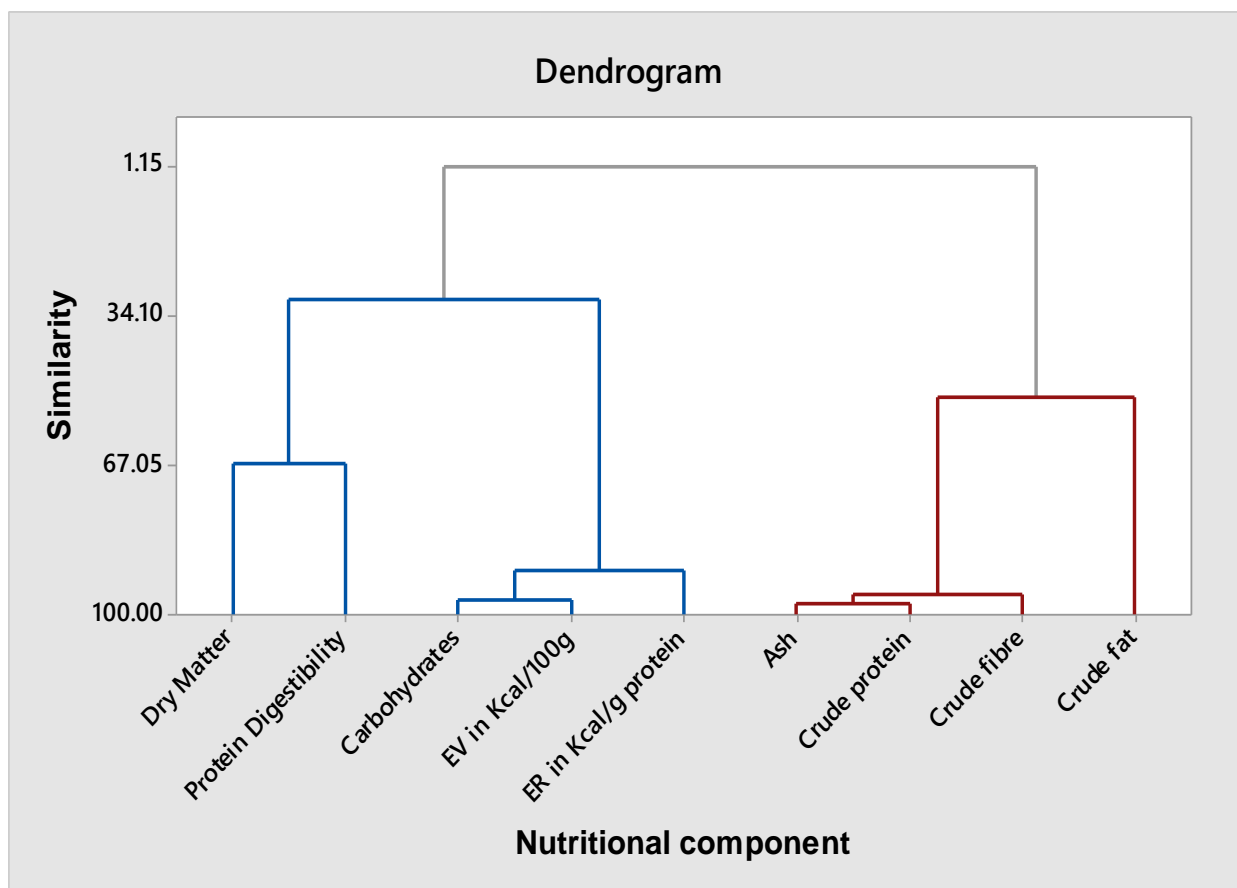


Figure 3.3 : Cluster groups for nutritional parameters in composite blends from rice, sorghum and bamboo shoot flours based on their similarities

3.3.5 Phytochemicals Content

Total phenolic and condensed tannin content for bamboo shoot, rice and sorghum are shown in Table 3.5. Sorghum had significantly higher mean total phenolic and condensed tannins of 45.512 (mg GAE/kg) and 2.512 (mg CE/g) respectively as compared to rice and bamboo. Rice had extremely low mean total phenolic and condensed tannins of 0.042 (mg GAE/kg) and 0.102 (mg CE/g), respectively. Fresh bamboo shoots had highest level content of HCN of 117.81 mg/kg. Other dried ingredients had a mean HCN content of 2.313, 1.584 and 0.066 mg/kg for dried bamboo, sorghum and rice respectively.

Table 3. 5 : Total phenolic and condensed tannin content of bamboo shoot, rice and sorghum use in the optimization

Ingredient	Total phenolic content (mg GAE/kg)	Condensed tannins content (mg CE/g)	Hydrogen cyanide (mg/kg)
Bamboo shoot	32.79±0.72 ^b	2.05±0.027 ^b	2.31±0.05
Rice	0.04±0.01 ^c	0.10±0.003 ^c	0.07±0.01
Sorghum	45.51±0.01 ^a	2.51±0.001 ^a	1.58±0.01
Bamboo shoots (fresh)	-	-	117.81±4.96

Key: GAE= Gallic Acid Equivalent; CE= Catetchin Equivalent; Std= Standard. Means with the same letter along the column are not significantly different at P<0.05

Total phenolic, condensed tannin and HCN content of composite blends used in the optimization process is shown in Table 3.6. It was found out that increasing the proportion of rice in the blend significantly reduced total phenolic and HCN content. Composite 2 which was 70% rice and 30% sorghum had the lowest mean content of total phenolic and HCN at 9.308 mg GAE/kg and 0.318 mg/kg.

Table 3. 6 : Total phenolic, condensed tannin and hydrogen cyanide of the different blend composites used during the optimization

Flour blend	Total phenolic content (mg GAE/kg)	Condensed tannins content (mg CE/g)	Hydrogen cyanide (mg/kg)
Composite 1	7.779±0.022 ^g	0.628±0.006 ^c	0.412±0.002 ^c
Composite 2	9.308±0.102 ^f	0.670±0.023 ^{de}	0.318±0.003 ^f
Composite 3	12.380±0.015 ^b	1.048±0.015 ^{bc}	0.672±0.001 ^a
Composite 4	10.838±0.001 ^d	0.860±0.042 ^c	0.528±0.001 ^c
Composite 5	9.362±0.031 ^f	0.768±0.007 ^d	0.487±0.007 ^d
Composite 6	10.352±0.234 ^{de}	0.755±0.001 ^d	0.425±0.000 ^e
Composite 7	10.048±0.001 ^e	0.813±0.003 ^d	0.582±0.008 ^b
Composite 8	12.333±0.057 ^b	1.094±0.001 ^b	0.573±0.001 ^b
Composite 9	11.675±0.018 ^c	1.539±0.016 ^a	0.567±0.002 ^b
Composite 10	10.386±0.029 ^d	0.819±0.010 ^{cd}	0.535±0.001 ^c
Composite 11	13.942±0.001 ^a	1.130±0.054 ^b	0.682±0.000 ^a

Key: GAE= Gallic Acid Equivalent; CE= Catetchin Equivalent; Std= Standard; Means with the same letter along the column are not significantly different at P<0.05

3.4 Discussion

3.4.1 Role of sorghum and bamboo shoots in enriching rice

Rice has high levels of carbohydrates that contribute to energy values in the diets as shown in Table 3.2. It is evident that rice is a poor source of other assessed nutrients that were of interest in this study. For example, rice has a very low content of crude fibre, disposing it to high glycaemic indices. Considering that rice is a major staple food for many communities around the world, over reliance on it as a food predisposes the respective consumers to malnutrition. Studies have linked consumption of rice with both macronutrient and micronutrient deficiencies (Kearns & Kagma, 2018; Nirmala *et al.*, 2016; Peña- Rosas *et al.*, 2019). Therefore, the call to enrich rice-based food products is vindicated. Conversely, rice has bland taste, attractive white colour, hypoallergenicity and ease of digestion which is a desired functional property when developing a nutrient rich flour blend (Omwamba & Mahungu, 2014). The criteria for selecting a particular raw material or usually a blend depend on low cost, easy availability, and protein content. It is suggested that the protein requirement of children with moderate protein-energy malnutrition should be at least 41.5 kcal/g of protein.

The blended/finished product is expected to contain a minimum of 26% protein per every energy content of 1000 kcal (Michaelsen *et al.*, 2009). Table 3.2 indicates that sorghum and bamboo shoots have lower values of energy per gram of protein compared to rice of 52.58 kcal/g of protein. Table 3.3 shows that increasing the proportions of bamboo shoot and sorghum in rice blends significantly reduces kcal/g of protein to below recommended values of 41.5.

Sorghum has a high content of most of the nutritional parameters of interest as shown in Table 3.2. Total carbohydrates in sorghum contribute to about 75% of the energy value, while protein, fat and fibre are contributing 11%, 9% and 5%, respectively. Though, sorghum remains to be one of nutritious foods that is still underutilized (Andrias *et al.*, 2019; Stefoska-Needham *et al.*, 2015;). Based on effect of lowering kcal/g of protein during blending as shown in Table 2.3, sorghum is heavily being used to enrich other food crops in addressing protein-energy malnutrition and micronutrient deficiencies (Delimont *et al.*, 2015; Okoth *et al.*, 2017; Zhao *et al.*, 2019). As a result, this has caused growing trend among developing countries advocating for consumption of sorghum as a measure to address food insecurity (Stefoska-Needham & Tapsell, 2020).

Unlike rice and sorghum, bamboo shoots are rich in protein, crude fibre and total minerals as shown in Table 3.2. Carbohydrates contribute about 27% of energy in bamboo shoots compared to protein and crude fibre that contribute 38% and 17%, respectively. Also, bamboo shoots had the least energy to protein ratio of 10.58 kcal/ g of protein, when compared to rice and sorghum which was 36.43 and 53.72 kcal/g of protein, respectively. Thus, incorporating bamboo shoots and sorghum into rice is anticipated to not only improve on nutrient density but also increase their consumption. This might explain why bamboo shoot consumption is being fronted as the next niche in tackling food insecurity due to the climate change (Basumatary *et al.*, 2015).

Optimization of the three ingredients caused significant changes on all nutritional components as shown in Table 3.3. Composite 3 that was composed of 50:20:30 for rice, sorghum and BSF respectively, had significantly high total minerals, crude proteins, fibre and fat but significantly lower carbohydrates. On the contrary, composite 2 that was composed of 70:30 respectively, had the significantly the lowest total minerals, crude proteins, fibre and fat but significantly highest carbohydrates. All the other composites are in between these two blends. These were the results that were used to empirically establish optimum points shown in Figure 3.2 by the Mixture Design Analysis system.

3.4.2 Optimization of protein, total minerals and crude fibre in rice blends

As shown in Figure 3.1, optimum protein, crude fibre and total minerals was established for the composite blend ratio of 50:27:23 rice, sorghum and bamboo shoots, respectively. This optimum blend has about 106%, 350% and 1200% more protein, fibre and total minerals than rice. Graphically, optimum composite blend is indicated as a white region in Figure 3.2a. The region is usually referred to as sweet spot. This sweet spot is a result of considering nutrient composition of each ingredient contributing to the overall blend. As shown Figure 3.2b, bamboo shoot contributed more of protein than sorghum. A similar trend is observed in Figure 3.2d for total minerals. However, it was observed that sorghum and bamboo shoots had almost equal contribution to crude fibre in the composite blend as shown in Figure 3.2c. These findings of the optimum composite ratio could contribute in addressing the major dietary risks for low-income countries. According to Afshin *et al.* (2019), consumption of diets low in whole grains and low in vegetables were identified as major dietary risks in low-income countries. Intakes of about 25-29 g per day of crude fibre could confer even greater benefit to protect against cardiovascular diseases, type 2 diabetes, and colorectal and breast cancer (Reynolds *et al.*, 2019).

Besides graphical representation of how and where the optimum blend lies in the mixture design, composition of each composite shown in Table 3.3 helps to understand how varying ingredients proportions affected nutrient density during optimization. Increasing the proportion of bamboo shoot had a negative implication on the energy value and energy to protein ratio of the composite blend. Regression analysis, shown in Table 3.4, indicates that the contribution of each ingredient to proximate composition of the blends was synergistic except bamboo's contribution to carbohydrates. It can be attributed to the fact that bamboo shoots are low in carbohydrates compared to rice and sorghum. Interaction between sorghum and bamboo shoots was also synergistic for crude fibre. This synergism is because both ingredients, sorghum and bamboo shoots are contributing towards total crude fibre in the blends. However, interaction between rice and sorghum contributed negatively/antagonistically for crude fibre and crude fat in the composite blends. Similarly, the interactions between rice-sorghum and rice-bamboo shoots had an antagonistic effect to contribution of crude fat in the blends. The antagonism could be attributed to rice being very low in crude fat and crude fibre thus increasing its proportions in the blends resulted in the reduction of these nutritional components. All interactions for protein, total minerals and carbohydrates were not significant at $p < 0.05$.

Cluster analysis was carried out to classify nutritional properties on the basis of similar contribution based on their degree of association during the optimization as shown in Figure 3.3. The three nutritional parameters under study: Protein, fibre and total minerals were observed to be greatly associated, in cluster 3. This implies that optimization of macronutrients to enrich rice using sorghum and bamboo shoots also greatly affects the micronutrient composition. Therefore, these three ingredients used in the optimization can be used not only to address protein-energy malnutrition but also micronutrient deficiencies. Cluster 1 featured dry matter and protein digestibility which are the only components that could be predicted during optimization as shown by regression analysis in Table 3.4. While cluster 2 had carbohydrates, energy value and energy-to-protein ratio which were the major nutritional parameters in base ingredient, rice. This means that the optimization process which was increasing nutrient intensity in composite blends affected cluster 2 by lowering their respective values. Lastly, cluster 4 had only crude fat. This is because all ingredients used are poor sources of fat in human diets.

3.4.3 Effect of optimization on phytochemicals

Besides nutritional components, this study also considered the effect of optimization on some phytochemicals. Both sorghum and bamboo shoots showed to be good sources of total phenolic compounds and condensed tannins as compared to rice as shown in Table 3.5. Reducing the rice proportion resulted in increase of phenolic compounds and condensed tannins in the composite blend during optimization. Presence of these phenolic compounds have been known to affect the sensory (appearance, taste and aroma) and oxidative properties of a food. Condensed tannins also called proanthocyanidins are known to bind proteins, carbohydrates and minerals thus decreasing their availability/digestibility. Phenolic compounds and tannins in foods have been associated with beneficial health effect such as being anti-oxidants, cholesterol-lowering, anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio-protective and vasodilatory properties (Dykes & Rooney, 2007; Shahidi & Peng, 2018). Thus, it can be inferred that sorghum and bamboo shoots also enriched rice with compounds that possess these functional properties.

Bamboo shoots are reported to contain very high levels of hydrogen cyanide (Bolarinwa *et al.*, 2019; Ferdiansyah *et al.*, 2019). In the current study, fresh bamboo shoots contained a mean of 117.81 mg/kg HCN as shown in Table 3.5. However, drying process of the shoots resulted in about 98.3% reduction to 2.313 mg/kg HCN content. Rice contained only trace amounts of HCN at 0.066 mg/kg. Similar to phenolic compounds and condensed tannins, reducing the rice

proportion in the composite blends during optimization resulted in effective increase of HCN. It is anticipated that further processing of the flour blends will reduce the HCN even further. World Health Organization recommends food for human consumption to have less than 10 mgHCN/kg (Omolara, 2014).

3.5 Conclusion and Recommendation

Based on the findings, the null hypothesis of this study that ingredients optimization has no significant effect on physical-chemical properties of composite blends is rejected. The findings of the current study ascertain the hypothesis that utilization of sorghum and bamboo shoot to enrich rice could be an alternative strategy to develop a nutrient dense flour. Adoption of this strategy would be worth considering the different enriched flour blends, besides the optimized blend, could be used for processing in domestic and industrial applications. Although, freshly harvested bamboo shoots contained very high contents of hydrogen cyanide, the drying process over a fire place greatly reduced the contents to a safe level. This means that composite blends that contain bamboo shoot flours are safe in relation to the risk of cyanide poisoning. Further thermal processing methods that could be applied on the composite blends are expected to eliminate the cyanides. Red sorghum on other hand influenced the colour of the composites.

Drying of *Yushania alpina* shoots over a fire place caused over browning and even darkening of the shoots as well as imparting a smoky flavour. Therefore, we recommend use of controlled solar drying or oven drying of *Yushania alpina* shoots to mitigate against these problems.

CHAPTER FOUR

FEED RATE, MOISTURE AND MIXTURE COMPOSITION FROM COMPOSITES CONTAINING RICE, SORGHUM AND BAMBOO SHOOTS NEXUS ROLE ON ALTERATIONS OF NUTRITIONAL PROPERTIES IN EXTRUSION OF INSTANT FLOUR

Abstract

Extrusion cooking impacts positively on the nutritional status of a food when compared to their raw form especially on nutrient availability. The extent of changes is influenced by both the extruder parameters and the properties of the feed. Hence, understanding the relationship between effect of ingredient variables and nutritional changes during extrusion will inform on novel product development. The objective of this study was to determine the effect of feed rate, water addition and composite composition of the flour containing rice (*Oryza sativa*), sorghum (*Sorghum bicolor*) and bamboo (*Yushania alpina*) shoots flour (BSF) on nutritional changes under extrusion cooking conditions. A commercial single screw dry extruder with a constant set barrel temperature of 250 °C and screw speed of 1480 rpm was used. Five different blends (100:0:0, 70:30:0, 60:30:10, 55:30:15 and 50:27:23 for rice, sorghum and BSF, respectively on dry weight basis) were extruded. The process had three levels of water addition (15, 20 and 25 kg/h) plus two feed rates (1800 and 2100 kg/h). Extrudates were milled to particle size of 2 mm to make instant flour. Crude protein, crude fat, crude fibre, total carbohydrates (CHO), *In-vitro* protein digestibility (IVPD), total phenolics (TP) and condensed tannins (CT) content were analyzed on both the raw blends and the resultant extruded instant flour. Overall, transformation of raw blends to instant flour through extrusion caused a significant increase in dry matter content by between 5.6% for 70:30:0 blend and 9.4% for 100% rice blend. This was accompanied by varying changes to nutrient composition. A blend of 100% rice recorded highest loss of protein at 1.41% and CT at 95%, while recording the least increase in CHO at 0.33%, IVPD at 8.60% and TP at 25.00% as compared to the other blends. Conversely, a blend with 23% BSF had the highest loss of fibre at 2.40% and highest increase in both CHO content at 4.04% and IVPD at 51.61%. Increase in water addition rate into the barrel from 15 to 25 kg/h was accompanied by reduced degradation of protein to 0.47% from 0.53% and fibre to 1.45% from 1.54%. However, the processed led to an increase for fat to 0.11% from 0.07%. As the rate of water addition increased, the CHO increased from 1.41% to 2.19%, the TP mean concentration from 17.69 to 18.49 mg GAE/kg and CT from 0.07 to 0.10 mg CE/g. Increasing the feed rate from 1800 to 2100 kg/h resulted in an increase in fat content from 1.25% to 2.10%.

However, it had a slight protective impact on proteins with a decrease of 0.48% compared to 0.54% at 1800 kg/h. The same observation was made for changes in TP at 17.8 mg GAE/kg at 1800 kg/h compared to 18 mg GAE/kg at 2100 kg/h. These alterations on nutritional properties emanates from ingredient variables which determines the magnitude of mixing and shear impact, mass moisture and specific mechanical energy thus influence on degree of bio-reactions.

Key Words: Extrusion, Bamboo shoots, Nutritional changes, Ingredient variables

4.1 Introduction

Extrusion cooking technology has vast possible applications in the manufacturing of a variety of food products from limitless number of ingredients (Yağci *et al.*, 2020). An extruder is basically a bioreactor where numerous thermomechanical and biochemical processes usually take place. As a consequence, these processes significantly modify the nutritional constituents of the extrudates. This culminates in obtaining products that have better nutritional properties when compared to their respective raw materials due significant decrease of anti-nutritional factors and increased digestibility (Moreno *et al.*, 2017; Offiah *et al.*, 2018). According to Lillford (2008), the composition and properties of the extruder feed material is very vital to the entire process of transformation. White rice (*Oryza sativa* L.) has been fronted as the most suitable raw material for the development of a varied range of convenient food products. These include but are not limited to snacks, breakfast cereals and dietetic foods. Extrusion cooking is one of the techniques that has been used expansively to process instantized flour from cereal-based foods such as rice and others (Ibanoglu *et al.*, 1996).

Generally, the extrusion is the most practical way of fortifying rice to obtain the best quality convenient products (Arribas *et al.*, 2019). Information on the effect of extrusion cooking of rice enriched with legumes, vegetables and other cereals on nutritional changes readily available (Moreno *et al.*, 2017). But, information on the nutritional transformations that occur on extrusion of flour blends containing rice, sorghum (*Sorghum bicolor* L.) and bamboo shoots (*Yushania alpina*) is very limited. Thus, the current study was aimed at creating an understanding of the effect of extrusion on the composite flour containing rice, sorghum and bamboo shoots on nutritional changes. Protein-energy malnutrition and key micro-nutrient deficiencies were envisaged that such a process would mitigate against (Rao & Annadama, 2017). Bamboo shoots and sorghum which locally available and affordable were found to be the most important alternative ingredients to exploit in order to accomplish this task. High levels of quality protein, availability and affordability were the critical pointers that informed

the choice of bamboo shoots. Bamboo shoots are rich in functional ingredients including active peptides, amino acids, polysaccharide, flavonoids, phenolics and phytosterols (Ge *et al.*, 2017; Nirmala *et al.*, 2008). Therefore, including bamboo shoots into a blend ratio with rice and sorghum was expected to enhance the quality and content of protein, dietary fibre, minerals and bioactive compounds. Extrusion cooking of the rice blend enriched with sorghum and bamboo shoots was employed to process instant flour and projected to alter positively the nutritional quality of the blends. According to Muthukumarappan and Karunanithy (2012), the interactions between extruder and ingredient variables brings out better outcomes when developing novel extruded food products.

Therefore, the purpose of this experiment was to assess the role of three extruder ingredient variables on degree of alteration of proximate constituents, *in vitro* protein digestibility, total phenolic and condensed tannins content upon extrusion cooking. Feed ratios (different blend ratios), extruder water addition rate and feed rate were the independent variables in the study. Constant variables in the experiment were screw parameters, temperature and die configuration. Ingredients used for blending were rice, sorghum and bamboo shoot flours. The control for the study was flour having 100% rice. Results of this experiment have provided valuable evidence on the prospects of: 1) using bamboo shoots and sorghum to enrich rice and rice products; and 2) that extrusion processing could be used in nutritional improvement of such enriched blends for the innovative development of novel convenient foods. This could go a long way in contributing to the mitigation of food insecurity, especially in emerging economies.

4.2 Materials and Methods

4.2.1 Materials

Materials for this experiment are described in section 3.3.1 of Chapter three

4.2.2 Experimental Design

A completely randomized design in factorial arrangement with three independent variables: Feed mixture composition of rice flour, sorghum and BSF (100:0:0, 70:30:0, 50:27:23, 60:30:10 and 55:35:15), water addition rate (15, 20 and 25 kg/h) and feed rate (1800 and 2100 kg/h) was adopted for the experiment. Feed compositions were established to optimize protein content, crude fibre and minerals in the composites. Feed compositions were modified composites established to optimize protein content, dietary fibre and minerals as shown in Chapter 3. Feed rate levels and water to be added were established during the extrusion process

pre-trials. Moisture level of all the composites was adjusted to 12% before extrusion. Samples were analysed before (raw) and after extrusion cooking in three replicates.

The statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\beta\gamma_{ijk} + \varepsilon_l$$

Where:

Y_{ijk} = Observations made on the response variables

μ = Overall mean

α_i, β_j and γ_k = Effect of i^{th}, j^{th} and k^{th} main factors in the experiment

$\alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\beta\gamma_{ijk}$ = Effect of interaction of factors in the experiment

ε_{ijk} = Random error effect associated with the experiment

4.2.3 Extrusion and Milling

A commercial single screw extruder (InstaPro Model 2000R, Chelworth, UK) was used in for the study. The extruder barrel was 1120 mm long divided into three sections: feeding section 480 mm, metering section 380 mm and cooking section 260mm, length to diameter ratio (L/D) of 16:1 and screw compression ratio of 3:1. Barrel temperatures for each section were 100 °C, 180 °C and 250 °C for feeding section, metering, and cooking, respectively. Screw had a diameter of 132.5 mm with a constant rotational speed was constant at 1480 rpm while the die diameter was 8.5 mm. The exit at the die had a cutter that chopped the extrudates into small pieces of 1 cm³ each. Extrudates dried immediately upon exit at the die after losing water through steam flash. These chopped dried extrudates were conveyed to a disk mill (Rotor Diameter 450 mm, Speed of Rotation 3000 rpm; FFC-45, Zhengzhou Muchnang, Henan, China) to be milled followed by sieving the flour to particle of size of 0.2 mm using a sieve of similar pore size.

4.2.4 Proximate Analysis

Procedure for proximate determination is shown in section 3.3.4 of Chapter three.

4.2.5 Determination of Total Phenolic

Procedure for proximate determination is shown in section 3.3.5 of Chapter three

4.2.6 Calculation of net alteration of proximate components due to extrusion

Mass balance principles were applied in estimating the effect of extrusion on the net change of proximate components and it was based on the overall change on dry matter content. Assuming unit mass of each composite blend before and after extrusion, then net change to proximate components will be:

$$\Delta X_i = X_A - X_E \dots \text{Equation 4. 1}$$

Given that:

$$X_E = X_O \times CI \dots \text{Equation 4. 2}$$

$$CI = \frac{DM_f}{DM_i} \dots \text{Equation 4. 3}$$

Where: ΔX_i = The estimated change on i^{th} proximate component before and after extrusion, X_A = The actual proportion of the i^{th} proximate component in the extruded flour, X_E = The expected proportion of the i^{th} proximate component in the extruded flour based on corresponding change on dry matter, DM_i = Initial % dry matter content of raw blend before extrusion, DM_f = Final % dry matter content of resultant extruded flour, X_O = The original proportion of the i^{th} proximate component in the raw feed blend and CI = Concentration Index.

The assumption is that:

$$\Delta X_i = 0 \dots \text{Equation 4. 4}$$

Such that if $\Delta X_i > 0$, then extrusion caused increase in the i^{th} proximate component, but if $\Delta X_i < 0$ extrusion caused a loss of the i^{th} proximate component.

4.2.7 Data analysis

Data obtained was first tested for normality via the PROC UNIVARIATE using SAS software version 9.1 (SAS Institute and Cary, NC) before analysis (example given in Appendix V). Analysis of variance (ANOVA) was carried out to investigate the effect of each factor: Feed rate, moisture and mixture ratios on nutritional properties using PROC GLM. Also, HOVTEST=LEVENE (example given in Appendix VI) option was used to test for homogeneity of variance among the observations. Two sample means test of nutritional components between raw and extruded flour was carried via the PROC T-TEST (output shown in Appendix VII). *Post-hoc* analysis of the data was done using Scheffe method at $p \leq 0.05$.

4.3 Results

4.3.1 Effect of extrusion cooking on nutritional components of feed mixture

The effect of extrusion on concentration of dry matter between feed blend and the resultant flour is shown in Figure 4.1. Highest concentration index of 1.094 was observed in blends that contained 100% rice while the lowest concentration index of 1.056 was observed in blend that contained 70% rice and 30% sorghum.

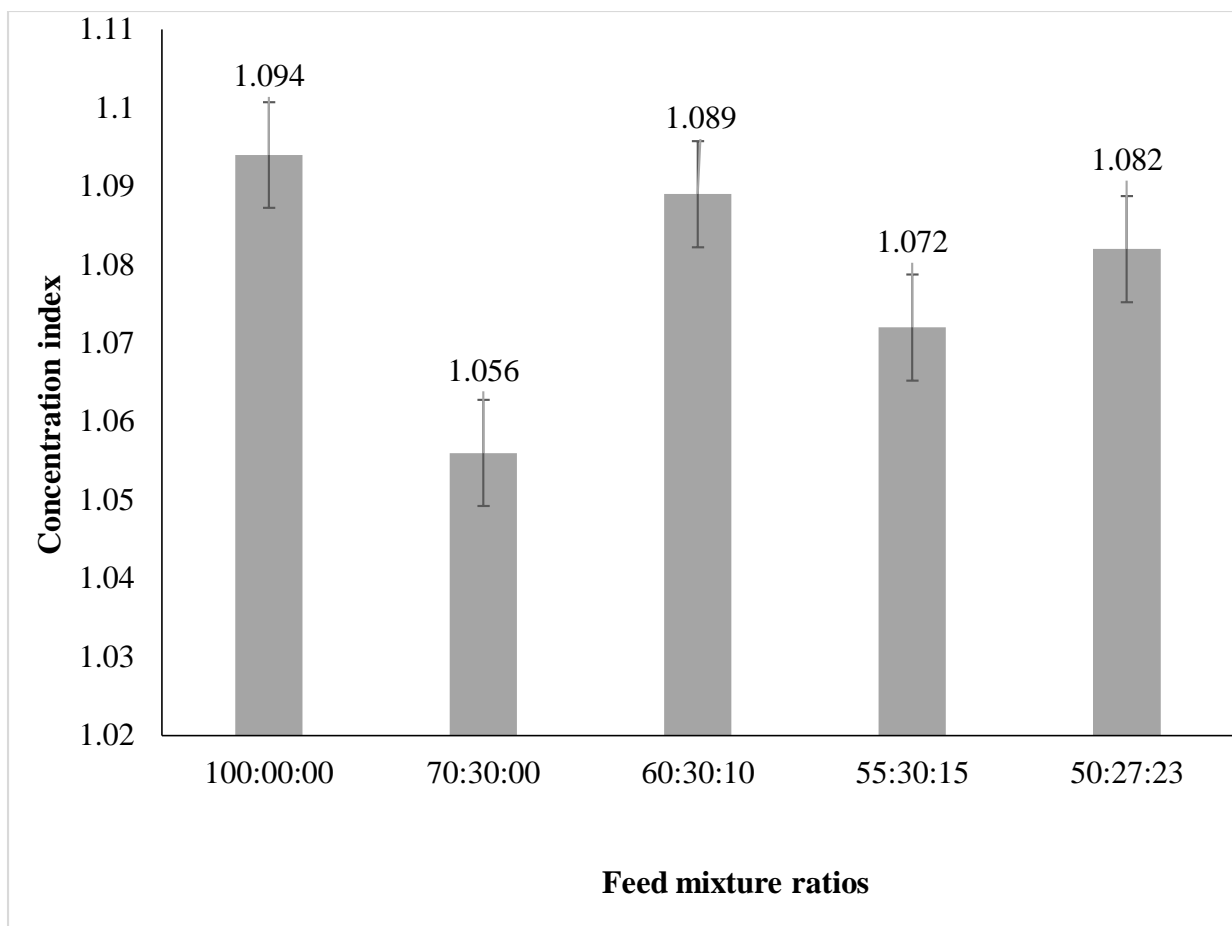


Figure 4. 1: Concentration indices due to extrusion cooking on blends

The effect of extrusion cooking on the nutritional components of rice, sorghum and BSF blend mixtures at different proportions are presented in Table 4.1. Moisture content significantly decreased upon extrusion cooking between feed ratio and resultant flour. The control (100% rice) recorded the highest decrease of moisture content from 13.65 to 5.52% while least decrease from 12.06 to 7.18% was in a blend containing 70% rice and 30% sorghum respectively. Protein content of the control significantly reduced by 8.13% while it increased significantly by 5.76 and 3.98% for blends containing 60:30:10 and 55:30:15 for rice, sorghum and BSF respectively. But proteins increased by 1.90 and 0.88% though not significantly for blends having 70:30:00 and 50:27:23 for rice, sorghum and BSF respectively. Crude fat also significantly increased by between 3.63-15.67% upon extrusion cooking except in the control where the increase was not significant. Total carbohydrates, *in vitro* protein digestibility and total phenolic content increased significantly upon extrusion by between 6.09- 11.71%, 8.60- 51.61% and 25.00- 146.96% respectively among the different mixture blends. Total dietary fibre significantly decreased by 15.57-20.49% for all the blends except in the control where it

increased by 6.96%, though not significantly. Condensed tannins significantly decreased upon extrusion by 86.80-95% upon extrusion cooking in all the blends.

Table 4. 1: Nutritional composition of raw composite blend and their resultant extruded instant flour

Ratio	Parameter	MC	Protein	Crude fat	Fibre	CHO	IVPD	TP	CT
100:00:00	Raw	13.65±0.19	6.47±0.32	0.85±0.13	1.34±0.28	77.41±0.59	85.61±0.83	0.04±0.01	0.10±0.01
	Extruded	5.52±0.27	5.96±0.08	0.91±0.01	1.43±0.01	85.02±0.30	92.97±0.40	0.05±0.01	0.01±0.01
	t-value	12.01	-2.22	1.27	0.96	9.91	7.16	0.06	-59.03
	p-value	<0.0001	0.0387	0.2208	0.3489	<0.0001	<0.0001	0.9497	<0.0001
	Change (%)	10.25	-8.13	7.06	6.96	9.83	8.60	25.00	-95.00
70:30:00	Raw	12.06±0.23	6.60±0.20	1.82±0.01	5.66±0.01	73.11±0.06	60.08±0.58	9.31±0.11	0.67±0.02
	Extruded	7.18±0.53	6.73±0.06	1.88±0.01	5.50±0.03	77.56±0.50	90.90±0.40	18.01±0.13	0.07±0.01
	t-value	3.71	0.80	2.67	-2.20	3.90	30.55	30.53	-52.13
	p-value	0.0015	0.4360	0.0152	0.0405	0.0010	<0.0001	<0.0001	<0.0001
	Change (%)	5.56	1.90	3.63	-20.49	6.09	51.30	93.45	-89.11
60:30:10	Raw	13.24±0.08	9.55±0.20	2.04±0.02	7.61±0.01	65.92±0.13	65.16±0.71	10.35±0.23	0.75±0.01
	Extruded	5.44±0.31	10.10±0.03	2.36±0.01	5.85±0.02	73.64±0.42	89.70±0.42	22.22±0.12	1.10±0.01
	t-value	10.13	5.25	11.43	-37.86	7.17	22.86	39.34	-62.82
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Change (%)	8.99	5.76	15.41	-23.14	11.71	37.66	146.96	-86.80
55:30:15	Raw	12.89±0.06	10.31±0.07	2.31±0.02	8.75±0.09	63.43±0.27	60.43±0.14	12.33±0.06	1.09±0.01
	Extruded	6.66±0.56	11.02±0.07	2.64±0.04	7.17±0.06	69.80±0.46	89.26±0.42	24.08±0.15	0.12±0.01
	t-value	4.43	4.49	3.87	-10.32	5.48	26.58	30.88	-73.33
	p-value	0.0003	0.0002	0.0010	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Change (%)	7.17	6.88	14.60	-18.03	10.05	47.71	95.22	-89.62

50:27:23	Raw	13.46±0.03	12.32±0.13	2.51±0.01	10.12±0.04	58.18±0.11	58.39±0.04	13.84±0.01	1.13±0.05
	Extruded	6.38±0.29	12.43±0.07	2.90±0.03	8.55±0.03	66.99±0.23	88.53±0.46	25.73±0.14	0.13±0.01
	t-value	9.86	0.63	6.28	-19.99	15.13	25.96	32.03	-41.83
	p-value	<0.0001	0.5354	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Change (%)	8.19	0.88	15.67	-15.57	15.15	51.61	85.94	-88.67

Key: MC= Moisture Content; CHO= Carbohydrate; IVPD= *In vitro* Protein digestibility; TP= Total Phenolic; CT= Condensed Tannins;

-ve= Loss of the component; +ve= Increase of the component.

Estimated change in proximate components based on concentration indices between each feed blend and the resultant flour on extrusion cooking is shown in Table 4.2. Extrusion cooking significantly reduced protein content by 0.032 - 1.414%, reduced crude fibre by 0.036 - 2.437% but caused an increase of the total carbohydrates by 0.334 - 4.039%. Crude fat significantly decreased by 0.020 - 0.042% in blends without BSF but increased by 0.138 - 0.184% in blends that contained BSF

Table 4. 2 : Mean net changes in proximate composition of different blends

Blend ratio	Proximate components (%)			
	Protein	Crude fat	Crude fibre	Total CHO
100:00:00	-1.414±0.08 ^d	-0.020±0.01 ^d	-0.036±0.01 ^a	+0.334±0.03 ^c
70:30:00	-0.240±0.06 ^b	-0.042±0.01 ^e	-0.470±0.03 ^b	+0.356±0.04 ^c
60:30:10	-0.299±0.03 ^b	+0.138±0.01 ^c	-2.437±0.02 ^d	+1.853±0.43 ^b
55:30:15	-0.032±0.07 ^c	+0.164±0.03 ^b	-2.210±0.06 ^c	+1.803±0.46 ^b
50:27:23	-0.900±0.01 ^a	+0.184±0.02 ^a	-2.400±0.03 ^d	+4.039±0.23 ^a

Key: CHO= Carbohydrates; -ve= Loss of the component; +ve= Increase of the component;

Means with the same letter along the column are not significantly different at P<0.05.

4.3.2 Influence of water addition on nutritional components during extrusion

Effect of water addition rate to the extruder barrel on changes occurring to the proximate components of blend containing rice, sorghum and BSF on extrusion is shown in Table 4.3. All components except protein content were significantly affected by the rate of water addition to the extruder barrel during processing. Protein loss was highest at 15 kg/h by 0.528% and lowest at 25 kg/h by 0.469%. Crude fat loss was highest at water addition rate of 25 kg/h by 0.111% and lowest at 20 kg/h by 0.071%. Crude fibre content loss significantly decreased from 1.543% to 1.453% as water addition rate increased to 15 to 25 kg/h. Total CHO changed from 1.413% to 2.191% as water addition rate increased to 15 to 25 kg/h.

Table 4. 3 : Effect of rate of water addition rate on proximate components mean net change

Rate	Protein	Crude fat	Fibre	CHO
15 kg/h	-0.528±0.10 ^a	-0.074±0.02 ^a	-1.543±0.02 ^b	1.413±0.36 ^b
20 kg/h	-0.526±0.09 ^a	-0.071±0.03 ^a	-1.528±0.01 ^b	1.416±0.41 ^b
25 kg/h	-0.469±0.09 ^a	-0.111±0.02 ^b	-1.453±0.01 ^a	2.191±0.38 ^a

Key: CHO= Carbohydrate; -ve= Loss of the component; +ve= Increase of the component; Means with the same letter along the column are not significantly different at P<0.05.

Effect of the rate of water addition to the extruder barrel on total phenolic content and condensed tannins of instant flour is shown in Figure 4.2. Extrusion cooking significantly increased concentration of total phenolics and condensed tannins in flour from 17.69 to 18.49 mg GAE/kg (Figure 4.2a) and 0.07 to 0.10 mg CE/g (Figure 4.2b) respectively with increase in rate of water addition from 15 to 25 kg/h respectively.

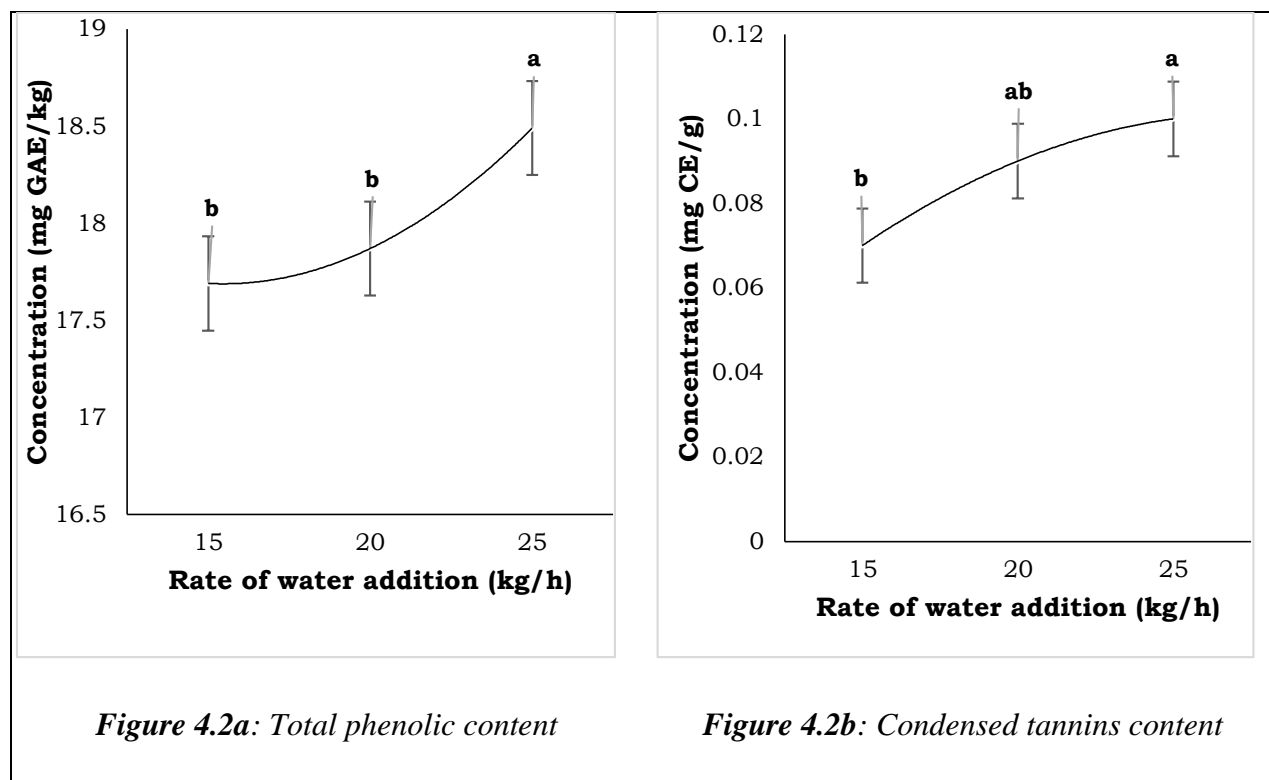


Figure 4. 2 : Effect of water addition rate to the extruder on total phenolics (4.2a) and condensed tannins (4.2b) in the extrudates

Key: Means with the same letter are not significantly different at P<0.05

4.3.3 Influence of feed rate on nutritional components during extrusion

Effect of extruder feed rate to the proximate components of blend containing rice, sorghum and BSF on extrusion is shown in Table 4.4. Total carbohydrates increase significantly increased from 1.248% to 2.098% with increase with feed rate from 1,800 kg/h to 2,100 kg/h. Changes

due to feed rate on protein, crude fat and crude fibre were not significant. Increase in feed rate from 1800 kg/h to 2100 kg/h caused a small increase in increase of crude fat from 0.084% to 0.087%. However, increase in feed rate from 1800 kg/h to 2100 kg/h caused a decrease in loss of protein and crude fibre from 0.537% to 0.478% and 1.511% and 1.505%, respectively.

Table 4. 4 : Effect of extruder feed rate on proximate components mean net change

Feed rate	Protein	Crude fat	Crude Fibre	Total CHO
1800 kg/h	-0.537±0.08 ^a	0.084±0.02 ^a	-1.511±0.16 ^a	1.248±0.29 ^b
2100 kg/h	-0.478±0.07 ^a	0.087±0.02 ^a	-1.505±0.05 ^a	2.098±0.32 ^a

Key: CHO= Carbohydrates; -ve= Loss of the component; +ve= Increase of the component; Means with the same letter along the column are not significantly different at P<0.05.

Effect of extruder feed rate to the extruder on total phenolic content and condensed tannins in instant flour is shown in Figure 4.3. Flour obtained from feed rate of 1800 kg/h had significantly higher mean total phenolics of 18.23 mg GAE/kg as compared to 17.8 mg GAE/kg of 2100 kg/h (Figure 4.3a). Concentration of condensed tannins in extrudates was not significantly affected by extruder feed rate though at 2100 kg/h though exhibited a marginally higher mean levels of 0.09 mg CE/g whereas 1800 kg/h had 0.08 mg CE/g (Figure 4.3b).

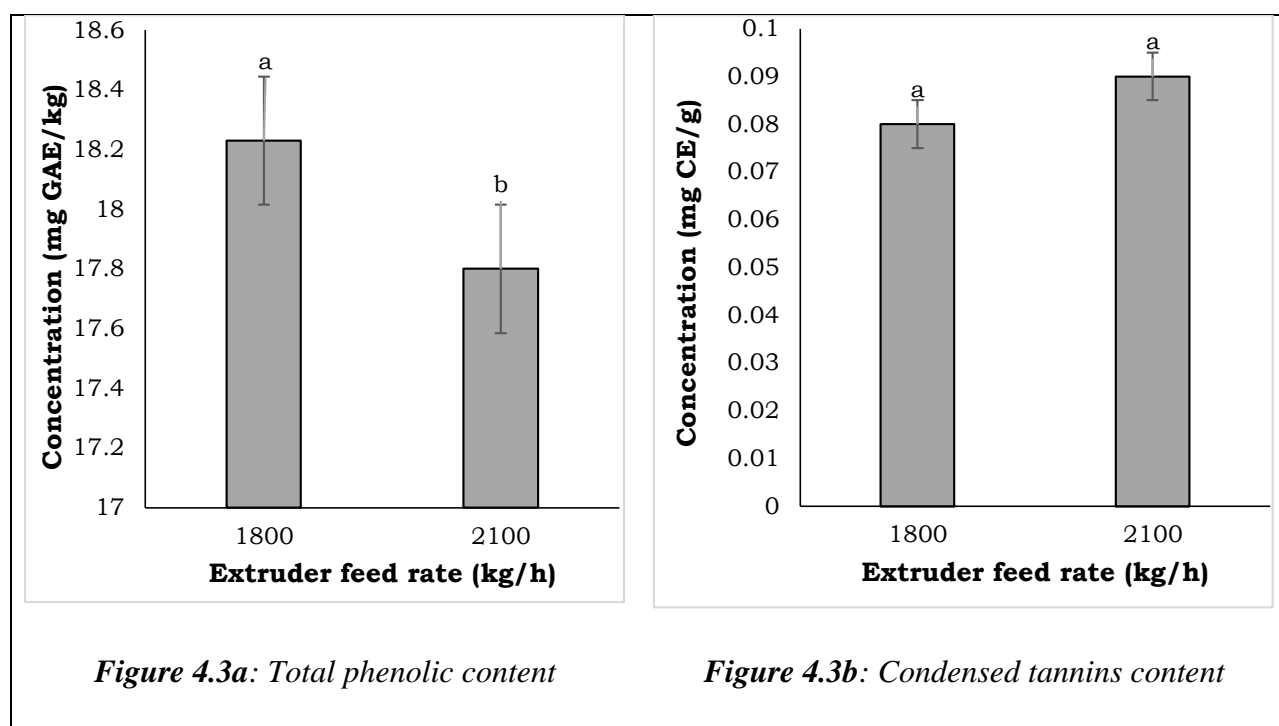


Figure 4. 3 : Effect of extruder feed rate to the extruder on total phenolics (4.3a) and condensed tannins (4.3b) in the extrudates

4.3.4 Interaction effect among ingredient variables on nutritional components on extrusion

Effect of extrusion on nutritional components due to interactions between feed composition, moisture level and rate is shown in Appendix VIII and IX. Three level interaction among all factors significantly affected all nutritional components during extrusion. The interactions between feed composition and moisture level and, feed composition and moisture had a significant effect on all nutritional components. The interaction between rate of water added to the barrel and feed rate had no significant effect on carbohydrate levels and total phenolic content.

4.4 Discussion

Different food ingredients are composed of biopolymers such as carbohydrates (starch and fibre), proteins and oils/ fat at varying proportions. Water, minerals, and vitamins are also part of these ingredients. Biopolymers in foods play an important role in influencing the nature of reactions that occur during processing and eventually the quality of the final product. Therefore, varying the composition of ingredients during blending is directly related to varying the biopolymers in the feed mix that undergoes extrusion processing. As found out in this study; (See ANOVA results in Appendices VIII and IX), feed composition significantly affected the extent of biopolymers reactions on extrusion cooking. Based on the ANOVA mean square values observed, feed composition accounted for >80% of changes observed in all nutritional components. Milling unit operation reduces particle size thus increasing the surface area for bio-reactions. Food solid powders can be transformed into a fluid (dough) through hydration if adequate water is present without the application of heat. This process is influenced by the: formula and particle size, moisture content, temperature, pressure, and mixing pattern (Yacu, 2011). Therefore, all these factors play a key role on the extent of chemical reactions in the bioreactor. In general, effects of extrusion cooking on nutritional quality include destruction of anti-nutritional factors, gelatinization of starch, increased soluble dietary fibre and reduction of lipid oxidation (Sreejith, 2019).

4.4.1 Role of mixture feed composition on nutritional components on extrusion

Feed composition, water added to the barrel and feed rate significantly contributed to the nutritional changes observed upon extrusion cooking as shown in Appendix VIII. Composite blends exhibited diverse effect on the nutritional components during extrusion cooking. During extrusion cooking, water contained in the melt is at very high temperatures and high pressure. Upon exit at the die, this water flashes into steam that evaporates away leaving behind a product

with a low moisture content than in the raw feed as shown in Figure 4.1 and Table 4.1. Overall, extrusion cooking caused increase in dry matter in instant flour as compared to the dry matter of its feed. Instant flour from 100% rice showed concentration total dry matter increase of 9.4% while flour from blend of 70% rice and 30% sorghum had the least increase of 5.6%. Blends that contained BSF showed a concentration increase of between 7.2% to 8.9%.

Results in Table 4.1 compares the proportions of nutritional components between raw blends and resultant instant flour. The change in the proportions of the nutritional components does not fully account for differences in moisture content of the raw blend and resultant flour. Hence, mass balance approach comes in hand to evaluate the actual change in nutritional components by accounting for differences in moisture levels. From mass balance findings shown in Table 4.2, it was observed that extrusion cooking reduced the contents of protein and crude fibre while increasing total carbohydrates. According to Singh *et al.* (2019) various thermomechanical and biochemical changes take place on the feed while it is cooked and sheared inside the barrel. This results in changes to the nature of bound and unbound water present in the extrudates. Consequently, the increased dry matter combined with low fat content can be associated with increased shelf life in the extrudates and resultant flour.

Crude protein content and *in vitro* protein digestibility behaved differently on extrusion cooking. Highest protein decrease of 1.414% was observed in blend with 100% rice while lowest decrease of 0.032% was in blend that had 55% rice, 30% sorghum and 15% BSF. This could indicate a possibility of shielding proteins from forming complexes called Maillard Reaction Products (MRP) (Potter *et al.*, 2013) during extrusion. Generally, this net decrease is caused by non-enzymatic reactions such as Maillard and caramelization. There was net increase in *in vitro* protein digestibility from raw blends to resultant flour. The blend of 100% rice had the lowest increase of 8.6% while the highest was for the blend containing 50% rice, 27% sorghum and 23% BSF. This increase in *in vitro* protein digestibility could be linked to shear impact and temperature causing denaturation which opens up the protein configuration to enzymatic activities and also the the destruction of anti-nutrients (Damodara *et al.*, 2017).

It was observed that as the content of crude fibre increased in the blends, there was a corresponding increase in loss on extrusion. Contrary to crude fibre, blends with lower carbohydrate content recorded the highest percentage increase on extrusion. This could point to the fact that extrusion cooking conditions could be disintegrating dietary fibres to smaller units, by breaking down the glycosidic bonds of the polysaccharides (Arribas *et al.*, 2017; Potter *et al.*, 2013). These smaller unit accounts for the increase in total carbohydrates. Also,

Yağci *et al.* (2020) suggested that the reduction in crude fibre during extrusion could be attributed to exposure of the blends to high shear stress and temperature of the extruder barrel causing chemical bond breakage, forming oligosaccharides. Thus, there is a transformation of some insoluble fibre components into soluble oligosaccharides. This occurs through the breakage of covalent and non-covalent linkages between carbohydrates and protein associated with the fibre components, resulting in small molecular fragments. According to Camire (1998), these smaller fragments may be soluble in aqueous ethanol. Thus, they are discarded during the extraction steps common to methods of fibre analysis contributing to the decrease in the overall fibre content. According to Riaz (2004), fibre has a negative effect on texturization of protein. A high level of fibre content will interfere with the texturization process by diluting the protein level and causing discontinuities in the texturized matrix. During the texturization process, fibre partially blocks some of the cross-linking of the protein macromolecules, which can affect structure and texture. To overcome this problem raw material can be ground very finely, so the fibre content will not interfere with the protein texturization.

The raw composite blend that contained 100% rice exhibited a low decrease of crude at 0.02%. A similar observation was made for the composite having 70% rice and 30% sorghum showing a decrease of 0.042%. The loss could be due the crude fat being involved in some biochemical reactions especially with starch and proteins. Starch polymers are degraded into smaller molecules during extrusion processing. However, this process impacts the amylopectin portion more than the amylose. As a result, there is formation of amylose-lipid complexes and starch-fatty acid esters during the extrusion process (Cui, 2005). Studies suggest that the lipid-carbohydrate or three-way lipid-carbohydrate-protein interactions may be responsible for the lipid binding observed. An interaction may involve either: (1) a physical entrapment or encapsulation mechanism, or (2) a molecular-level interaction. Fatty acid (FA) molecules have the ability to form inclusion complexes, which may be a mechanism for them to interact with amylose. Thus, FA bond with starch more efficiently than with the triacylglycerols (TAG). There is a direct molecular-level interaction between FA and amylose or amylopectin fractions of cornmeal during extrusion. The bonding involved is either hydrogen bonding or the hydrophobic interaction by helical inclusion complex formation or, most likely, a combination of the two. Amylose-FA complexes were produced with FA and monoacylglycerols (MAG), but not with TAG. Bonding of lipids protects them from oxidation (Pokorny & Kolakowska, 2003). Besides the biochemical reactions that cause lipids binding at elevated temperatures, the

lipids also undergo heat induced degradation into volatile compounds such as aldehydes and ketones. These volatiles will be released with the water after the exiting die (Gertz *et al.*, 2014; Grigorakis *et al.*, 2010; Reifsteck & Jeon, 2000). On the contrary, it was observed that blends with BSF registered increase in crude fat proportional to the level of BSF. Thus, the higher the proportion of BSF in the blend the higher the increase in crude fat. This probably means that shear pressure in the extruder is causing expulsion of fat from cells of the BSF by breaking down the cell wall (Sandrin *et al.*, 2018). The net increase was because lipids expelled were more than what was being involved in the biochemical reactions and heat induced degradations. Extrusion processing increased total phenolic content of the resultant instant flour when compared to the respective raw blends. This increase is due to the breaking of ester bonds with cell wall components such as cellulose and lignin. Thus, the releasing of bound phenolics such as flavonoids to free acids (Hu *et al.*, 2018). Increase of total phenolics can be associated with corresponding increase in antioxidant capability and other functional roles of the developed food product (Melini *et al.*, 2020; Shahidi & Ambigaipalan, 2015). Conversely, condensed tannins content greatly decreased on extrusion cooking of raw blends to their respective resultant products by between 88.80 to 95.0%. Tannins are among compounds known to be heat labile and therefore could not withstand high extrusion cooking temperatures. Hence, extrusion cooking can be said to improve on nutritional properties of a food where anti-nutrients are significantly reduced while increasing protein digestibility as shown in Table 4.1. Similar observations were reported by Nikolopoulou and Grigorakis (2008). These researchers demonstrated that extrusion cooking causes inactivation of heat-labile anti-nutritional factors such as tannins and phytic acids therefore increasing the digestibility of starch and protein.

The researchers also empirically showed that extrusion cooking of different types of beans resulted in protein reducing by 0-10%, fibre reducing by 3-27%, fat increasing by 0-22% and tannins reducing by up to 54%. The exception was lupins where an increase of up to 25% was reported (Nikolopoulou & Grigorakis, 2008). Despite apparent losses of some proximate components like protein and crude fibre, proportions of these components in extruded flour are higher per unit mass than in the respective raw blend. This observation can be linked to the fact that extruded flour has high dry matter content as compared to their respective raw blends as shown by concentration indices in Figure 4.1. Extrusion cooking also improved the properties of the flour matrix. This is because bamboo shoot belongs to grass family of plants that possess cellulose and hemicellulose which enhances their structural rigidity. According to Vincent (2008), these polysaccharides are the main components of dietary fibres. However, they have

intrinsic toughness which makes them difficult to chew by human beings. Therefore, extrusion processing transforms the food matrix containing bamboo shoots to be soft to chew by reducing the size of the structural fibres via bond breaking.

4.4.2 Influence of water addition on nutritional components in extrusion

Nutritional components were found to be affected by the rate of water addition to the feed during extrusion cooking as shown in Table 4.3 and Figure 4.2. According to Camire (1998), feed moisture influences shear, product viscosity in the barrel and residence time. This is because water plasticizes the matrix of the feed, decreasing its stiffness and increasing the extension possible before irreversible changes such as gelatinization sets in (Vincent, 2008). Water acts as a plasticizer, reducing the glass transition and melting temperature of starch. Heating the formed dough above a certain temperature will result in starch gelatinization/melting. The latter phenomena involve starch granular swelling (due to an increase in water uptake), resulting in a significant viscosity rise (Yacu, 2011). High moisture level leads to the formation of protein aggregates by cross-linking of disulphide bonds which modify the starch-protein morphology in the extrudate (Dalbhat *et al.*, 2019).

Increasing the rate of water added to feed during extrusion significantly increased the total carbohydrates while reducing crude fat and crude fibre from raw blends to extruded flour as shown in Table 4.3. Though not significantly, protein content decreased with increase with increase in water addition rate. This observation, could be attributed to residence time where the higher the rate of water addition barrel, the less viscous the melt. Consequently, the low viscosity of the melt causes short residence hence less time for biochemical reactions. Besides residence time in the extruder, increase in total carbohydrates could be due to increase in solubility of the soluble fibres and starch digestibility (FAO, 2008). Level of moisture has been shown to affect degree of macromolecular degradation during extrusion cooking. Oligosaccharides and starch are broken when cereal products are extruded through liquefaction of carbohydrates without the use of enzymes. Liquefaction occurs due to the development of high pressure and high shear affecting the $\alpha(1,6)$ linkages hence breaking down into small molecular weight sugars (Gray & Chinnaswamy, 1995).

Both total phenolics and condensed tannins concentration showed an increase with increase in the rate of water addition into the extruder as shown in Figure 4.2a and 4.2b. By increasing water to the feed in the barrel, heating phenomenon changes from dry heat to moist heat. Studies have shown that high feed moisture protects phenolic compounds from degradation thus maintaining their stability (Patil & Kaur, 2018). This is because dry heat is more lethal on

phenolic compounds when compared to moist heat. These findings are contrary to results reported by Camire (2011) which indicated that increasing feed moisture reduced the tannin levels during extrusion. However, it was acknowledged that the experiment was done by using small extruders and effect of scale-up was not known.

4.4.3 Role of feed rate on nutritional components on extrusion cooking

Extruder feed rate directly influences the extent of mixing of ingredients, rate of heat transfer to the material in the barrel and residence time (Sisay *et al.*, 2017). Hence feed rate controls degree of cooking and the accompanying reactions that take place during extrusion. Increasing the feed rate generally reduces the average retention time in the extruder. This results in a lower specific mechanical energy input, lower product temperature, higher melt viscosity at the die, and higher extrudate density. The motor torque increases but not linearly with the feed rate. The drop in the specific mechanical energy input could be explained by the fact that the total melt length does not linearly increase with feed rate. It increases only in the forward conveying sections. The restrictive sections fill length remains constant regardless of the feed rate. However, if a larger proportion of the forwarding elements become full (for example, small pitch screws getting choked), the rate of mechanical energy input increases with increasing feed rate above a certain threshold (Yacu, 2011).

The role of feed rate on nutritional components is shown in Table 4.4 and Figure 4.3. Among proximate components, the total carbohydrates content was significantly affected by feed rate between raw composite blends and extruded instant flour. The increase of total carbohydrates with increase in feed rate could be due to increase in shear rate that enhances breakdown of plant fibres/polysaccharides to soluble fibres and simple sugars. Similarly, the marginal increase in crude fat by increase in feed rate could be attributed to increase in expulsion of bound oils from plant cells due to increase in shear rate. The increase in feed rate caused a decrease in the loss of protein and fibre due to reduced residence time for bio-reactions leading to complex formation. According to Singh *et al.*, (2007), at mild or moderate conditions, extrusion cooking does not significantly change crude fibre content but it solubilises some fibre components. At more severe conditions, the dietary fibre content tends to increase, mainly owing to the increases in soluble fibre and enzyme- resistant starch fractions.

Contrary, increase in feed rate significantly decreased *in vitro* protein digestibility and total phenolic content. This observation could be attributed to the higher feed rate which lowers the shear impact required to break bound and conjugated phenols so as to render them as free phenolic acids. Though feed rate did not significantly differ on the effect for condensed tannins,

extrudates from 2100 kg/h had higher concentrations than at 1800 kg/h as shown in Figure 4.3b. This is because higher feed rate offers reduced residence time for thermal degradation.

4.4.4 Relationship between ingredient variables and nutritional changes during extrusion

Findings of this study have shown that the feed composition, moisture level and rate significantly influenced nutritional changes during extrusion as summarized in Figure 4.4 and Appendix VIII. Interaction between feed blends and water addition occasioned changes to mass moisture of the matrix in the barrel. Moisture directly affects the degree of reactions such as starch gelatinization, dextrinization and shielding effect on total phenolics and condensed tannins. Feed blends composition interaction with feed rate determined the extent of mixing and shear impact which affects reaction rate of biopolymers. The interaction between feed rate and water addition caused changes to specific mechanical energy (SME) of the extruder. Similar studies have demonstrated that SME is dependent on feed moisture and feed rate (Shruthi *et al.*, 2017). Mass moisture, mixing, shear impact and SME collectively determine the degree of plasticization, chemical reactions and residence time of the mass in the barrel. According to Pansawat *et al.* (2008), increasing water addition and reducing feed rate will cause lowering of SME, reduced mixing, increased residence time distribution and decreased die pressure which culminates to the degree of product transformation. a negative effect of an increased flow rate on SME (Seker, 2011). However, interaction between rate of water added to the barrel and feed rate which define SME significantly affect change to proximate components except for carbohydrates. This suggests that since carbohydrates increase is associated mainly by dietary fibres breakdown the chemical reactions are independent of the SME factors. The SME factors include extruder torque, efficiency, mass flow rate and motor power. An increase of SME and the high mechanical shear degrades macromolecules such molecular weight of starch granules decreases and hence increasing the water solubility index of the product (Altan & Maskan, 2011). SME also decreases with an increase in moisture content of feed due to reduced shear, torque and viscosity (Seker, 2011).

Feed stock composition and hydration properties affect the extent of mixing, rheological properties and flow behaviour of the melt in the barrel. The basic mechanism of mixing in polymer extruders is by a convective motion in the laminar flow region. The mixing action generally takes place by shear and elongational flow. It is called distributive mixing when the mixed components do not exhibit a yield point. Distributive mixing can be described by the extent of deformation or strain the fluid elements are exposed to. Dispersive mixing is critical in compounding some food extrusion applications. The breakdown stress of agglomerates

depends on their size, shape, and nature. The stresses acting on the agglomerate will depend on the flow and rheological properties of the polymer field. The higher the viscosity, the greater will be the dispersive mixing (Yacu, 2011).

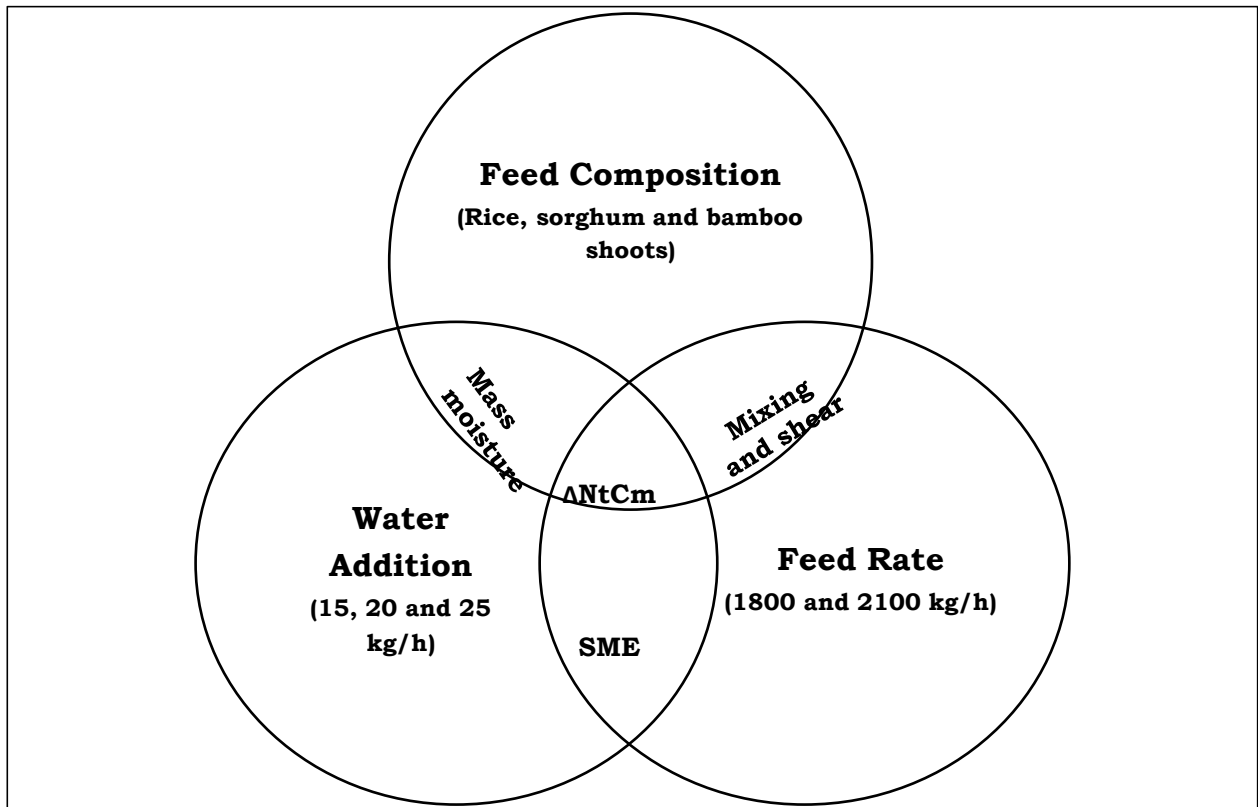


Figure 4. 4 : Nexus role of feed composition, rate and water addition in alteration of nutritional properties of instant flour during extrusion processing

Key: SME= Specific Mechanical Energy; $\Delta NtCm$ = Changes in Nutritional components in the product

The application of chemical reactions in the barrel is in the production of food products with desired functional properties such as gelatinization during the production of instant foods. According to Cui (2005) pre-gelatinized starches have been widely used in instant foods. They have more recently been prepared mainly by extrusion cooking, because of various advantages of this process over traditional methods. This is due to the ability or versatility to optimize the degree of chemical reactions in the extruder barrel so as to obtain product(s) with desirable functional properties. Extruded starches are easily dispersible, improved solubility, and have a lower viscosity. The partial degradation of appropriately heated amylose shows that chemical changes also occur at temperatures of 185–200°C (Belitz *et al.*, 2009). The degree of starch gelatinization of the extrudate mainly depends on extrusion conditions, such as the initial moisture content of materials. Water is generally added to the ingredients to provide enough

moisture content for gelatinization. However, excessive initial moisture content may reduce the degree of starch gelatinization of the extrudate. The heating level and retention time in an extruder are also important factors affecting the gelatinization of starches. This degree of starch gelatinization increases gradually along the cooking zone. In a low-fat-content treatment, increasing the screw speed increases the length of the cooking zone, while an opposite result was observed when using a higher fat content. The lubricating effect of lipid at a higher fat content reduces the friction generated at a higher screw speed, and therefore results in a lower degree of starch gelatinization (Devahastin, 2011).

4.5 Conclusion and Recommendation

Based on the findings of this study, the null hypothesis that extrusion cooking has no significant effect on nutritional composition of the blends is therefore rejected. Generally, extrusion cooking caused reduction in protein, crude fibre and condensed tannins but increased total carbohydrates, *in vitro* protein digestibility and total phenolics content. Raw materials composition, water addition rate and loading rate to the extruder were shown to affect the extent of alteration of the nutritional components during cooking. This observation was made when the operating parameters were held constant. Increase of bamboo shoots proportions in the blends caused decreased loss of protein, increased loss of crude fibre, increased total carbohydrates and increased protein digestibility. Loss of protein and crude fibre decreased with increase in feed rate and water addition rate. Therefore, in innovative development of novel food products through extrusion, information about ingredient variables should be factored besides extruder operation parameters.

All composite blends formulated in this study are recommended for any processing method but for extrusion we recommend *Yushania alpina* to be below 15% of the blends proportion. While undertaking this study, it was observed that composite blends with *Yushania alpina* above 15% interfered with the efficiency and mechanical operation of the extruder. This triggered frequent breakdowns and very high rate of screws wearing. High fibre content in blends with high proportion of *Yushania alpina* was linked to this problem.

CHAPTER FIVE

POST-EXTRUSION MICROBIAL LOAD AND ITS INFLUENCE ON SHELF-LIFE AND THERMODYNAMICS OF INSTANT FLOUR

Abstract

Post processing handling of a food pose a risk of spoilage and foodborne illnesses due to microbial contamination if hygiene is not prioritized. Profile of microbial communities in the food after contamination are influenced by the nature of the food matrix. Aim of this study was to determine post-extrusion microbial contamination, shelf life and thermodynamics of instant flour due to extruder ingredient variables: Feed rate, water addition and composite blend containing rice, sorghum and bamboo shoots flour (BSF). A commercial dry type single screw extruder with a constant set barrel temperature of 250°C and screw speed of 1480 rpm was used. Feed composition had five levels (100:0:0, 70:30:0, 60:30:10, 55:30:15 and 50:27:23 for rice, sorghum and BSF, respectively on dry weight basis). Water addition had three levels (15, 20 and 25 kg/h) while feed rate was at two levels (1800 and 2100 kg/h). Extrudates were collected in storage gunny, then milled and flour stored into their respective bags. Samples were aseptically for drawn from storage gunny for determination of total viable count (TVC), total coliform count (TCC), yeasts and moulds (YM), *Staphylococcus aureus* and accelerated shelf-life testing. Flour from 100% rice had the highest mean microbial contamination at log₁₀ 2.89, 2.18 and 2.57 cfu/g for TVC, TCC and YM, respectively. Overall *Staphylococcus aureus* prevalence was at 23.3%. As water addition rate increased from 15 to 25 kg/h, TVC reduced from log₁₀ 2.78 to 1.85 cfu/g, TCC reduced from log₁₀ 1.73 to 0.83 cfu/g and YM reduced from log₁₀ 1.73 to 1.59 cfu/g in flour. Increase of feed rate from 1800 to 2100 kg/h significantly reduced TCC from log₁₀ 1.65 to 0.97 cfu/g and YM from log₁₀ 1.79 to 1.63 cfu/g. The most prevalent moulds were *Cladosporium* spp. at 32.65% while the least at 4.08% was *Trichothecium* spp. Most prevalent yeasts were of *Candida* spp. at 80.28% and *Rhodotorula* spp. at 2.82% was the least. Shelf life of the flours ranged between 65- 408 days while spoilage was due to extracellular enzymes. Q₁₀ values for fungi were 0.76-1.01, activation energy 0.7-66.6 kJ/K/mole, enthalpy energy -1.82- 64.15 kJ/mole, entropy energy -41.98- -10.44 kJ/mole and Gibbs free energy 10.57- 67.23 kJ/mole. Hygiene programmes are needed to control microbial contamination during post extrusion processing.

Key words: Extrusion, Microbial contamination, Shelf life, Thermodynamic properties

5.1 Introduction

Food processing essentially encompasses one or a set of unit operations that transform the properties of raw materials with the purpose of obtaining product(s) that satisfy the needs of a consumer. Such transformations of natural raw food materials are directed to obtain products with no risk to cause foodborne illnesses, greater acceptance in the market and with better possibilities of storage and transport (De Boeck *et al.*, 2015; Granato *et al.*, 2020). The ability of a processed food to meet these functions is usually influenced by the nature of the food material due to inherent properties and post-processing handling. Therefore, understanding this concept in food products development is of paramount importance. This is because any alteration of ingredients and/or processing parameters has a consequence on the microbial communities, sensory parameters and shelf life of the new food product.

Bamboo shoots are not only delicious but are rich in nutrient components, mainly proteins, carbohydrates, minerals, dietary fibre, phenolic compounds and are low in fat and sugars (Mustafa *et al.* 2016). Bamboo shoots as an ingredient has gained popularity in development of novel products such as breakfast cereals, fruit juices, bakery and meat products, sauces, shredded cheeses, cookies, pastas, snacks, frozen desserts, and many other food products (Nirmala *et al.*, 2009). Inclusion of bamboo shoots in cereal crops such as rice, wheat, maize, sorghum and many others, with processing, will not only affect nutritional components but also influence microbial profile in resultant products. This is because change in factors that affect microbial growth in foods such as moisture content, water activity, pH, redox potential, nutrients and inhibitory substances will definitely occur. However, very limited information on microbial properties and shelf life of extruded products developed from blending rice, sorghum and bamboo shoot is available.

The length of time that food remains edible and nutritious depends on temperature, moisture, and other factors that affect the biochemical processes especially growth rates of organisms that cause spoilage (Hammond *et al.*, 2015). According to Haouet *et al.* (2018) all foods are composed by biological components that undergo degradation or spoilage with time from factors that cannot be fully blocked. Hence, shelf life is the time in which the product remains, after manufacture and packing, safe and suitable to use, in defined storage conditions. The food product must preserve in that period its own chemical, physical, microbiological and sensorial characteristics and, where appropriate, comply with any label declaration of nutritional information when stored according to the recommended conditions. Shelf life is commonly estimated by two different stability testing procedures: real-time stability tests and accelerated

stability tests. In real-time stability testing, a product is stored at recommended storage conditions and monitored until it fails the specification. In accelerated stability tests, a product is stored at elevated stress conditions (such as temperature, humidity, and pH). Degradation at the recommended storage conditions can be predicted using known relationships between the acceleration factor and the degradation rate. Temperature is the most common acceleration factor (Calligaris *et al.*, 2012). Measurable chemical, physical, and microbiological properties of a food can be used as quality or critical indicators to evaluate extent of deterioration (Calligaris & Manzocco, 2012).

The most critical aspect that must be given utmost priority during handling of food is hygiene. Poor hygiene poses risk of contaminating a food with spoilage and pathogenic microorganisms (Joshi, 2019). Subsequently this will result in shortened shelf life to the product and increased chances of food borne illnesses transmission to the consumer (Liu *et al.*, 2020; Ricci *et al.*, 2020). Although extruded cereal-based products enriched with bamboo shoots are high key macronutrients such as crude protein and total carbohydrates, if stored properly their low a_w will curb the growth of all microbes. But sometimes these conditions of low a_w favour growth and development of *Bacillus* spp. bacteria and several genera of yeasts and moulds. *Bacillus* spp. bacteria which belong to aerobic spore formers who are capable of producing extra-cellular enzymes, which enables them to breakdown flour and related products and use it as sources of energy and other nutrients. The microbial flora in cereal-based foods and related products such as convenience foods may be expected to from the soil, storage environments and those picked up during the processing of these commodities (Jay, 2012; Ricci *et al.*, 2020). Hence, the aim of this study was to determine the degree of variability in post processing microbial contamination levels, shelf life and thermodynamic properties due to extruder ingredient variables on resultant instant flour. Shelf life and thermodynamic properties were predicted based on chemical kinetics principles and Arrhenius model obtained derived from rate of fungi growth during storage at difference temperature in relation to a reference temperature. Ingredient variables: Feed mixture composition (different blend ratios), extruder water addition rate and feed rate were the independent variables in the study. The control for the study was blend having 100% rice. All other extruder variables were held constant. Findings of this study have provided insightful information on the nature of spoilage that occur in flour and predominant fungi that could be responsible for the spoilage.

5.3 Materials and Methods

5.3.1 Materials

Materials were prepared as described in section 3.3.1 of Chapter three.

5.3.2 Experimental Set-up

The experimental design is described in section 4.2.2 of Chapter four except that analysis was done in duplicates for each sample.

5.3.3 Extrusion and Milling

Extrusion was done as described in section 4.2.3 of chapter four.

5.3.4 Sampling for Microbial and Shelf-Life Analysis

Prior to milling as described in section 4.3.3, the chopped dried extrudates were transported using screw conveyors to a point of collection after exiting the die. Collection was done manually in storage nylon gunny bags purchased from a local open air market and stored for an overnight. The following morning extrudates were milled and resultant flour was collected in their respective storage gunny bags. Flour samples of about 100g were aseptically obtained from the storage gunny bags into kraft bags and immediately taken to the laboratory for microbial load determination and accelerated shelf-life testing. A total of 60 samples (30 samples in two replicates) were for analysis of initial microbial load and 360 samples for shelf-life analysis (each sample was sampled 12 times and divided into 3 batches of 4 for each storage temperature).

5.3.5 Microbial Analysis

Microbial load was determined according to AOAC (2000), where 10 g of each sample was accurately weighed and homogenized with 90 mL of sterile buffered peptone water (Oxoid, UK). The samples were then serially diluted before inoculating 1 mL on petri dish in duplicates by pour plating technique. Microorganisms were cultured as follows:

- a. Total viable counts (TVC) was obtained by inoculating samples with Plate Count Agar (PCA) (Oxoid, UK) and incubating at 37°C for 48 hours.
- b. Total Coliform Counts (TCC) was obtained by inoculating samples with MacConkey agar (Oxoid, UK) and incubating at 37°C for 24 hours.
- c. Yeasts and moulds were obtained by inoculating samples with Potato Dextrose Agar (PDA) supplemented with 0.01% chloramphenicol (Oxoid, UK) and incubating at 25°C for 7 days.

- d. Finally, for determination of *Staphylococcus aureus*, inoculation was done with Baird Parker agar enriched with egg yolk (Oxoid, UK) and incubated at 37°C for 48 hours.

Colonies of TVC, TCC, yeasts and moulds were manually counted after their respective incubation period, transformed by \log_{10} and reported as \log_{10} colony forming units per gram of sample (\log_{10} cfu/g). Black colonies formed on Baird Parker agar were considered to be *Staphylococcus aureus* and reported as either present or absent in the sample (prevalence). Colonies of yeasts and moulds were further phenotypically characterized to determine the predominating genuses.

5.3.6 Phenotypic Characterization of Fungi

Phenotypic (morphological and physiological) characterization of fungi to genus level was done according to procedures described by Benson (2001) and Pitt and Hocking, (2009). Identification of moulds was done in two steps. First step was based on observation of cotton-like colonies surface and backside colour as they appeared on the petri plate. Second step involved isolating the colony, mounting on a slide and microscopically observing hyphal structure and type of spores of the colony. Identification of yeasts involved isolation of colonies, mounting on a slide, staining the slide with methylene blue and observing under microscope to describe the cells. Summary of the phenotypic traits for the fungi isolated in this study are presented in Appendix X.

5.3.7 Shelf-Life Tests

To determine shelf life, samples were packaged in 'sugar' bags were divided into three batches, each containing 30 samples in quadruplets. First batch was stored under refrigeration at $4\pm 0.5^\circ\text{C}$, the second batch stored in an incubator at $25\pm 0.5^\circ\text{C}$ while the last batch was stored in an incubator held at $40\pm 0.5^\circ\text{C}$ for 28 days. Each sample from every batch was collected for enumeration of yeasts and moulds according to AOAC (2000) method on days 7, 14, 21 and 28.

Yeasts and moulds were used as indicators of quality during storage of extruded flour. To predict the shelf life in number of days, steps suggested by Taoukis *et al.* (1997) (graphically demonstrated in Appendix XI) were used, where:

1. First, yeasts and moulds data in \log_{10} cfu/g for every sample was plotted against storage time for each of the three storage temperatures to determine the kinetics order of reaction (Labuza, 1984).

- Second, after establishing that the order of reaction to be first order, a regression plot of $\frac{[A]}{[A_0]}$ against storage time carried out to get a linear model (linear model equations are given in Appendix XI), according to the following equation:

$$\ln \left(\frac{[A]}{[A_0]} \right) = kt$$

Where $[A]$ is the yeasts and mould count on a given storage day while $[A_0]$ is the initial counts of day 0 and t is the storage time in days.

- Third, was to establish a regression plot of semi-logarithmic scale for the rate constant for each sample at the three temperatures ($\ln k$) against absolute inverse of temperature ($\frac{1}{T}$) to obtain an Arrhenius equation based on:

$$\ln k = \ln k_o - \frac{E_a}{R} \left(\frac{1}{T} \right)$$

$$\frac{1}{T} = \frac{1}{T^*} - \frac{1}{T_{ref}}$$

Where k is the reaction rate constant; R is the molar gas constant (8.314 J/K/mol), T is the absolute temperature (K); E_a is the apparent activation energy (J/mol), and k_o is the pre-exponential factor; T_{ref} , corresponding to the average of the temperature range used during the experiment where in this case was taken to be 25°C (298.15⁰K) and T^* is the temperature to which prediction of shelf life is done, in this case it was 22°C (295.15⁰K).

- Last step was to use the Arrhenius parameters obtained to estimate the shelf life according to formula described by Calligaris *et al.* (2012):

$$Shelf\ life = \frac{[A_{lim}] - [A_0]}{k_{ref} \exp \left[-\frac{E_a}{R} \left(\frac{1}{T^*} - \frac{1}{T_{ref}} \right) \right]}$$

Where $[A_0]$ is the initial yeasts and moulds counts at time zero; k_{ref} is the rate constant at the reference temperature; E_a is the apparent activation energy (J/mol); T^* is the temperature to which prediction of shelf life is done, in this case it was 22°C (295.15⁰K); $[A_{lim}]$ is the standard acceptable limit for yeasts and moulds count in processed flour which is log₁₀ 4.00 cfu/g according to KS ISO 16649 (2018); T_{ref} , corresponding to the average of the temperature range used during the experiment where in this case was taken to be 25°C (298.15⁰K).

NB: Maximum expected shelf life of the samples was estimated on the assumption that the initial yeasts and moulds was zero, $[A_0] = \log_{10} 0.00$ cfu/g.

5. Also, Q_{10} values from the Arrhenius model were calculated as follows:

$$Q_{10} = 10^{bT^*}$$

Where b is the gradient of the Arrhenius equation and T^* is the temperature to which prediction of shelf life is done, in this case it was 22°C (295.15⁰K)

5.3.8 Estimation of Thermodynamic properties

Thermodynamic parameters were derived based on absolute reaction rate theory using the mathematical expressions based on Eyring's equation (Taoukis *et al.*, 1997):

$$k = \frac{K_B T}{h} \times e^{\frac{\Delta S^*}{R}} \times e^{\frac{-\Delta H^*}{RT}}$$

$$\Delta H^* = E_a - RT$$

$$\Delta G^* = \Delta H - T\Delta S$$

Where K_B is Boltzmann's constant, 1.38×10^{-23} J/°K; h Planck's constant, 6.63×10^{-34} J.S; ΔS^* entropy of activation, J/mol °K; ΔH^* enthalpy of activation, J/mol and ΔG^* is the Gibbs free energy of activation, kJ/mol.

5.3.9 Data Analysis

Microbial counts of the samples underwent \log_{10} transformation before analysis. Transformed data was then subjected to normality of distribution and homogeneity of variance tests to satisfy requirements of parametric analysis (example is shown in Appendix XII). Analysis of variance (ANOVA) was carried out to investigate the main and interaction effects of experimental factors microbial contaminations levels (as shown in Appendix XIII). Regression modelling was applied in determining rate of chemical kinetics and Arrhenius equation in prediction of shelf life and thermodynamics due to yeasts and moulds. Coefficient of determination (R^2) from regression model were used as measure of fit between predictor and response variables. Post *hoc* analysis of means employed use of Scheffe method at 95% confidence level.

5.4 Results

5.4.1 Microbial contamination levels

Effect of feed composition, moisture level and rate on extent of microbial contamination is shown in Appendix XIII. It found out that all the three factors significantly affected levels of TVC, TCC and YM in instant flour, except feed composition on YM. From the means squares of ANOVA, TVC was greatly influenced by moisture level (5.688) than composition and feed

rate. TCC was influenced more by feed rate (7.059) while YM was influenced feed composition (3.436).

1) Effect of feed composition on microbial load of instant flour

Microbial load of extruded instant flour from different blends is shown in Table 5.1. Flour from the control showed significantly highest counts of TVC, TCC and yeasts and moulds at \log_{10} 2.89, 2.18 and 2.57cfu/g respectively. Least TVC of \log_{10} 1.82 cfu/g it was recorded in a flour blend that contained 50:27:23 for rice, sorghum and bamboo shoot respectively. Least TCC of \log_{10} 0.76 cfu/g and YM of \log_{10} 1.10 cfu/g was recorded in recorded in a flour blend that contained 55:30:15 for rice, sorghum and bamboo shoot, respectively.

Table 5. 1: Mean microbial load of instant porridge flour influenced by blends

Feed Composition	Log ₁₀ TVC	Log ₁₀ TCC	Log ₁₀ YM
100:00:00	2.89±0.11 ^a	2.18±0.16 ^a	2.57±0.10 ^a
70:30:00	2.68±0.21 ^b	1.53±0.25 ^b	1.66±0.17 ^b
60:30:10	2.22±0.14 ^c	1.26±0.31 ^b	1.51±0.24 ^b
55:30:15	2.71±0.30 ^b	0.76±0.21 ^c	1.10±0.15 ^c
50:27:23	1.82±0.17 ^d	0.83±0.18 ^c	1.73±0.10 ^b

Key: TVC= Total Viable Count; TCC=Total Coliform Count; YM= Yeast and Moulds Count. Means with the same letter along the column are not significantly different.

2) Prevalence of *Staphylococcus aureus*

Prevalence of *Staphylococcus aureus* among different flour composition is shown in Figure 5.1. Overall prevalence of *Staphylococcus aureus* was 23.3% (14/60) was observed in the flour samples. Flour from blend composition of 55:30:15 for rice, sorghum and bamboo shoot respectively, had 0% (0/12) prevalence while blends of 100:00:00, 70:30:00 and 60:30:10 had 33.3% (4/12) each.

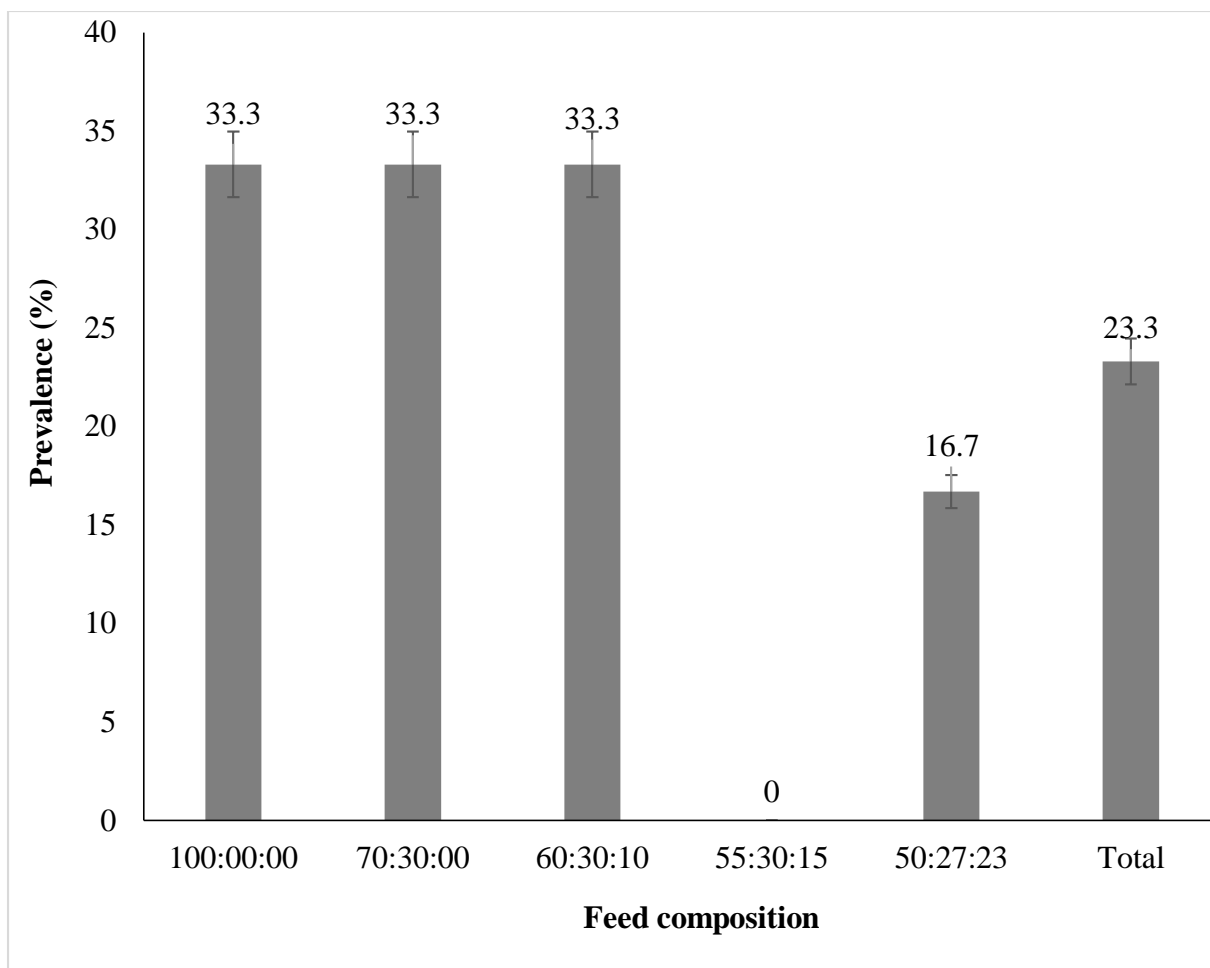


Figure 5. 1: Prevalence of *Staphylococcus aureus* in extruded flour from different composite blends

3) Effect of extruder water addition rate on microbial load of instant flour

Effect of water addition into extruder barrel on microbial load of instant flour is shown in Figure 5.2. As water addition rate increased from 15 to 25 kg/h, TVC significantly reduced from \log_{10} 2.78 to 1.85 cfu/g, TCC significantly reduced from \log_{10} 1.73 to 0.83 cfu/g and YM significantly reduced from \log_{10} 1.73 to 1.59 cfu/g in flour.

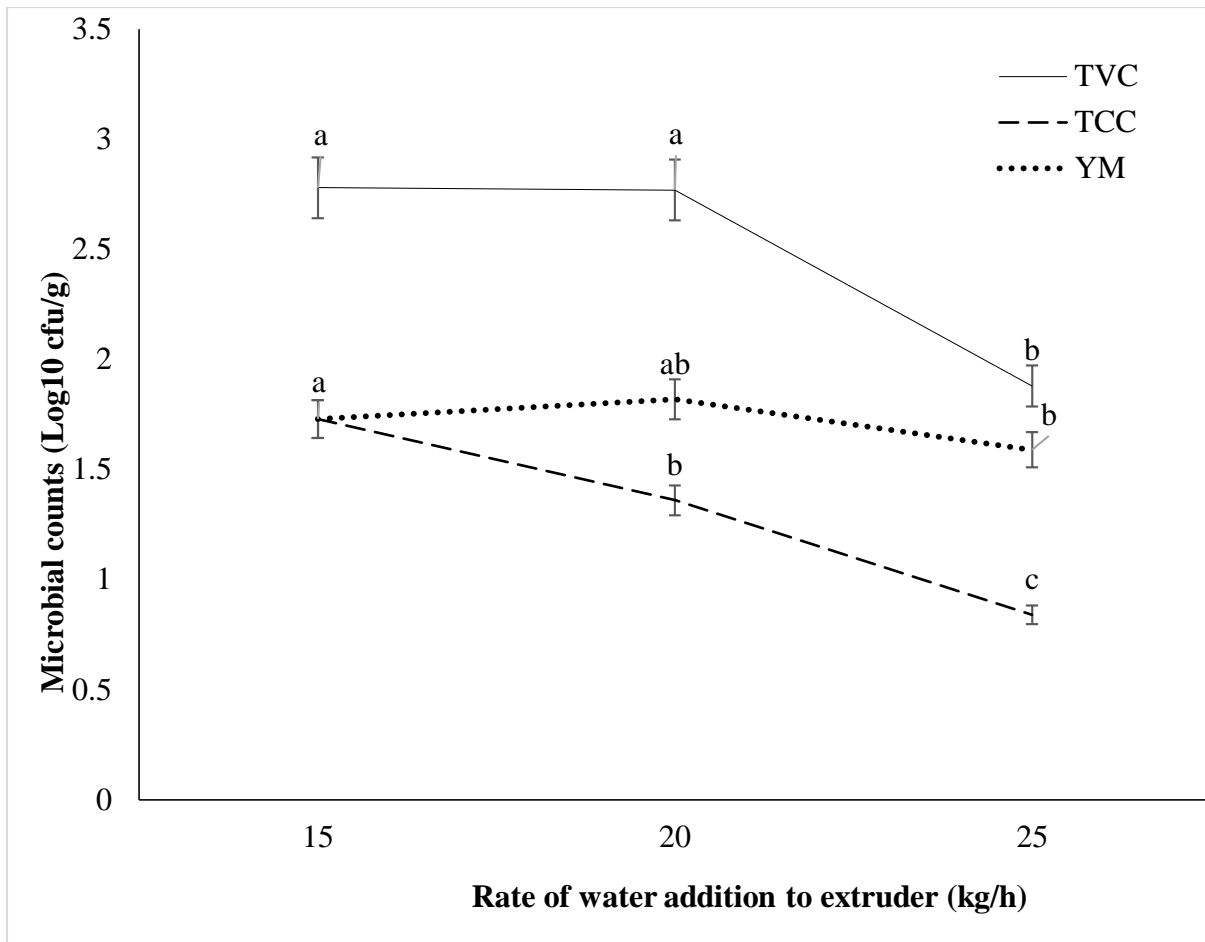


Figure 5. 2 : Effect of water addition into extruder barrel on microbial load of instant porridge flour

4) Effect of feed rate on microbial load of instant flour

Effect of extruder feed rate on microbial load of instant flour is shown in Figure 5.3. Increase of feed rate from 1800 to 2100 kg/h significantly reduced TCC from log₁₀ 1.65 to 0.97 cfu/g and YM from log₁₀ 1.79 to 1.63 cfu/g.

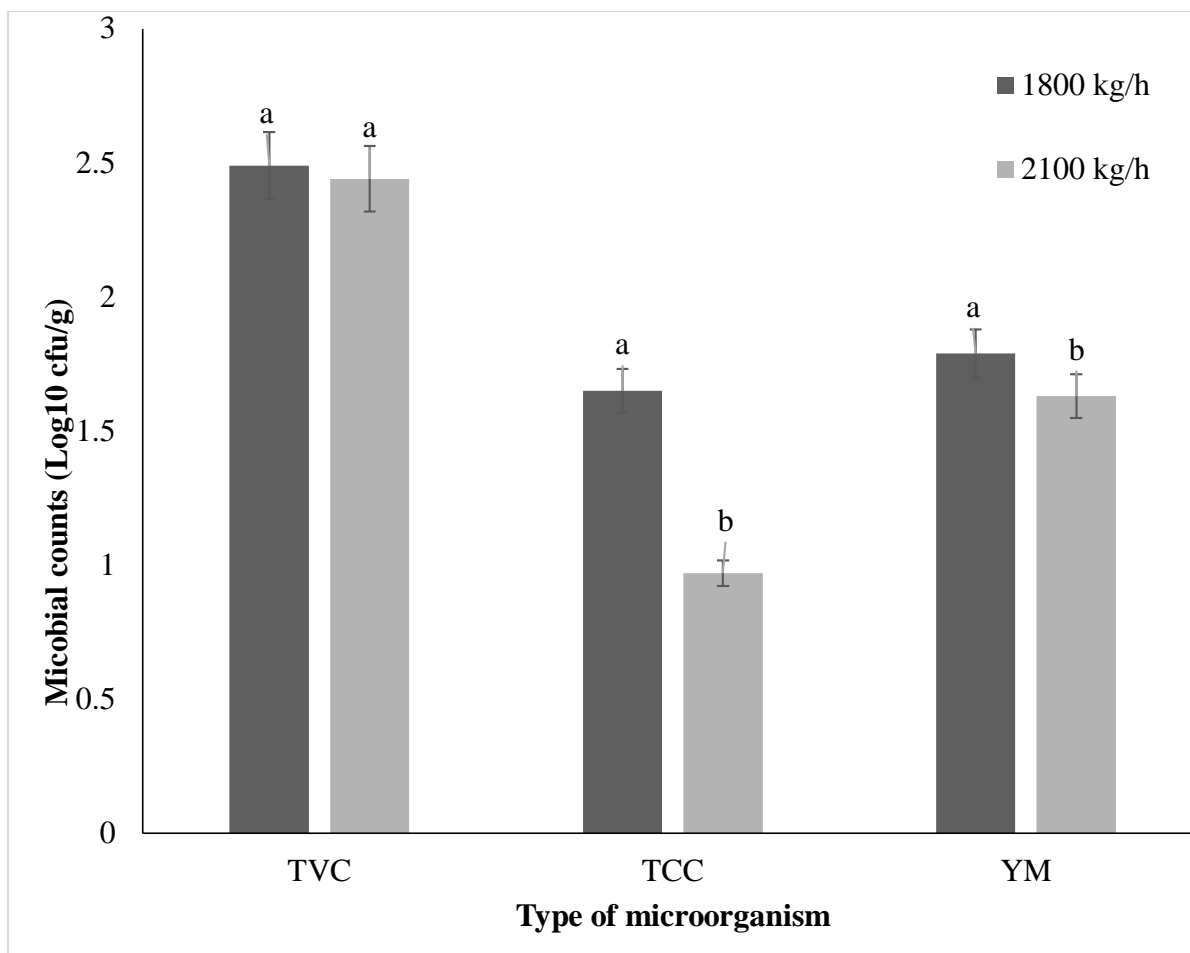


Figure 5. 3 : Effect of extruder feed rate on microbial load of instant flour

5) Interaction effect of factors on microbial load

Effect of two level and three level interaction of factors is shown in Appendix XIII. All interactions significantly affected the extent of load of TVC, TCC and YM in instant flour except interaction between feed moisture and rate did not affect TVC and YM. The mean microbial load for the three-level interaction.

6) Predominant moulds isolated in flour during storage

Prevalence of predominant genera of moulds in instant flour during storage is shown in Figure 5.4. The most prevalent moulds were *Cladosporium* spp. at 32.65%, followed by *Paecilomyces* spp. at 18.37% while the least prevalent at 4.08% was *Trichothecium* spp.

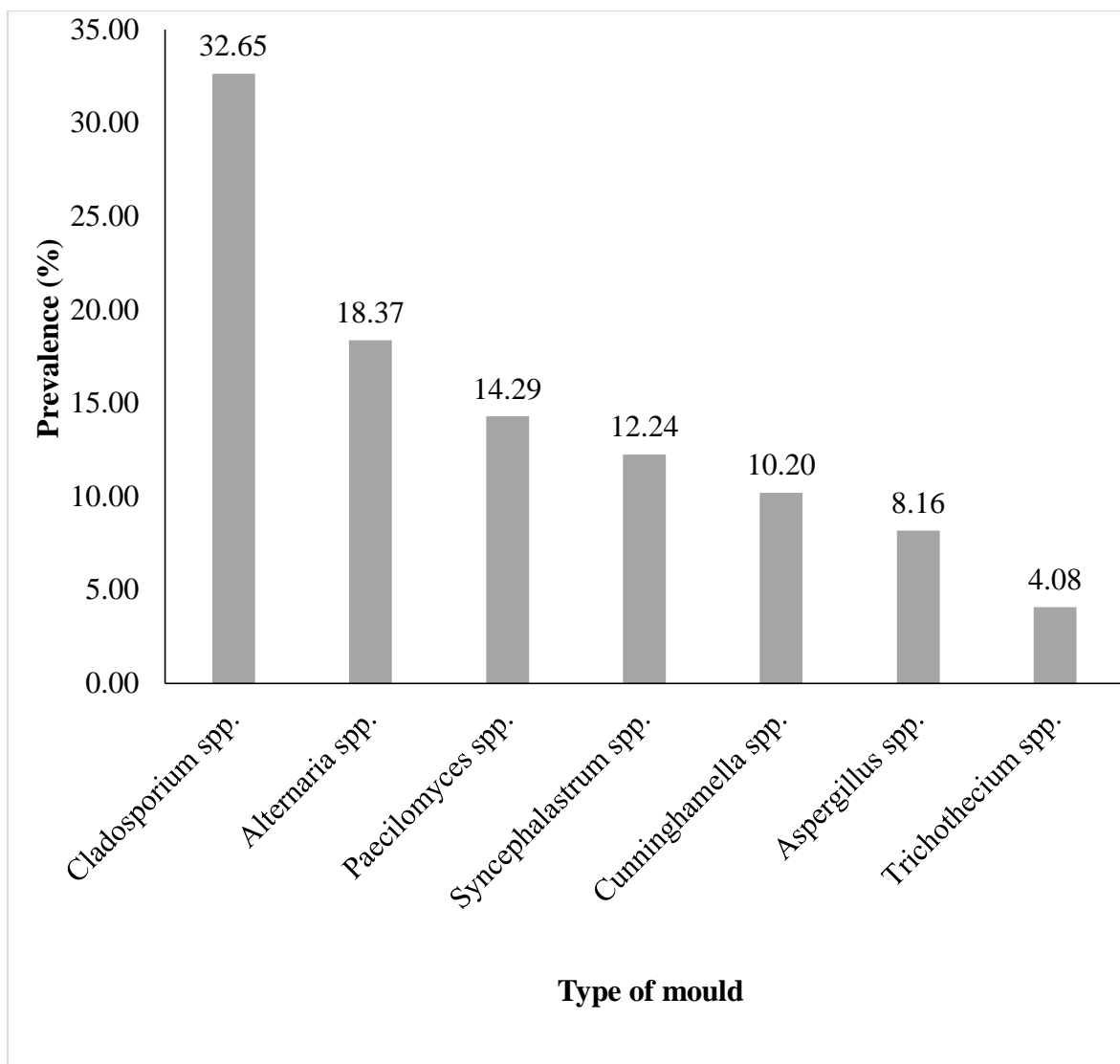


Figure 5. 4 : Predominant moulds isolated from instant flour during storage

7) Predominant yeasts isolated in flour during storage

Prevalence of predominant genera of yeasts in instant flour during storage is shown in Figure 5.5. The most prevalent yeasts were *Candida* spp. at 80.28%, followed by *Saccharomyces* spp. at 11.27% and *Rhodotorula* spp. at 2.82% was the least prevalent.

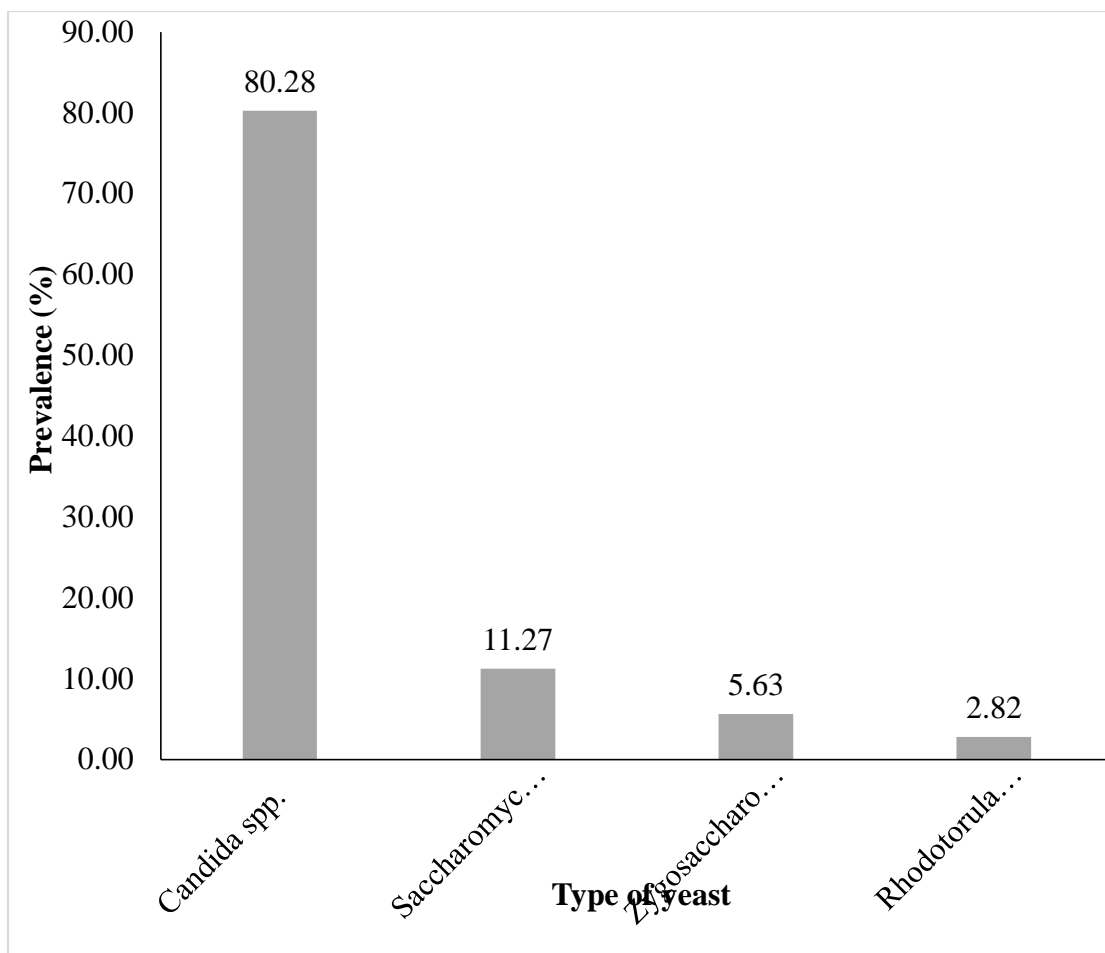


Figure 5. 5: Predominant yeasts isolated from instant flour during storage

5.4.2 Shelf Life and Q_{10}

Predicted shelf life, activation energy and Q_{10} for each of flour samples if stored at 22 °C based on yeasts and moulds growth using the Arrhenius regression equations is shown in Table 5.2. The Arrhenius equations are related to the linear regression equations given in Appendix XI. It was found out that Q_{10} values for yeasts and moulds were between 0.76 and 1.01. Shelf life of the flour was observed to be between 65 days and 408 days while maximum possible shelf life that could be attained was between 79 to 697 days.

Table 5. 2 : Predicted shelf life of instant flour at storage temperature of 22°C

Feed Composition	Added water (kg/h)	Feed rate (kg/h)	Arrhenius equation	R²	SL (Days)	Max. SL (Days)	SLD (%)	Q₁₀
100:00:00	15	1800	Y= -2756.4X-4.046	0.9834	81.66	284.03	71.2	0.91
		2100	Y= -3298.6X-4.095	0.8753	153.97	311.04	50.5	0.89
	20	1800	Y= -3957.6X-3.930	0.9384	100.74	277.90	63.7	0.87
		2100	Y= -2876.1X-4.179	0.9998	79.41	327.46	75.7	0.91
	25	1800	Y= -3778.8X-4.121	0.9945	119.38	331.60	64.0	0.88
		2100	Y= -2209.8X-3.734	0.9026	80.45	199.87	59.7	0.82
70:30:00	15	1800	Y= -2282.9X-3.495	0.9832	104.48	157.70	33.7	0.93
		2100	Y= -1289.0X-3.650	0.9718	71.51	170.27	58.0	0.96
	20	1800	Y= -162.47X-3.846	0.7826	78.57	184.87	57.5	1.01
		2100	Y= -168.78X-2.974	0.7521	65.46	79.34	17.5	0.99
	25	1800	Y= -108.7X-3.622	0.7277	83.49	148.42	43.7	1.01
		2100	Y= -462.85X-3.534	0.7909	87.38	142.09	38.5	0.98
60:30:10	15	1800	Y= -2151.7X-3.725	0.9993	128.69	196.45	34.5	0.96
		2100	Y= -3649.3X-3.670	0.9980	164.71	209.15	21.2	0.88
	20	1800	Y= -4544.9X-4.211	0.9178	196.59	385.47	49.0	0.86
		2100	Y= -1274.9X-4.427	0.7554	115.65	370.06	68.7	0.96
	25	1800	Y= -75.71X-4.154	0.7836	145.46	256.32	43.3	1.02
		2100	Y= -917.43X-3.020	0.7105	80.41	88.12	8.7	0.97

55:30:15	15	1800	$Y = -2523.0X - 4.002$	0.9181	135.89	262.59	48.3	0.93
		2100	$Y = -648.1X - 3.125$	0.7943	79.05	95.82	17.5	0.98
	20	1800	$Y = -765.9X - 2.971$	0.9861	68.36	82.86	17.5	0.97
		2100	$Y = -1254.4X - 3.328$	0.7409	101.55	123.10	17.5	0.96
	25	1800	$Y = -3426.7X - 3.603$	0.8781	136.96	192.23	28.8	0.89
		2100	$Y = -4631.9X - 3.984$	0.8357	197.84	309.14	36.0	0.85
50:27:23	15	1800	$Y = -2659.7X - 3.792$	0.9273	131.14	218.56	40.0	0.91
		2100	$Y = -1830.6X - 3.980$	0.9417	108.11	247.10	56.2	0.94
	20	1800	$Y = -3052.1X - 3.884$	0.9286	129.07	247.02	47.7	0.90
		2100	$Y = -8010.8X - 4.533$	0.9751	408.17	697.73	41.5	0.76
	25	1800	$Y = -5728.6X - 3.826$	0.8240	201.38	287.69	30.0	0.82
		2100	$Y = -6017.0X - 3.299$	0.7415	121.65	173.78	30.0	0.82

SL= Shelf life; R^2 =Coefficient of determination; Max.= Maximum ; SLD=Shelf life difference between predicted and max. shelf life

Thermodynamic properties of yeasts and moulds growth in instant flour during storage is shown in Table 5.3. Activation energy was found to be between 0.7 and 66.6 kJ/K/mole of the flour, enthalpy energy -1.82 to 64.15 kJ/mol, entropy energy -41.98 to -10.44 kJ/mol and Gibbs free energy 10.57 to 67.23 kJ/mol. Flour from a blend of 60:30:10 for rice, sorghum an BSF respectively had the highest Q_{10} of 1.02 and the least E_a , ΔH^* , ΔS^* and ΔG^* of 0.63 kJ/K/mol, -1.82 kJ/mol, -41.98 kJ/mol and 10.57 kJ/mol, respectively. While Flour from a blend of 50:27:23 for rice, sorghum an BSF respectively had the lowest Q_{10} of 0.76 and the highest E_a , ΔH , ΔS and ΔG of 66.60 kJ/K/mol, 64.15 kJ/mol, -10.44.98 kJ/mol and 67.23 kJ/mol, respectively.

Table 5. 3 : Thermodynamic properties of fungi in instant flour during storage

Feed Composition	Added water (kg/h)	Feed rate (kg/h)	E_a (kJ/K/Mol)	ΔH^* (kJ/Mol)	ΔS^* (J/K/Mol)	ΔG^* (kJ/Mol)
100:00:00	15	1800	22.92	20.46	-29.30	29.11
		2100	27.42	24.97	-27.29	33.02
	20	1800	32.90	30.45	-24.87	37.79
		2100	23.91	21.46	-28.86	29.98
	25	1800	31.42	28.96	-25.53	36.50
		2100	18.37	15.92	-31.38	25.18
70:30:00	15	1800	18.98	16.53	-31.10	25.70
		2100	10.72	8.26	-35.04	18.60
	20	1800	1.35	-1.10	-40.92	10.98
		2100	1.40	-1.05	-40.87	11.01
	25	1800	0.90	-1.55	-41.51	10.70
		2100	3.85	1.39	-38.86	12.86
60:30:10	15	1800	17.89	15.44	-31.60	24.76
		2100	30.34	27.89	-26.00	35.56
	20	1800	37.79	35.33	-22.75	42.05
		2100	10.60	8.15	-35.10	18.50
	25	1800	0.63	-1.82	-41.98	10.57
		2100	7.63	5.17	-36.64	15.99
55:30:15	15	1800	20.98	18.52	-30.18	27.43
		2100	5.39	2.93	-37.90	14.12

	20	1800	6.37	3.91	-37.33	14.93
		2100	10.43	7.98	-35.18	18.36
	25	1800	28.49	26.04	-26.82	33.95
		2100	38.51	36.06	-22.43	42.68
50:27:23	15	1800	22.11	19.66	-29.67	28.42
		2100	15.22	12.77	-32.85	22.46
	20	1800	25.38	22.92	-28.20	31.24
		2100	66.60	64.15	-10.44	67.23
	25	1800	47.63	45.17	-18.50	50.63
		2100	50.03	47.57	-17.48	52.73

Key: E_A = Activation Energy; ΔH^* = Change in enthalpy of activation; ΔS^* = Change in entropy of activation and ΔG^* = Change in Gibbs free energy activation

5.5 Discussion

5.5.1 Microbial load in instant flour

As shown Table 5.1, flour from all composite blends had microbial contamination. There was significantly varying levels of microbial contamination among the flour from these composite blends. The flour from 100% rice had at the highest levels for TVC, TCC and YM. These differences in levels could be linked to the inherent properties of each composite blends such a_w and presence of anti-microbial compounds. Composite blend that had 55% rice, 30% sorghum and 15% BSF (55:30:15) had the least levels of TCC, YM and 0% presence of *Staphylococcus aureus*. Prevalence of *Staphylococcus aureus*, as shown in Figure 5.1, indicates that microbial contamination occurred during handling of the product by the personnel during post-extrusion operations. Furthermore, presence of coliforms indicates unhygienic contact surfaces, such as gunny bags and handling equipment, while YM spores could be from the miller and air in the factory environment (Al-Defiery & Merjan, 2015; Ballata *et al.*, 2019; Marriot, 2006).

Besides composite blends, rate of water addition to the extruder significantly affected the microbial in extruded flour as shown in Figure 5.2. As the rate of water addition to the extruder increased, the level of microbial load reduced in the resultant flour. Water is a plasticizer in extrusion cooking that dictates the degree of melt and eventually rheological properties of the product. Therefore, increase in rate of water addition to the feed in the barrel causes resultant product to possess intrinsic properties that are unfavourable to microbial activity from

increased degree of plasticization. However, increasing the extruder feed rate significantly reduced the microbial load for only TCC and YM in the resultant flour as shown in Figure 5.3. This observation could be due to less contact time between the product and sources of contamination through increased throughput from increased mass flow rate.

Due to low moisture content of the product and by extension low a_w because of flash water evaporation at the die, yeasts and moulds are the microorganisms of importance in relation to spoilage. Mould isolated from the flour show that *Cladosporium* spp. was the prevalent while *Trichothecium* spp. was the least prevalent as shown in Figure 5.4. Apart from spoilage, some of the moulds isolated are known to produce mycotoxins in food. These are *Paecilomyces* spp. that produce paecilotoxins, *Aspergillus* spp. produce aflatoxins and *Trichothecium* spp. produce trichothecene toxins. Conidia of *Cladosporium* spp. are particularly well adapted to aerial dispersal since they are small, dry, heavily pigmented and highly resistant to humid conditions (Ohta *et al.*, 2006). Heterogeneity encountered in conidia is relevant in the area of food spoilage; the strongest spore will define if spoilage occurs. In nature, the strongest spore enlarges the limits of growth of a species. Fungal spoilage of processed foods cause waste, economic losses and foodborne illnesses (Mom *et al.*, 2020; Van den Brule *et al.*, 2019;). Food storage has also been associated with incidences of foodborne illnesses (Ricci *et al.*, 2020).

Another type of fungi isolated from the flour are yeasts as shown in Figure 5.5. *Candida* spp. was the most predominant yeast. The possible source of these fungi into the flour could be the spores from the air and gunny bags that were for storage. Fungi produce numerous spores that are distributed by water, air and other vectors such as insects and soil. Airborne spores are present in every cubic meter of air and cause fungal colonization in all environments (van den Brule *et al.*, 2019).

Water activity of the food describes the energetics of water in a food system, and hence its availability to act as a solvent and participate in chemical or biochemical reactions. Pathogenic bacteria cannot grow below a water activity of 0.85–0.86, whereas yeast and moulds are more tolerant to a smaller water activity of 0.8 (Chen, 2010). According to Yousef and Balasubramaniam (2013) bound portion of water in a food matrix adheres to food components by physical or chemical binding mechanisms. There are many sites that bind water; these include hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and salt ions. On other hand, unbound water is usually present within the pores or the interstitial tissue of the food. The amount and form of water influence microbial behaviour, as well as the physicochemical and biological reactions in food. The presence of microbial contaminants in

a food having favourable a_w , in combination with other factors such as low acidity, can lead to quality deterioration or compromise the safety of foods. An example are fungi which are not only spoilage microbes but some also produce potent toxic and carcinogenic agents. These fungi cause various degrees spoilage by producing enzymes breakdown lipids, proteins and ferment carbohydrates to forth unpleasant odours in foods (Marriot, 2006).

5.5.2 Shelf life of flour

From Table 5.2, instant flour from composite blends of 70% rice and 30% showed to have shorter shelf life of between 65.5 to 104.5 days while flour from blends of 50% rice, 27% sorghum and 23% BSF had the longest shelf life of 108.1 to 408.2 days. Shelf life of flour in number of days and also difference with maximum attainable shelf life were heavily dependent on the initial contamination load of yeasts and moulds. Largest difference of about 75.7% between predicted shelf life of the composite blend and the maximum possible shelf life that can be attained was observed in flour from 100% rice extrude at a feed rate of 2100 kg/h and water addition rate of 20 kg/h. Smallest difference of about 8.7% was observed in flour from a blend of 60% rice, 30% sorghum and 10% BSF extruded at 2100 kg/h with a water addition rate of 25 kg/h. This prediction of shelf life can help in coming up with measures that prevent deterioration due to microbial activities. This agrees with Leng *et al.* (2017) who urges that to prevent performance deterioration, it is important to estimate the biological growth kinetics, which are mainly based on the fundamental relationships between growth rate and substrate utilization rate.

The difference between predicted shelf life of each flour and its corresponding maximum shelf life that can be attained was calculated as shelf-life difference (SLD) and expressed as a percentage as shown in Table 5.2. Maximum shelf life was predicted on the assumption that optimum hygienic standards are observed during processing and initial yeasts and moulds is zero. Flour from 100% rice extruded at feed rate of 2100 kg/h and water addition rate of 20 kg/h registered the biggest SLD of 75.7%. While Flour from a blend of 60% rice, 30% sorghum and 10% BSF extruded at feed rate of 2100 kg/h and water addition rate of 25 kg/h registered the least SLD of 8.70%. In general, resultant flour composite blends with bamboo shoots had longer shelf life with a mean of about 148 days, 100% rice produced flour with mean shelf life of 103 days and flour from blend of 70% rice and 30% sorghum had the least shelf life with mean shelf life of 82 days. This infers that besides feed rate and water addition to the feed during extrusion, inherent properties of blends are influencing shelf life and SLD.

All Q_{10} values were for fungi growth when reference storage temperature was set at 25°C (298.15 °K) ranged from 0.76 to 1.01. These values are low because this reference temperature falls within the optimum temperature of growth for yeasts and moulds. Also, Q_{10} values observed fall below values reported for spoilage microorganisms of 2.3 to 4.1 (Hammond *et al.*, 2015). However, according to McMeekin (2004), Q_{10} can be said not to hold good in biological reactions because some of the temperature constants used in chemistry. Therefore, Arrhenius equation which is temperature dependent model for microbial growth are often thought to have a greater mechanistic basis than other models. But according to Hough (2010), stable foods such as these instant flours after relatively prolonged storage, may be microbiologically safe to eat but rejected due to changes in their sensory properties.

5.5.3 Thermodynamic properties of fungi in flour during storage

Extrusion of different composite blends at different moisture level and feed rate greatly affected nature of the resultant food matrix in the flour, consequently affecting the thermodynamic properties as indicated in Table 5.3. Instant flour from composite blends of 70% rice and 30% sorghum and 60% rice, 30% sorghum and 10% BSF extruded at feed rate of 1800 kg/h and water addition at a rate of 25 kg/h showed the least activation energy, E_a , of 0.90 and 0.63 kJ/K/mol. respectively. Flour from composite blend of 57% rice, 27% sorghum and 23% BSF extruded at feed rate of 2100 kg/h and water addition at a rate of 20 kg/h showed the least activation energy, 66.60 kJ/K/mol. Activation energy is energy change required to elevate a chemical entity from a ground state to a transition state, whereupon reaction can occur (Damodaran, 2017). The higher the values E_a , the longer the predicted shelf life of the flour as shown in Table 5.2.

The activation energy values highly correlated with change in enthalpy, Gibbs free energy and entropy (Table 5.3). Where the flour that had very low E_a values exhibited negative enthalpy energy change, very low Gibbs free energy and very low entropy and vice versa. Enthalpy energy change values for yeasts and moulds in instant flour were found to be positive except for flour from two groups of composite blends. First group of composite blends are from 70% rice and 30% sorghum extruded with water addition rate of 20 kg/h at both feed rates and with water addition rate of 25 kg/h at feed rate of 1800kg/h. Second group of blend is from 60% rice, 30% sorghum and 10% BSF extruded at feed rate of 1800 kg/h and water addition at a rate of 25 kg/h. The repercussion of positive values for change in enthalpy energy is that the growth rate of fungi in flour is dependent on storage temperature and light exposure except for the two groups of flour. Increasing temperature increases free energy in the system; the net

result is the lowering of the energy barrier for reactions to occur and they are accelerated. The term endergonic rather than the normal chemical term endothermic is usually used to indicate that a process is accompanied by gain of free energy in any form, not necessarily as heat. All organisms, including fungi, must obtain supplies of free energy from their environment in order to maintain living processes (Murray *et al.*, 2003). Therefore, storing this flour at lower temperatures than optimum for fungi of 25⁰C will increase shelf life.

The negative change in activation entropies for all the reactions studied indicate that activated complex in these reactions are well ordered structures. Negative entropy is unfavourable for yeasts and moulds growth because hydrophobic effect arises as a result water forming a hydration shell (clathrate hydrate) around a nonpolar substance. When water surrounds a hydrophobic molecule, the optimal arrangement of hydrogen bonds results in a highly structured shell, or solvation layer, of water in the immediate vicinity making it unavailable for microbial activity (Bhunkar *et al.*, 2014). Similar to trend for E_a , composite blends of 70% rice and 30% sorghum and 60% rice, 30% sorghum and 10% BSF extruded at feed rate of 1800 kg/h and water addition at a rate of 25 kg/h showed the least entropy energy, of -41.51 and -41.98 J/K/mol. respectively. While flour from composite blend of 57% rice, 27% sorghum and 23% BSF extruded at feed rate of 2100 kg/h and water addition at a rate of 20 kg/h showed the least activation energy, -10.44 J/K/mol. This signifies that many yeasts and moulds thrive well in flour when water molecules are ordered (bound) than unordered (free molecules) (Oren, 2008).

On other hand, free Gibb's energy had positive values indicating that yeasts and moulds that thrive in flour are aerobic. Anaerobic reactions occur in when the net Gibbs free energy (ΔG^*) is negative (Leng *et al.*, 2017). Moulds have an require absolute oxygen for optimum growth and are inhibited in an environment that has high levels of carbon dioxide (5% to 8%). Moulds ability to grow at very low levels of oxygen including in vacuum packages has enabled them to function as oxygen scavengers (Marriott, 2006). According to von Stockar and Liu (1999), Gibbs free energy for microbial growth should be negative because of involvement in formation of biomass, as in case of fermentation. Therefore, positive Gibbs free energy indicates that growth is coupled with other exothermic reactions other themselves endergonic based on positive enthalpy energy change. This claim is supported by Snyder *et al.* (2019) who suggest that fungi are prolific food spoilage organisms that are saprobic, adapted to nutrient derivation environment and are chemoheterotrophic where each species express a suite of extracellular enzymes that, collectively in a complex food matrix, are capable of digesting

structural biopolymers. A study by Nadeem *et al.* (2015) points out that fungi produce extracellular enzymes, for example, yeasts produce β -fructosidase type of invertase whereas the moulds invertase is an α -glucosidase. Also, several genera of moulds have been known to producing amylase enzyme that facilitate their access to nutrients in flour (Jay, 2012). Other extracellular enzymes from fungi include cellulases, lipases, proteases and pectin enzymes (Banwart, 1989).

5.6 Conclusion and Recommendation

Findings of this study infer that we fail to reject the null hypothesis that extrusion cooking conditions have no significant effect on microbial load and shelf life. Initial microbial load in flour was due to contamination from handlers as indicated by presence of *Staphylococcus aureus*, unhygienic contact surfaces as indicated by presence of coliforms and air as indicated by aerobic spore-formers fungi. The consequence of microbial contamination is on the safety of the consumers and shortened shelf life. Safety issues arises due to presence of foodborne pathogen bacteria *Staphylococcus aureus* and mycotoxin producing moulds such *Paecilomyces* spp., *Aspergillus* spp. and *Tricothecium* spp. The shortened shelf life could be due to spoilage associated with extracellular enzymes produced by predominant fungi isolated from the flour. Besides degree of initial microbial load contamination, inherent properties of individual ingredients used in blending, feed rate and moisture level significantly influenced shelf life of the instant flour. This is because these extruder ingredient variables alter intrinsic properties of flour which then affect energy requirements of spoilage fungi. Therefore, hygienic conditions should always be observed during and post processing. As much fungi growth rate during storage period was useful quality indicator of predicting shelf life of the products, we also recommend use of a chemical indicator and/or sensory score as means of monitoring quality.

CHAPTER SIX
INFLUENCE OF FEED RATE, WATER ADDITION AND MIXTURE
COMPOSITION ON PHYSICAL PROPERTIES OF EXTRUDED FLOUR AND
MASS TRANSFER

Abstract

Raw material composition, loading rate and moisture level in extrusion processing have a direct bearing on physical properties of the product besides other extruder operational parameters. These properties greatly influence the consumer appeal, package design and shelf-life of the product. This study aimed at evaluating the effect of sorghum and rice flour containing bamboo shoot flour (BSF) and other ingredients on resultant flour and product throughput. A commercial single screw extruder with a constant barrel temperature of 250°C and screw speed of 1480 rpm was used. Five different blends (100:0:0, 70:30:0, 60:30:10, 55:30:15 and 50:27:23 for rice, sorghum and BSF respectively, on dry weight basis) were extruded at three levels of water addition (15, 20 and 25 kg/h) and two feed rates (1800 and 2100 kg/h). Extrudates were analyzed for expansion ratio (ER). Extrudates were then milled to particle size of 0.2 mm and flour samples were analyzed for hydration properties, colour, bulk density (BD) and oil holding capacity (OHC). Product throughput was calculated using mass balance equation based on dry matter content of the raw materials and the corresponding extruded product. Mixture composition significantly affected all physico-chemical properties analyzed for the products except water solubility index (WSI). Feed rate significantly affected all physico-chemical of the product properties except WSI and colour lightness (L*). Water addition significantly affected product mass transfer, ER, BD, colour and swelling capacity (SC). Therefore, BSF can be blended with other ingredients and extrusion parameters be manipulated to give forth a product with desirable properties.

Key words: Bamboo shoots, Extrusion, Physical properties, Mass transfer

6.1 Introduction

Extrusion cooking has been defined as the thermomechanical process in which heat transfer, mass transfer, pressure changes and shear are combined to produce unit operations such as cooking, sterilization, drying, melting, cooling, texturizing, conveying, puffing, mixing, kneading, conching (chocolate), freezing, and forming (Berk, 2018). Extrusion processing has been used extensively in the production of convenience foods owing to Due to its versatility nature of its ease of operation and ability to produce a variety of physical properties which are both appealing to consumers and nutritious (Dalbhagat *et al.*, 2019; Offiah *et al.*, 2019).

Predominant physical properties that influence consumer appeal for a product include colour, structure, density, viscosity shape, size and texture (Barrett *et al.*, 2010). Many studies have looked at how manipulation of extruder operational parameters such as barrel temperature, screw speed, screw configuration and die dimensions affects physical properties of different types of extruded foods (Kristiawan *et al.*, 2020; Meng *et al.*, 2010; Panyoo & Emmambux, 2019; Sue *et al.*, 2015; Yang *et al.*, 2020). Manipulation of extruder ingredients variables: Feed rate, moisture level, mixture composition and particle size remain to be of interest to researchers.

Blending of different food ingredients to be extruded has recently gained popularity among food processors and researchers. The main purpose of blending is to enrich extruded products so as to address nutrient deficiencies and use of natural substances having high nutritional value as additives makes the product more acceptable (Dalbhagat *et al.*, 2019). According to Bravo-Núñez and Gómez (2018) besides the nutritional aspects, there is an emergent interest in polysaccharide-protein systems, as they can function as gelling agents, thickeners, emulsifiers, texture modifiers or stabilisers in foods. This is derived from knowledge on capability of proteins have on the behaviour of flours. When elaborating starchy products, the hydration, pasting, thermal, rheological and gelling properties of flours are important parameters that will influence the behaviour of flours when they are applied to products. Sometimes, these properties are not optimal in native starch and therefore need to be modified by various techniques to better suit the relevant end product.

Rice is one of the major cereal crops that act as an attractive material for the manufacture of ready-to-cook (RTC) products like pasta, noodles, ready-to-eat (RTE) breakfast cereals, modified starch, weaning foods, snack foods, pet foods, and dried soup, because of its colour, bland taste, flavour as well as good processing characteristics. Due to low nutritional value, many studies have been carried out to enrich rice with proteins, minerals and fibre from eggs, vegetables, legumes, fruit wastes and other cereals like sorghum and wheat (Alam *et al.*, 2016; Arribas *et al.*, 2017; Awolu *et al.*, 2019; Dalbhagat *et al.*, 2019). However, very little information is available on effect of incorporating bamboo shoots into rice and sorghum on the physical properties of extruded products.

Bamboo shoots consumption is gaining popularity globally due to increasing awareness about it being rich in main nutrients, such as proteins, carbohydrates, minerals and fibre and are low in fat. Beyond nutritional, bamboo shoots have been found to confer other health benefits attributed to its components exhibiting prebiotic capability, nutraceutical capacity, free radical

scavenging ability and other functional properties (Chongtham *et al.*, 2011; Kumar *et al.*, 2017). So as to increase consumption of bamboo shoots, there is concerted effort by researchers to incorporate it in major staple foods. Besides blending with other ingredients, different processing methods are being used such fermentation, pickling, baking, roasting and canning (Satya *et al.*, 2010) to increase bamboo shoot consumption. As a result of exploiting bamboo shoots in different formulations with staple foods, it is expected that the impact will not be only nutritional but also of physico-chemical nature of the blends.

However, very little information is available on impact of incorporating bamboo shoots in rice and sorghum coupled with extrusion processing on physico-chemical properties. Rice and sorghum are among the key cereal crops that are staple food among many communities around the world. Therefore, the aim of this study was to evaluate the influence of raw material containing bamboo shoot flour, sorghum and rice at different moisture level and feed rate in a single screw extruder on physical-chemical properties of resultant flour. We theorized that formulating the ingredients at different ratios and extruding these ratios at different moisture level and feed rate will not only affect the hydration properties, colour, density and expansion but also mass transfer during extrusion. Mass balance equations were employed in estimating the rate of product throughput and flashed steam. Extruder barrel temperature, screw speed and die dimensions were held constant.

6.3 Materials and Method

6.3.1 Materials

Materials for this study are described in section 3.3.1 of Chapter 3

6.3.2 Experimental design

Experimental design for the study is described in section 4.2.2 of Chapter 4.

6.3.3 Extrusion Cooking and Milling

Extrusion processing and milling is described in section 4.2.3 of Chapter 4.

6.3.4 Physical Properties Analysis

1) Expansion Ratio

Expansion ratio (ER) was determined by dividing the average cross-sectional diameter of the extrudates with the cross-sectional diameter of the extruder die which was 8.55 ± 0.01 mm. The cross-sectional diameters were measured in millimetres with a Vernier calliper (Mituyoto, Japan).

$$ER = \frac{\text{Diameter of extrudate}}{\text{Diameter of die}} \dots \text{Equation 5. 1}$$

2) Flour Hydration Properties

a) Water Absorption Index (WAI) and Water Solubility Index (WSI)

Water absorption index (WAI) and Water solubility index (WSI) were determined as described by Makoswka *et al.* (2018). One gram of a ground sample was accurately weighed, put into test tubes and 20 ml of distilled water added. The tubes were shaken on a vortexer and left to stand for 15 min. The tubes were then shaken and centrifuged at 1000 g for 10 min. The supernatant portion was decanted into an evaporating dish of known weight and dried at 105°C for 12 hours. The weight of the remaining gel in the tube was taken as the WAI.

$$WAI \text{ (g/g)} = \frac{\text{Weight of wet residue} - \text{Weight of dried residue}}{\text{Weight of the sample}} \dots \text{Equation 5. 2}$$

$$WSI \text{ (g/g)} = \frac{\text{Weight of soluble}}{\text{Weight of sample}} \dots \text{Equation 5. 3}$$

b) Swelling Capacity (SC)

The swelling capacity (SC) was determined according to the method described by Ge *et al.* (2017) with small modifications. Accurately 10.0 g of the sample was weighed and gradually mixed with 100 ml of distilled water. The suspension was held at room temperature (22±2°C) for 24 hours. Volume of the absorbent treated sample was recorded and then dried in an oven at 105°C for 12 hours. Each test was carried out in triplicate and the readings reported as means. Swelling capacity (SC) was expressed as ml of water per gram of dry sample as given in equation below

$$SC \text{ (g/ml)} = \frac{\text{Volume of absorbent treated sample} - \text{Volume of dry sample}}{\text{Weight of dry sample}} \dots \text{Equation 5. 4}$$

c) Water Holding Capacity (WHC)

The water holding capacity (WHC) was determined according to the method described by Song *et al.* (2018). Accurately 1.0 g of sample was weighed and mixed with 25 ml of distilled water at room temperature (22±2°C) and left to stand for 24 hours. Centrifugation at 4000 g for 15 min was done and the residue collected, weighed and dried at 105°C. Each test was carried out in triplicate and the readings reported as the mean. The WHC was expressed as grams of water per gram of dry sample as given in equation below:

$$WHC \text{ (g/g)} = \frac{\text{Weight of wet residue} - \text{Weight of dry residue}}{\text{Weight of dry residue}} \dots \text{Equation 5. 5}$$

3) Oil Holding Capacity

The oil holding capacity (OHC) was determined according to the method described by Song *et al.* (2018). Accurately 1.0 g of sample was weighed and mixed with 25 ml of distilled water at room temperature (22±2°C) and left to stand for 24 hours. Centrifugation at 4000 g for 15 min was done and the residue collected, weighed and dried at 105°C. Each test was carried out in triplicates and the readings reported as the mean. The OHC was expressed as g of oil per mg of dry sample as given in the equation below:

$$\text{OHC (g/g)} = \frac{\text{Weight of sample with oil} - \text{Weight of dry sample}}{\text{Weight of dry sample}} \dots \text{Equation 5. 6}$$

4) Bulk Density

Bulk density of flour samples was estimated by gently filling into 10 ml graduated cylinders and the bottom of each cylinder tapped gently on a laboratory bench several times until reduction of the sample level ceases after filling to the 10 ml mark (Maninder *et al.*, 2007). Bulk density was then calculated as weight per unit volume of sample (g/cm³)

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Mass of the flour sample}}{\text{Volume of the flour sample}} \dots \text{Equation 5. 7}$$

5) Colour Determination

Colour properties of samples were measured using a HunterLab Colour Difference Meter (Minolta, Tokyo, Japan). The results were expressed using the L* a* b* system, where L* values range from black (0) to white (100), a* values range from green (-60) to red (+60), and b* values range from blue (-60) to yellow (+60). The hue angle and Chroma values were calculated according to Pathare *et al.* (2013):

$$\text{Chroma, (C *)} = \sqrt{(a^*)^2 + (b^*)^2} \dots \text{Equation 5. 8}$$

$$\text{Hue angle (h *)} = \tan^{-1} \left(\frac{b^*}{a^*} \right) \dots \text{Equation 5. 9}$$

6.3.5 Mass Transfer Calculation

1) Rate of Product Throughput and Flash Steam Evaporation

Estimation of the rate of product throughput and flash steam being evaporated at the die was done in two steps. Stem one was to determine the dry matter content of the five feed mixture and the extruded flour samples. The oven method (AOAC, 2000 Method 934.01) was used. About 2.0 g of samples was accurately weighed and transferred into aluminium dishes. The samples were dried in a dry air oven at 105°C for 24 hours and cooled in a desiccator before weighing.

The second step was to use mass balance equation to determine the rate of mass transfer as follows:

$$TMB: F + W = S + P \dots \text{Equation 5. 10}$$

$$DMB: FX_f + WX_w = SX_s + PX_p \dots \text{Equation 5. 11}$$

The assumption made was that water being added to the feed and steam being liberated does not contain any dry matter component, therefore:

$$DMB: FX_f = PX_p \dots \text{Equation 5. 12}$$

Where: F= Extruder feed rate at kg/h; W= Rate of water addition to the extruder at kg/h; S= Rate of flash steam being liberated at kg/h; P= Rate of product throughput at kg/h; X_f= Dry matter content in the feed; X_w= Dry matter content in water; X_s= Dry matter content in steam; X_p= Dry matter content of the product; TMB= Total mass balance and DMB= Dry matter balance.

2) Specific Mechanical Energy (SME)

Specific Mechanical Energy (SME) was calculated using the equation below according to (Fayose & Huan, 2015; Muthukumarappan & Karunanithy, 2012;):

$$SME = \frac{T \times \omega}{M_f} (W \cdot h/kg) \dots \text{Equation 5. 13}$$

Where: T=Torque of the extruder (N.M); ω = Angular velocity (radians/s) and M_f= Extruder feed rate (Kg/h). Given that:

$$\omega = \frac{2\pi N}{60} \text{ (radians/s) } \dots \text{Equation 5. 14}$$

$$T = \frac{60P}{2\pi N} \text{ (N.m) } \dots \text{Equation 5. 15}$$

Where N=Screw speed of the extruder which was 1480 rpm; P= Power rating of the extruder in kW which was 75 kW.

Assumption made was that torque remained constant due to constant screw speed while SME changed based on changing feed rate of the extruder.

6.3.6 Data Analysis

Data obtained was tested for normality using PROC UNIVARIATE using SAS software version 9.1 (SAS Institute and Cary, NC) (example given in Appendix IV). Analysis of variance (ANOVA) was carried out to investigate the effect of feed rate, moisture and mixture ratios on physical properties using PROC GLM. In addition, HOVTEST=LEVENE (example is given in Appendix VI) option was used to test for homogeneity of variance among the observations. Means separations were done using Scheffe method at $p \leq 0.05$.

6.4 Results

Effect of each ingredient variable: Feed composition, rate of water addition to the barrel and feed rate on physical and mass flow properties as determined by analysis of variance (ANOVA) is shown in Appendix XV. WSI was the only physical property of the instant flour that was not significantly affected by all ingredient variables at $p < 0.05$. Apart from WSI, rate of water addition of the also did not significantly affect WHC, WAI, OHC, BD and b^* physical properties of the instant flour. Also, besides WSI, feed rate did not significantly affect L^* only while feed composition significantly affected all other physical properties. All ingredient variables significantly affected the mass flow properties: Product flow rate and steam flush evaporation rate.

6.4.1 Physico-chemical properties of the ingredients

Hydration properties and bulk density of ingredients is shown in Table 6.1. Rice flour had significantly highest mean BD of 0.93 g/cm^3 and sorghum flour had the least with 0.57 g/cm^3 . BSF recorded significantly highest mean SC of 2.37 g/ml and WSI of 0.27 g/g as compared to other ingredients. All ingredients did not differ significantly for WHC and WAI though BSF had highest mean values.

Table 6. 1 : Physico-chemical properties of the ingredients

Ingredients	BD	Hydration properties			
		SC	WHC	WAI	WSI
BSF	0.63±0.01 ^b	2.37±0.03 ^a	0.31±0.04 ^a	4.98±0.60 ^a	0.27±0.01 ^a
Rice	0.93±0.05 ^a	0.97±0.01 ^b	0.20±0.02 ^a	3.73±0.31 ^a	0.03±0.01 ^c
Sorghum	0.57±0.01 ^b	0.87±0.07 ^b	0.26±0.01 ^a	4.23±0.28 ^a	0.15±0.02 ^b

Key: Means with same letter within a factor are not significantly different at $p < 0.05$. BD= Bulk Density; SC= Swelling Capacity; WHC=Water Holding Capacity; WAI= Water Absorption Index; WSI= Water Solubility Index

6.4.2 Effect of extruder mixture composition on hydration properties of flour

Effect of extruder feed composition on hydration properties of flour is shown in Figure 6.1. Feed composition had a significant effect on WHC, WAI and SC of extruded flour. Flour from feed ratio with 60:30:10 for rice, sorghum and bamboo shoots respectively had significantly highest values for WHC, WAI and SC. Flour from feed ratios with 55:35:15 and 50:27:23 for rice, sorghum and bamboo shoots respectively were not significantly different but had the least values compared to other feed ratios. On other hand, WSI was not significantly affected by feed composition, though flour from feed ratio of 60:30:10 had the highest solubility while flour from 55:35:15 and 50:27:23 had the least.

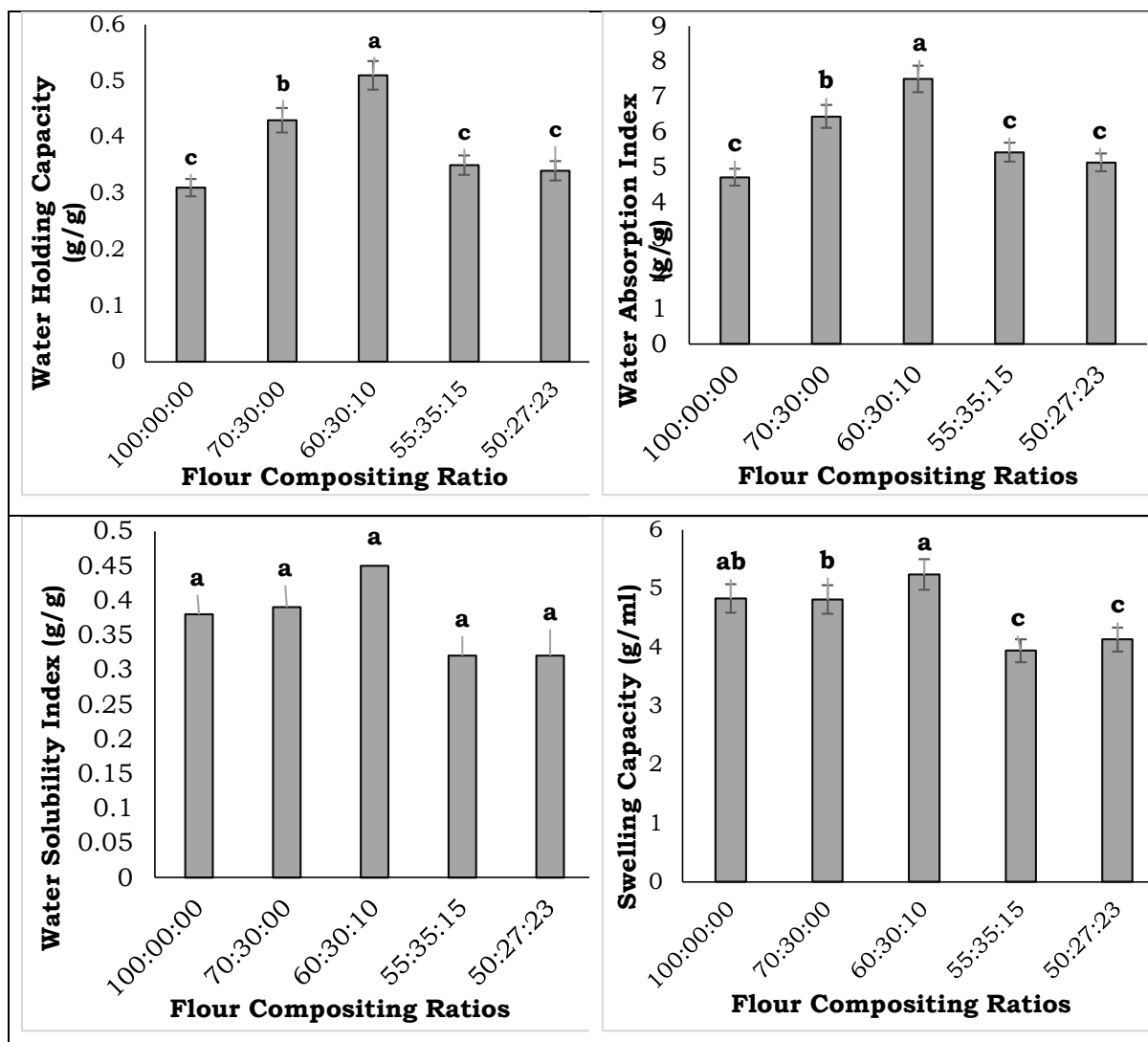


Figure 6. 1 : Effect of extruder feed composition on flour hydration properties

Key: Mean error bars with same letter not significantly different at $p < 0.05$.

6.4.3 Effect of water addition to the feed on hydration properties of the flour

Effect of extruder rate of water addition on hydration properties of the flour is shown in Figure 6.2. Rate of water addition to the extruder had a significant effect on only SC of extruded flour. Water addition at a rate of 20 kg/h had significantly highest values of SC. While WHC, WAI and WSI were not significantly affected by rate of water addition, 20 kg/h had the highest values for WHC and WAI.

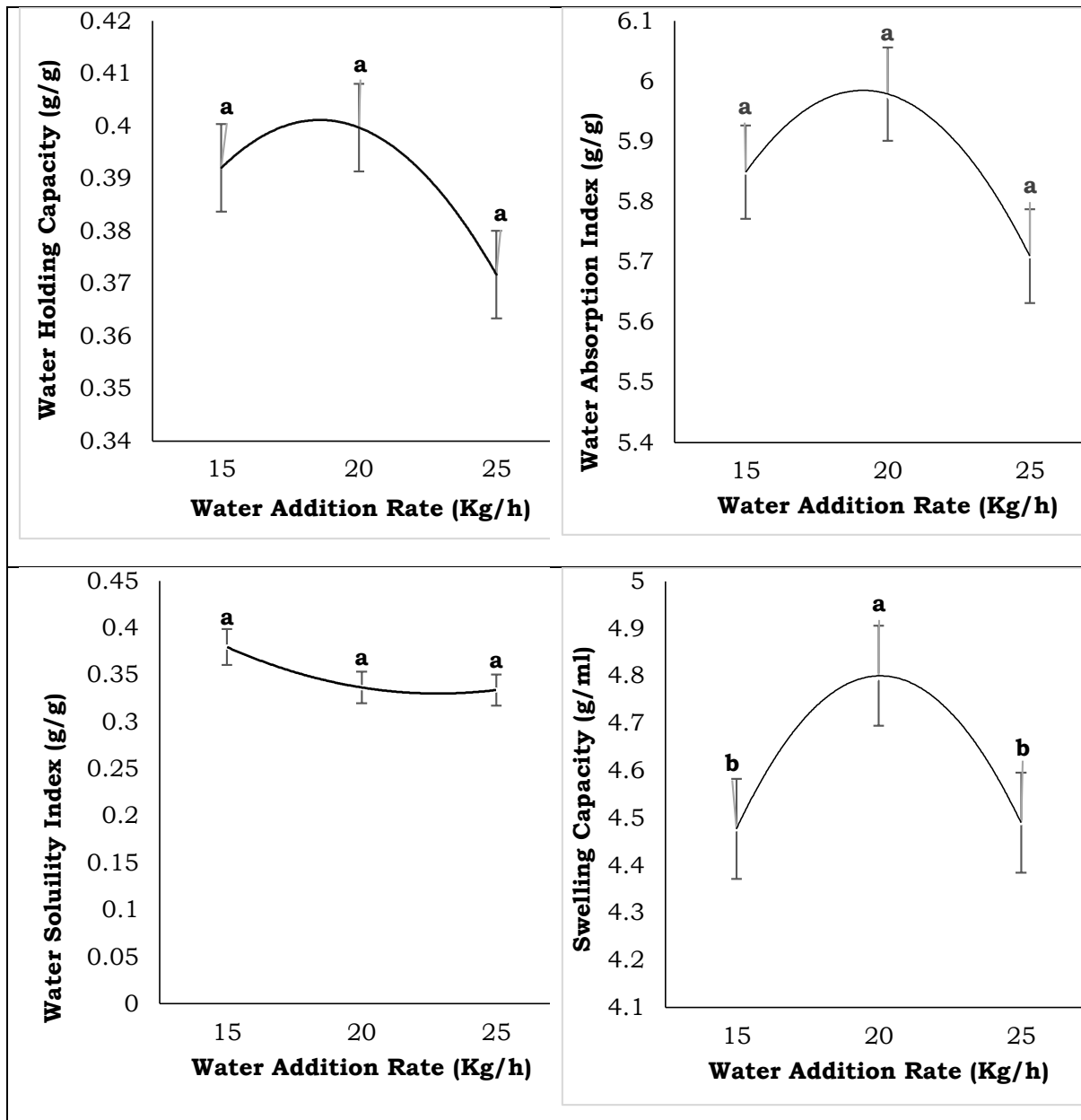


Figure 6. 2 : Effect of extruder water addition rate on flour hydration properties.

Key: Mean error bars with same letter not significantly different at $p < 0.05$.

6.4.4 Effect of feed rate on hydration properties of flour

Effect of extruder feed rate on hydration properties of flour is shown in Figure 6.3. Extruder feed rate had a significant effect on WHC, WAI and SC of extruded flour. Feed rate of 2100 kg/h had significantly highest values for all water hydration properties except WSI.

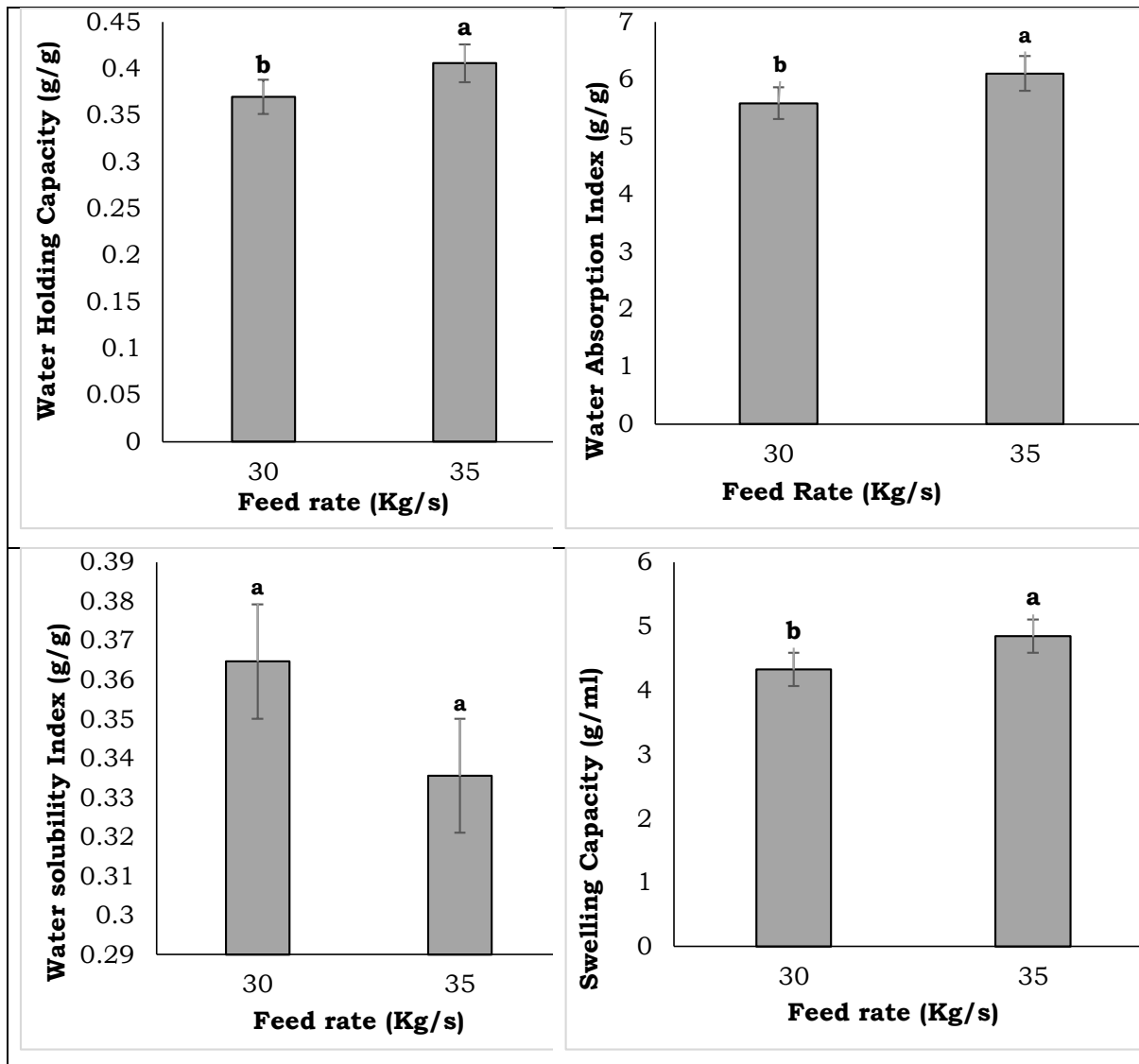


Figure 6. 3 : Effect of extruder feed rate on hydration properties of the flour.

Key: Mean error bars with same letter not significantly different at $p < 0.05$.

6.4.5 Effect of ingredient variables on physical properties of extrudates and the resultant flour

Effect of extruder feed composition, feed rate and water addition rate on other physical properties of extrudates and the flour is shown in Table 6.2. Feed composition significantly affected all physical properties with the control having the highest values for ER, OHC, BD and SR. The rate of water addition significantly affected physical properties except OHC and BD with water addition at 25 kg/h having the values expect for PR. Extruder feed rate significantly affected all physical properties except with feed rate of 2100 kg/h having the highest values except for OHC.

Table 6. 2: Physical and mass transfer properties of extrudates and flour

Factor	Level	ER	OHC	BD	PR	SR
Feed ratio	100:00:00	4.21±0.21 ^a	2.51±0.10 ^a	0.56±0.02 ^a	1782.0±32.4 ^c	22.80±1.07 ^a
	70:30:00	2.37±0.12 ^b	2.21±0.10 ^b	0.42±0.03 ^e	1848.0±35.4 ^b	21.70±0.92 ^b
	60:30:10	2.09±0.06 ^c	2.01±0.08 ^{bc}	0.45±0.01 ^d	1844.4±31.2 ^b	21.76±0.97 ^b
	55:35:15	1.94±0.06 ^c	2.15±0.12 ^b	0.47±0.04 ^c	1876.2±40.2 ^a	21.23±1.14 ^c
	50:27:23	1.57±0.11 ^d	1.92±0.03 ^c	0.52±0.02 ^b	1842.6±31.2 ^b	21.80±1.05 ^b
Added water	15 kg/h	2.37±0.15 ^b	2.16±0.07 ^a	0.48±0.02 ^a	1843.2±26.4 ^a	16.78±0.14 ^c
	20 kg/h	2.20±0.18 ^c	2.19±0.08 ^a	0.48±0.02 ^a	1848.6±27.6 ^a	21.69±0.15 ^b
	25 kg/h	2.73±0.24 ^a	2.13±0.08 ^a	0.49±0.02 ^a	1823.4±26.4 ^b	27.11±0.14 ^a
Feed rate	1800 kg/h	2.31±0.17 ^b	2.25±0.07 ^a	0.46±0.02 ^b	1701.6±6.0 ^b	21.64±0.65 ^b
	2100 kg/h	2.56±0.15 ^a	2.07±0.06 ^b	0.51±0.02 ^a	1975.8±7.8 ^a	22.07±0.65 ^a

Key: Means with same letter within a factor are not significantly different at $p < 0.05$. ER= Expansion Ratio; OHC= Oil Holding Capacity; BD= Bulk Density; PR= Product Throughput rate; SR= Flashed Steam Evaporation rate.

6.4.6 Effect of feed rate on specific mechanical energy

Effect of feed rate on SME is shown in Figure 6.4. Feed rate of 1800 kg/h had significantly higher mean for SME, 150.0 (W.h/kg) compared to feed rate at 2100 kg/h, 128.6 (W.h/kg).

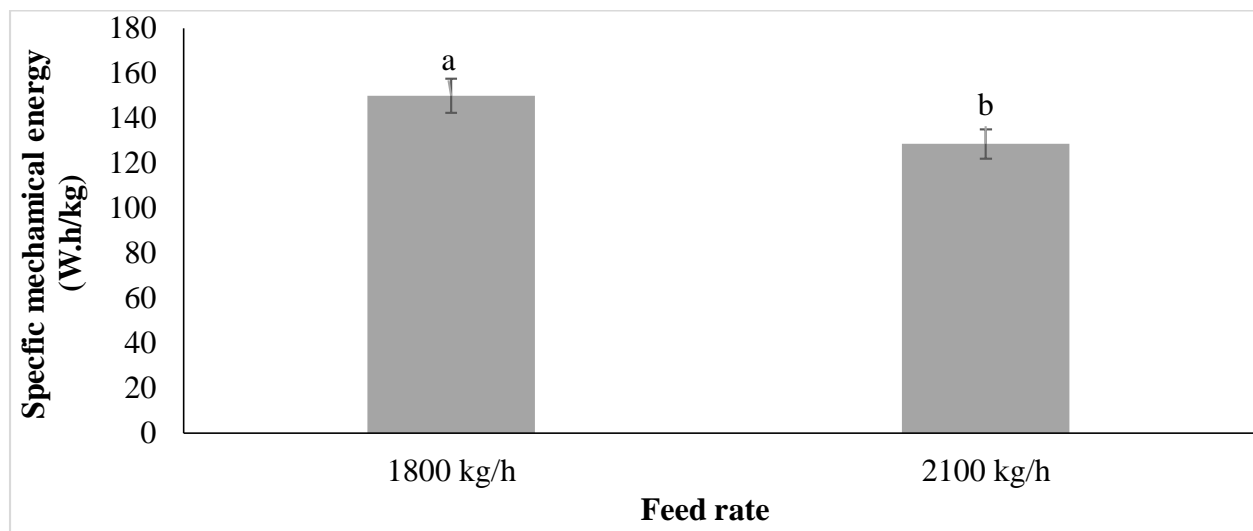


Figure 6. 4 : Effect of extruder feed rate on specific mechanical energy.

Key: Mean error bars with same letter not significantly different at $p < 0.05$.

6.4.7 Effect of extruder ingredient variables on colour of the resultant flour

Effect of extruder feed composition, feed rate and water addition rate on calorimetric properties of extrudates and the flour is shown in Table 6.3. The feed mixture ratio significantly affected

all calorimetric parameters. Rice had the least mean values for a*, b* and chroma while the highest for L* and hue angle. Amount of water added to the extruder significantly affected L* and a*. Water addition at 20 kg/h had the highest L* mean values while 15 kg/h had the least. The feed rate to the extruder significantly affected all calorimetric parameters except L*. Feed rate 1800 kg/h had significantly highest mean values for b*, chroma and hue angle and least for a*. Physical appearance of the extruded products is shown in Appendix XVI adopted from Wanjala *et al.*, (2020).

Table 6.3 : Calorimetric properties the extruded flour

Factor	Level	L*	a*	b*	Chroma	Hue angle
Feed ratio	100:00:00	69.53±1.40 ^a	1.93±0.25 ^d	16.33±0.44 ^c	16.47±0.44 ^c	83.34±0.87 ^a
	70:30:00	46.95±1.60 ^b	9.05±0.15 ^a	19.37±0.08 ^b	21.39±0.12 ^a	64.98±0.30 ^d
	60:30:10	44.51±1.01 ^b	6.88±0.14 ^c	19.21±0.29 ^b	20.42±0.29 ^b	70.26±0.42 ^c
	55:35:15	43.86±1.02 ^b	6.98±0.15 ^{bc}	20.76±0.26 ^a	21.91±0.25 ^a	71.38±0.43 ^b
	50:27:23	37.87±1.48 ^c	7.27±0.10 ^b	19.54±0.38 ^b	20.86±0.36 ^{ab}	69.54±0.45 ^c
Added water	15 kg/h	47.13±1.68 ^b	6.43±0.36 ^{ab}	19.38±0.34 ^a	20.49±0.39 ^a	71.90±0.88 ^a
	20 kg/h	50.32±2.41 ^a	6.27±0.50 ^b	18.80±0.34 ^a	19.95±0.41 ^a	72.18±1.35 ^a
	25 kg/h	48.17±2.56 ^b	6.57±0.51 ^a	18.95±0.40 ^a	20.19±0.48 ^a	71.59±1.34 ^a
Feed rate	1800 kg/h	48.46±1.63 ^a	6.29±0.34 ^b	19.55±0.22 ^a	20.64±0.26 ^a	72.43±0.90 ^a
	2100 kg/h	48.62±2.03 ^a	6.56±0.41 ^a	18.53±0.33 ^b	19.78±0.41 ^b	71.35±1.05 ^b

Means with same letter within a factor are not significantly different at p<0.05. L=Lightness scale of colour where black is 0 and white is 100; a*= (+) is red and (-) is green; b*= (+) is yellow and (-) blue.

6.4.8 Interaction of mixture composition, added water and feed rate on physical and mass flow rate properties

The interaction effect between the ingredient variables: Feed composition, rate of water addition to the barrel and feed rate on physical and mass flow properties as determined by analysis of variance (ANOVA) is shown in Appendix XVI. WSI was the only physical property of the instant flour that was not significantly affected by any interaction between ingredient variables at p<0.05. Apart from WSI, interaction between feed composition and feed rate significantly affected all other physical and mass flow properties. The three-level interaction between all ingredient variables significantly affected all physical and mass flow properties except b* and WSI. Similarly, the interaction between feed composition and rate of water addition to the barrel significantly affected all physical and mass flow properties except, OHC,

b* and WSI. However, interaction between water addition rate to the barrel and feed rate did not significantly affect WHC, WHI, L*, b*, PR, SR and WSI.

6.5 Discussion

6.5.1 Influence of mixture composition, added water and feed rate on hydration properties of extruded flour

On composition of the ingredients used, rice is about 78.87-82.94% carbohydrates, 6.87-9.51% protein, 0.48-0.85% crude fibre and 0.06-0.92% crude fat on dry weight basis (Verma & Srivastav, 2017). Sorghum is about 6.23 - 13.81% protein, 65.57 - 76.28% carbohydrates, 3.60 - 10.54% crude fat and 1.65 - 7.94% crude fibre (Jimoh & Abdullahi, 2017). Bamboo shoots from *Y. alpina* contains 26.0-31.33% protein, 12.17-18.9% crude fibre, 1.6-5.5% crude fat and 18-19% carbohydrates (Karanja *et al.*, 2015; Mulatu *et al.*, 2019). Therefore, increasing the proportion of bamboo shoots in the blend subsequently increases protein and crude fibre content while at the same time reducing the carbohydrates. Major carbohydrate in these three ingredients is starch. The difference in composition of ingredients is also manifested in physico-chemical properties as shown in Table 6.1. Bamboo shoots flour recorded highest values for hydration properties compared to rice and sorghum flour. This could be probably attributed to BSF possessing hydrophilic amino acids, water soluble components such as minerals and relatively higher apparent porosity credited to lower bulk density.

WHC, WAI and SC mean values for the extruded flour significantly increased from the control with only rice to an optimum in feed blend with 60:30:10 for rice, sorghum and bamboo shoot, respectively, as shown in Figure 6.1. Beyond the feed blend of 10% bamboo shoots, WHC, WAI and SC of extruded flour mean values significantly reduced. Feed blends with 15 and 23 % bamboo shoot had the least mean values for WHC and WAI which did not differ significantly from the control. WSI was not significantly affected probably due to hydrophobic nature. Unlike feed mixture composition, SC of the extruded flour was the only hydration property significantly affected by rate of water addition as shown in Figure 6.2. However, water addition at a rate of 20 kg/h still had higher mean values for WHC and WAI but the least for WSI. Total amount of water in the feed (inherent and added water) has a direct influence on the rate of bio-reaction that happens in the extruder. The increasing moisture content induces plasticizing effect which retards shearing and lowers starch gelatinization and degradation, thereby, increasing the hydration of rice-based extrudates (Bravo-Núñez & Gómez, 2018). Effect of extruder feed rate had a similar effect on hydration properties flour. Flour from an extruder fed at 2100 kg/h had significantly highest mean values for WHC, WAI and SC as compared to

1800 kg/h. The rate of loading the extruder affects the residence time of a product in the extruder and consequently the extent of cooking. Slower the feeding rate results in longer the residence time and higher biochemical reactions such as gelatinization, caramelization and maillard reactions.

WHC, WAI and SC can be defined as the ability of flour to absorb water and swell for thus improving consistency in food. It is a desirable property in food systems to improve yield and consistency and give body to the food. There is a direct relationship between WAI of the flour with amylose and amylopectin in the native granules of starch and weaker associative forces maintaining the granules' structure. Usually used as a measure of the strength of starch inter-granular bond where low water binding is a characteristic to fit tightly while high water binding capacity is to specify the slack association of starch polymers or low lipid content (Offia-Olua, 2014; Rao *et al.*, 2016). According to Cui (2005) the water absorption index (WAI) is the weight of gel formed per gram of dry sample. The water solubility index (WSI) is the percentage of the dry matter in the supernatant from the water absorption determination. Extruded starch shows an increase in water solubility, but a decrease in water absorption (less able to form a gel). Increasing severity of thermal treatment in the extruder will progressively increase WSI, indicating that more starch polymers have been degraded into smaller molecules. Also, according to Altan and Maskan, (2011) water absorption depends on the availability of hydrophilic groups that bind water molecules and, on the gel-forming capacity of macromolecules while water solubility gives information about degradation. These changes are related to the modification of fibre, due to extrusion, and the release of low molecular weight compounds that cause. Water absorption (WAI) and solubility (WSI) indices have been used to estimate the functional characteristics of extrudates developed from by-products. The WAI measures the amount of water absorbed by starch and can be used as an index of starch gelatinization (Ajita, 2018).

ER increased with decreasing BSF proportion in the feed stock but BD showed increasing trend with increase in proportion of BSF in the blends except in the control sample as shown in Table 6.2. According to Altan and Maskan (2011) the expansion of extrudates are complex even for products based on a single component. ER depend on the viscoelastic properties of the melt, the mechanism of bubble nucleation and growth, as well as the plasticizing properties of water in the transition from fluid (melt) to viscoelastic and subsequently to a glassy state, which are all important for the expansion. Product bulk density is directly related to the extent of extrudate expansion and is a very important parameter in the production of expanded and formed food

products. Increase in bulk density was attributed to the increasing fibre content of the feed material. This is because the presence of fibre particles tended to rupture the cell walls before the gas bubbles had expanded to their full potential. Increasing the fibre content produced extrudates with a denser structure of reduced average cell size, an increased number of holes on the cell wall, as well as an increased number of apertures on the surface of extrudates. They concluded that the overall effect was a decrease in radial expansion and an increase in bulk density. This is due to the fact that high protein content influences density since friction and shear during extrusion cause extensive interlacing between proteins and lead to their texturization. High protein content extrudates are denser and more rigid (Shruthi *et al.*, 2017).

6.5.2 Influence of mixture composition, added water and feed rate on other physical properties of extrudates and resultant flour

Expansion ratio of the extrudates was significantly affected by the feed mixture ratio, rate of water addition to the extruder and the feed rate as shown in Table 6.2. Increasing proportion of bamboo shoots in the feed mixture significantly reduced the expansion rate. This could be attributed to the decreasing content of carbohydrates. With increase in rate of water addition, expansion ratio of extrudates significantly reduced and the increased. Also, higher feed rate resulted in higher expansion ratio. Increase in rate of gelatinization has been found to reduce expansion ratio. According to Altan and Maskan (2011), increased feed moisture content during extrusion may reduce the elasticity of the dough through plasticization of the melt and have a negative impact on the starch gelatinization, causing low shear impact thereby reducing the product's expansion. Low moisture content causes high shear, which results in higher expansion. increasing the level of feed moisture content resulted increase in expansion of extrudate as sufficient water available for expansion of the extrudate. This could be explained by the fact that when materials are forced through an extruder die their water content vaporized, and simultaneous vapour flash-off expands their starch content, producing a porous, sponge-like structure in the extrudate (Shruthi *et al.*, 2017). The expansion of the extruded products is inversely related to the moisture level of the raw material. It has been established that as moisture increases, extrudates expansion occurs when the product exits the die, but the structure collapses before the necessary cooling, resulting in a dense and hard product (Sreejith, 2019). Expanded volume of extrudates decreases with increasing amounts of proteins in the feed material, but increases with increasing starch content (Ajita, 2018).

OHC of the extruded flour was significantly only affected by feed mixture ratio and feed rate as shown in Table 6.2. Similar to expansion ratio, progressive increase in proportion of bamboo

shoots in the mixture caused significant decrease in OHC. Increase in feed rate resulted in decrease in OHC of the extruded flour. OHC could be attributed to the lipophilic nature of the constituents of the flour. Flours which have high OAC can retain the flavour, improve the palatability, and extend the shelf life of bakery products (Seena & Sridhar, 2005). According to Chandra and Samsher (2013), OHC depend upon the intrinsic factors like amino acid composition, protein conformation and surface polarity or hydrophobicity. The ability of the proteins of these flours to bind with oil makes it useful in food system where optimum oil absorption is desired. This makes flour to have potential functional uses in foods such as sausage, whipped toppings, chiffon dessert, angel and sponge cakes. WAI increased at higher moisture contents and this could be explained that a moderate extrusion treatment disrupts structures and therefore creates pores that water can penetrate (Altan & Maskan, 2011).

BD of the extruded flour was also significantly affected by feed mixture ratio and feed rate as shown in Table 6.2. Bulk density (BD) significantly reduced with increase in proportion of bamboo shoots in the mixture up to 10% level, after which it then increases. On the other hand, slower feed rate of the extruder resulted in higher bulk density of the extruded flour. Increase in BD has been postulated to the alteration of the structure of amylopectin which reduces the melt viscosity and elasticity of dough through plasticizing effect. Bulk density indicates the porosity of a product which influences package design and type of packaging material. The high dependence of bulk density on feed moisture would reflect its influence on elasticity characteristics of the starch-based material. Increased feed moisture content during extrusion may reduce the elasticity of the dough through plasticization of the melt, therefore increasing the density of extrudate (Shruthi *et al.*, 2017). Increased feed moisture promotes a sharp increase in extrudate density (Ajita, 2018).

6.5.3 Influence of mixture composition, added water and feed rate on mass transfer properties

Rate of product throughput and flash steam evaporated at the exit of the extruder was significantly affected by the feed mixture ratio, rate of water addition and feed rate as shown in Table 6.2. Increase in proportion of bamboo shoots in the mixture up to 15% level caused an increase in product throughput, after which it started to decrease. On contrary, rate of steam evaporated decreased with increase in bamboo shoot proportion in the feed mixture up to 15% and then it increased.

Also, increase in rate of water addition caused an increase in product throughput but decreased afterwards. Conversely, increase in rate of water addition resulted in increase in rate of steam

evaporation at the die. A higher feed rate caused higher product throughput and rate of steam evaporation. Lower feed rate had a significantly higher SME and vice versa as shown in Figure 6.4. This could be credited to the lower product throughput that required more time and definitely energy, to mix ingredients in the metering section of the extruder. According to Muthukumarappan and Karunanithy (2012), SME is the indication of the viscous dissipation of mechanical energy provided by the screw drive shaft, into the dough due to frictional resistance. The increased moisture in feed increases the mass flow rate as it increases moisture available for melting hence reducing viscosity (Shruthi *et al.*, 2017).

6.5.4 Influence of mixture composition, added water and feed rate on colorimetric properties of extruded flour

Feed mixture ratio significantly affected all aspects of extruded flour colour as shown in Table 6.3. Lightness of the product reduced with reduction of rice proportion in the mixture. Rate of water addition only affected L* and b* aspects of colour. Feed rate affected all aspects of colour of the extruded flour except L*. Increase in feed rate had caused lower mean values for b*, chroma and hue angle. Many reactions occur during extrusion that affects the colour of the extrudate. The most common reactions are non-enzymatic browning such as Maillard reaction and caramelization and pigment degradation (Martínez-Bustos *et al.*, 2011). According to Suzanne (2010) colour is three dimensional, and any colour-order system will need to address hue, what we instinctively think of as colour (e.g., red, blue, green), value, which represents lightness and darkness, and chroma or saturation which indicates intensity. Chroma (C*) is considered the quantitative attribute of colourfulness, is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness. The higher the chroma values, the higher the colour intensity of samples perceived by humans. Hue angle (h*), considered the qualitative attribute of colour, is the attribute according to which colours have been traditionally defined as reddish, greenish, etc., and it is used to define the difference of a certain colour with reference to grey colour with the same lightness. An angle of 0° or 360° represents red hue, whilst angles of 90°, 180° and 270° represent yellow, green and blue hues, respectively (Pathare *et al.*, 2013).

Colour intensity depends on both the physico-chemical characteristics of the raw materials and the operating conditions during processing. Colour of the extruded foods together with the formed Maillard reaction products such as furosines are responsible for the organoleptic properties of this type of products. However, darkening step which affects L* starts when the product temperature reaches a critical value and also at advanced level of non-enzymatic

browning, compounds with fluorescence properties are also produced affect negatively on organoleptic properties (Soria & Villamiel, 2012).

6.6 Conclusion and Recommendation

Based on the findings of this study, we reject the null hypothesis that extrusion cooking has no significant effect on physical properties. Extruder ingredient variables have a significant effect on the resultant products as well as mass transfer within the extruder during cooking. More energy per kg is required when extruding blends at lower feed rate of 1800 kg/h than at 2100 kg/h. Extrusion cooking of blends made from BSF, sorghum and rice could possess a wide range of applications in the food processing. A blend that had 60:30:10 for rice, sorghum and BSF respectively, is recommended for the applications because it had optimal hydration properties which indicated that it has the potential to be used in baking, soup thickening and sausage making.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Rationale for the Study

Exploitation of locally available under-utilized food crops in many regions to enrich staple foods has been demonstrated as one of the approaches to attaining food and nutrition security (Chandra *et al.*, 2020; Singh *et al.*, 2018;). Therefore, this strategy has been highly recommended to emerging economy countries that are struggling with burden of food insecurity and malnutrition. Besides enriching the staple foods, this approach also assures sustainable food availability and affordability (Graham *et al.*, 2001). For instance, *Yushania alpina* is a native species of bamboo that covers thousands of hectares of land of the East African highlands and contributes significantly towards afforestation. Increase in consumption of this bamboo species, identified as non-timber forest product (NTFP), could go a long way in prompting sustainable conservation measures that could result increased afforestation as well as food security (Karki & Chowdhary, 2019). Sorghum on other the hand is a crop that has been fingered to assuage food insecurity and malnutrition in developing world due to its ability to tolerate drought conditions of arid and semi-arid land (ASAL) (Shumetie & Alemayehu, 2019). Cultivation of bamboo shoots should be explored and encouraged, as the bamboo shoots could serve as a food source as well as a source of income for their families (Kong *et al.*, 2020).

The urge to understand the composition of different materials that could be used as food or ingredients for food formulations and their behaviour when subjected to different processing conditions has given rise to a discipline known as food material science (Fellows, 2009). There is a growing adoption rate of this discipline among scientists dedicated in developing novel food products that meet different and dynamic consumer demands. Incorporation of food processing technologies with aspects of food enrichment has demonstrated the potential of not only nutritional improvement and but value addition of food and food products (Augustin *et al.*, 2016). Among many known food processing technologies, extrusion has proven to be very versatile in terms of ability to produce a wide range of products (Qamar, 2018). According to Arora *et al.* (2020), to wholly exploit opportunities offered by extrusion for functionalization of food materials it is important to understand the conformational and biochemical changes induced in the feedstock. Where the term ‘functionalization’ is used in this context to mean the process of physico-chemical changes or conformational modification of food constituents so as to achieve desirable attributes in resultant food matrices. In general, functionality can be

induced or altered in food through conformational changes such as denaturation of protein, or degradation of starch, as well as dextrinization and/or formation of peptides

This study therefore investigated the possibility of enriching rice, which is an important staple food globally, with sorghum and *Yushania alpina* to improve on protein and dietary fibre. Consequently, effect of extrusion processing on properties of products from different formulations of enriched rice was also investigated.

The Thesis addresses four main research hypotheses which include: (i) Ingredients optimization has no effect on the physico-chemical, nutritional and anti-nutritional properties of the composite flour blend containing rice, sorghum and bamboo shoots, (ii) Optimization of the extrusion cooking processing conditions has no effect on the, nutritional and anti-nutritional properties of the extrudates from composite flour blend containing rice, sorghum and bamboo shoots, (iii) Optimization of the extrusion cooking processing conditions has no effect on the microbial load, shelf life and sensory acceptability of extrudates from composite flour blend containing rice, sorghum and bamboo shoots, (iv) Optimization of the extrusion cooking processing conditions has no effect on the physico-chemical properties of extrudates from composite flour blend containing rice, sorghum and bamboo shoots. Findings that test these hypotheses have been presented in Chapters 3, 4, 5 and 6 respectively. In this Chapter therefore the methodological approaches used to investigate these hypotheses, discussion of the main findings in Chapters 3, 4, 5 and 6, and their implication on nutritional, physical and shelf-life properties are highlighted.

7.2 Methodological Approach

A two-step approach was used in this study. First step was to optimize protein and dietary fibre using study ingredients in formulating composite blends and investigate how this optimization was affecting physical and nutritional properties. Second step was specifically to evaluate the effect of extrusion processing on nutritional and physical properties of resultant products from the composite blends. A statistical method that empirically optimize mixtures was employed to guide the process of enriching rice flour with sorghum and *Yushania alpina* in order to obtain a composite blend that had optimum protein and dietary fibre as demonstrated in Chapter 3. Optimization process was based on the nutritional evaluation of the individual ingredients as determined beforehand. Besides the optimized blend, there were other 11 composites with different varying proportions of the ingredients.

Due to practical and technical difficulties encountered during pre-trials to establish conditions for extruding the formulated blends, only a few blends were purposively selected to undergo processing. The composite blend with optimum protein and dietary fibre and other four selected blends from the 11 composites obtained during the optimization were subjected to different extrusion cooking conditions. The major technical difficulty was the nature of the extruder used in this study that only allowed varying ingredient variables and not extruder variables. This is because extruder operational parameters were pre-set to fixed level to meet conditions suitable for manufacturing commercial products for the organization. Therefore, ingredient variables: Feed composition, rate of water addition to the feed and feed rate formed the basis for the study of extrusion process on blends of rice, sorghum and *Yushania alpina* as demonstrated in Chapters 4, 5 and 6. Limits for feed rate and the rate of water to be added into the barrel were established during pre-trial experiment. After extrusion, extrudates were milled to obtain instant flour. Products obtained were analyzed for nutritional properties and physical-chemical properties.

Assumption of equal variances also known as assumption of homoscedasticity assumes that different samples have the same variance despite the different treatments used. This assumption is very important in many statistical tests such as Analysis of Variance (ANOVA). Running ANOVA without checking for equal variances can have a significant impact on results and may even invalidate them completely. Also, results are affected by the type of homoscedasticity test used and how sensitive that test is to unequal variances. Levene's test is one of the very robust method that recommended to check that variances are equal for all samples (Brown & Forsythe, 1974; Erceg-Hurn & Mirosevich, 2008). Scheffé's method was used carry out means separation for main factors and their interactions. Scheffé's method is a *post-hoc* single-step multiple comparison procedure which performs simultaneous and joint pairwise comparisons for all possible pairwise combinations of each group mean. Scheffé's method is preferred when theoretical background for differences between groups is unavailable or previous studies have not been completely implemented (exploratory data analysis) (Lee & Lee, 2018).

7.3 Design and Implementation of the Study

Mixture Design Analysis (MDA) was employed to carry out empirical optimization of protein and dietary fibre in composite blend of the three ingredients as demonstrated in Chapter 3. The base ingredient was rice which had to be at least 50% and above of the blends proportion. Main reason why MDA was chosen for this study was to reduce cost and time required to come up with formulas (Jeirani *et al.*, 2012). Output from this empirical method was presented in form

of triangular shaped figures where a blend with optimum protein and dietary fibre lied within a segment called sweet spot within the figure, which is the white region as shown in Figure 3.2a.

For Chapters 4, 5 and 6 employed a completely randomized design in a factorial experimental arrangement of the three ingredient variables to investigate effect of individual ingredient variable and effect due to their interactions on response variables. Analysis of variance method was used to test the hypotheses that the main factors had no effect and also that there was no interaction among the factors. Chapter 4 main focus was to quantify changes/alterations of nutritional components of the selected composite blends due to the ingredient variables used in the extrusion cooking. Chapter 5 was centred around extent of microbial contamination of the extruded products due to post-extrusion within ingredient variables and how it impacts on shelf life. Chapter 6 looks at quantifying changes of physical components of the selected composite blends due to the ingredient variables used in the extrusion. Principles of thermodynamics and material balance were applied in Chapters 4, 5 and 6 to elucidate the technological aspects of the designed/developed products due to the ingredient variables used in the extrusion processing. Chapter 4 assumed steady state thermodynamics while Chapter 6 assumed transient/unsteady state thermodynamics with clear assumptions for each given in their respective Chapters.

7.4 The Results

In Chapter 3, increase of sorghum and *Yushania alpina* proportions in the blends caused a significant decrease in total carbohydrate content while significantly increasing all other nutritional components in composite blends. Therefore, the intended goal of enriching rice flour was found to be practically feasible when sorghum and *Yushania alpina* are used. Similarly, total phenolic content and condensed tannins in blends also significantly increased with the nutritional enrichment of rice flour. Though fresh *Yushania alpina* recorded very high levels of hydrogen cyanide (HCN), drying and milling unit operations carried out to obtain flour significantly reduced the HCN by about 95%.

In Chapter 4, extrusion cooking of the raw composite blends to products significantly resulted in: (i) Increase of dry matter, protein digestibility, total carbohydrates and total phenolic content, and (ii) Decrease of protein content and condensed tannins. These alterations were brought about by the biochemical reactions between and within the nutritional components such as Maillard and caramelization among others. In general, extrusion processing improved nutritional state of the blends because of increased protein digestibility and reduced contents

of condensed tannins that are anti-nutritional components. All the ingredient variables under the study significantly affected the above alterations of nutritional components at varying degrees. These ingredient variables influenced the level of mass moisture in the barrel, degree of mixing and shear impact and specific mechanical energy and consequently the degree of bio-reactions.

Chapter 5 demonstrates that handling practices after extrusion and the environment where extrusion was done contributed to the contamination of the products. The consequence of these high microbial was not only on compromising the safety, but also storage time of the extruded products. The assumption when using Arrhenius modelling to predict the shelf life of products was that growth rate of yeasts and moulds at different storage temperatures occurs as same as in chemical reactions. It found that yeasts and moulds cause d spoilage of the instant flour product by probably producing extra cellular enzymes that helped in breaking down of nutritional components for their nourishment.

In Chapter 6, it was demonstrated that all the ingredient variables significantly affected physico-chemical properties of the instant flour except water solubility index property and mass flow rate. Physico-chemical properties of the instant flour are play an important role in determining the possible applications of utilization on domestic and industrial levels. Variability in physico-chemical properties could also be linked to biochemical reactions happening during extrusion as elucidated in Chapter 4. These reactions include Maillard reaction, caramelization, formation and breakdown of disulfide bonds, formation of iso-peptides, other polymerization, degradation, and crosslinking reactions (Arora *et al.*, 2020).

7.5 Conclusions

This study provides vital information needed in developing of novel products, especially nutritious convenient foods from bamboo shoots, sorghum and rice through extrusion processing. Formulation in itself enriches rice with nutrients from sorghum and bamboo shoots while extrusion improved the nutritional status of the blends. The different instant flour obtained from the different composite blends could have a wide range of domestic and industrial applications. The findings further demonstrated that if hygienic conditions were observed during post-extrusion handling, the products have the potential of a longer shelf life.

7.6 Recommendations

The instant flours developed in this study are recommended for use in preparation of instant porridge formulas, soup thickening agents and snack bars. However, quality assurance

programmes such as Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Programmes (GMP) should be observed during processing of these instant flours.

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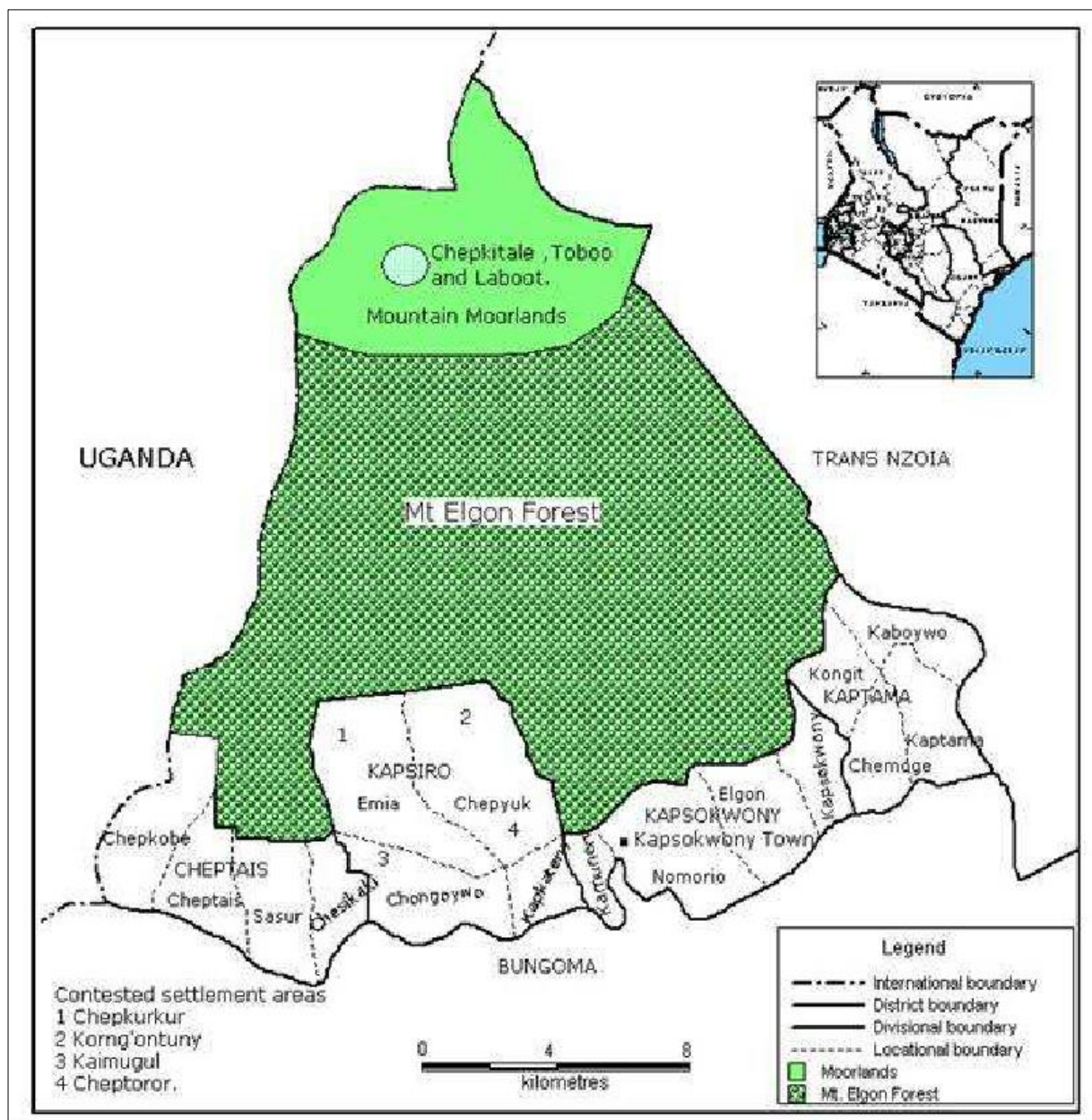
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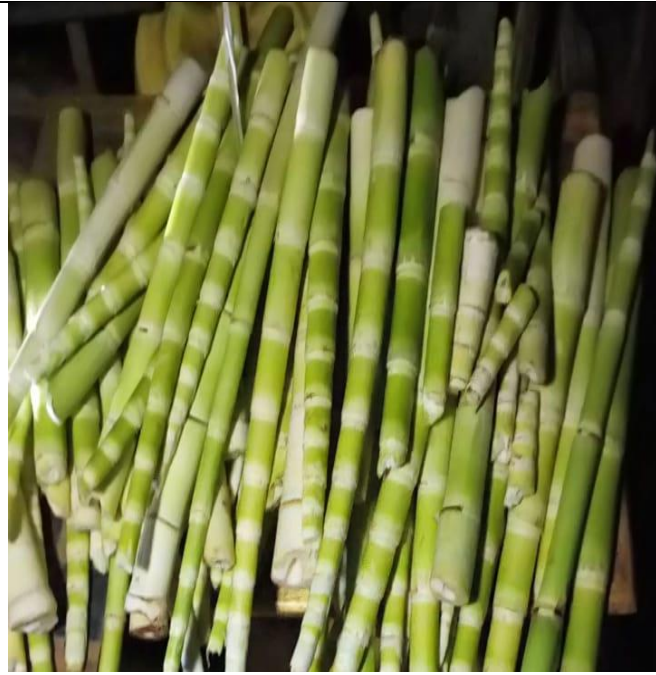
APPENDICES

APPENDIX A: Map of Mt. Elgon



Source: Kiptum *et al.* (2011)

APPENDIX B: Photos of bamboo shoots



A. Freshly harvested shoots of *Yushania alpina*



B. Drying of the *Yushania alpina* shoots over a fire place in a hut

APPENDIX C: Mixture design outputs:

a) Total minerals

Analysis of Variance for ASH (component proportions)						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	56.5085	56.5085	11.30169	115.53	0.000
Linear	2	55.7870	3.2040	1.60201	16.38	0.000
Quadratic	3	0.7215	0.7215	0.24050	2.46	0.084
Rice*Sorghum	1	0.0270	0.2165	0.21652	2.21	0.148
Rice*Bamboo	1	0.3580	0.1042	0.10416	1.06	0.311
Sorghum*Bamboo	1	0.3365	0.3365	0.33651	3.44	0.075
Residual Error	27	2.6413	2.6413	0.09783		
Lack-of-Fit	5	1.2322	1.2322	0.24644	3.85	0.012
Pure Error	22	1.4091	1.4091	0.06405		
Total	32	59.1498				

S = 0.312771 PRESS = 3.28707
R-Sq = 95.53% R-Sq(pred) = 94.44% R-Sq(adj) = 94.71%

b) Crude protein

Analysis of Variance for CRUDE PROTEIN (component proportions)						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	116.032	116.0323	23.2065	47.58	0.000
Linear	2	114.502	7.0418	3.5209	7.22	0.003
Quadratic	3	1.530	1.5303	0.5101	1.05	0.388
Rice*Sorghum	1	0.113	1.1676	1.1676	2.39	0.133
Rice*Bamboo	1	1.052	0.4990	0.4990	1.02	0.321
Sorghum*Bamboo	1	0.365	0.3650	0.3650	0.75	0.395
Residual Error	27	13.169	13.1694	0.4878		
Lack-of-Fit	5	8.329	8.3285	1.6657	7.57	0.000
Pure Error	22	4.841	4.8409	0.2200		
Total						

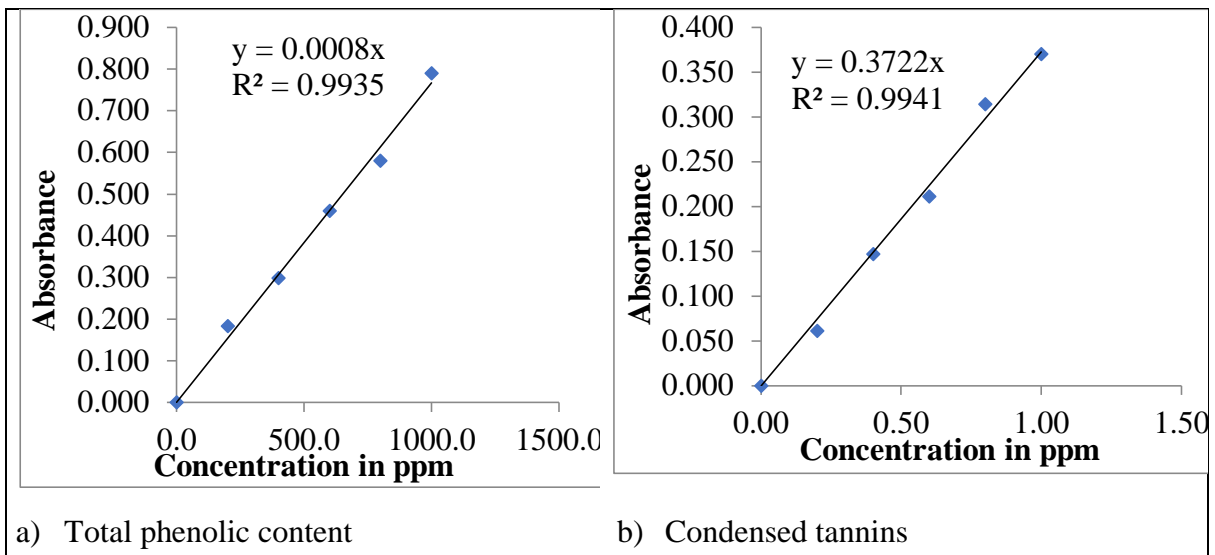
S = 0.698395 PRESS = 17.3245
R-Sq = 89.81% R-Sq(pred) = 86.59% R-Sq(adj) = 87.92%

c) Crude fibre

Analysis of Variance for CRUDE FIBRE (component proportions)						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	59.1187	59.11868	11.82374	50.17	0.000
Linear	2	56.5269	5.52976	2.76488	11.73	0.000
Quadratic	3	2.5918	2.59180	0.86393	3.67	0.025
Rice*Sorghum	1	0.1409	0.00089	0.00089	0.00	0.951
Rice*Bamboo	1	1.3883	2.21486	2.21486	9.40	0.005
Sorghum*Bamboo	1	1.0626	1.06258	1.06258	4.51	0.043
Residual Error	27	6.3629	6.36288	0.23566		
Lack-of-Fit	5	2.0009	2.00087	0.40017	2.02	0.116
Pure Error	22	4.3620	4.36201	0.19827		
Total	32	65.4816				

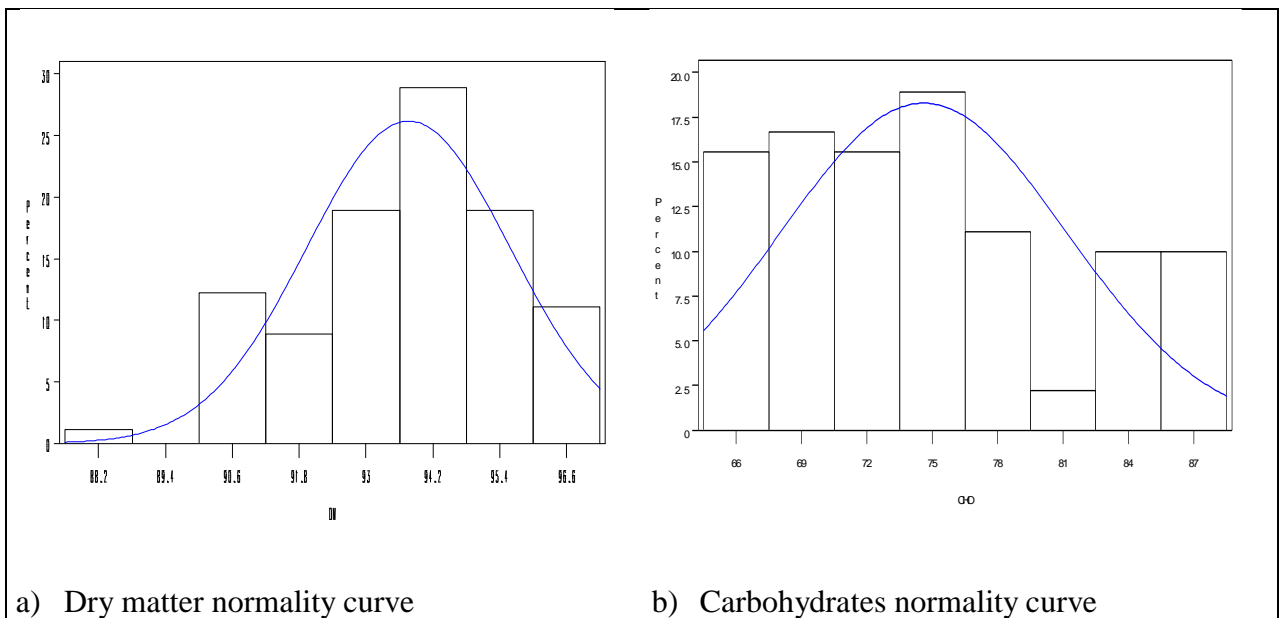
S = 0.485451 PRESS = 8.54384
R-Sq = 90.28% R-Sq(pred) = 86.95% R-Sq(adj) = 88.48%

APPENDIX D: Standard calibration curves

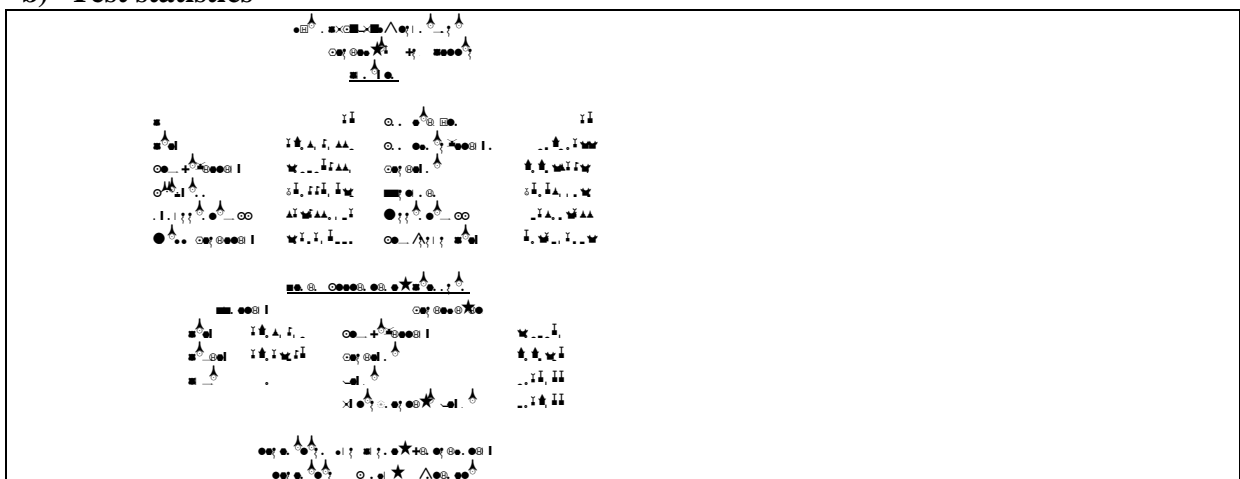


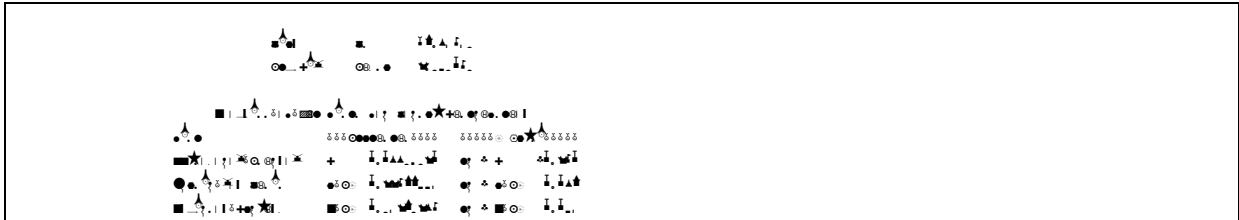
APPENDIX E: Examples of normality tests nutritional components

a) Normality curves

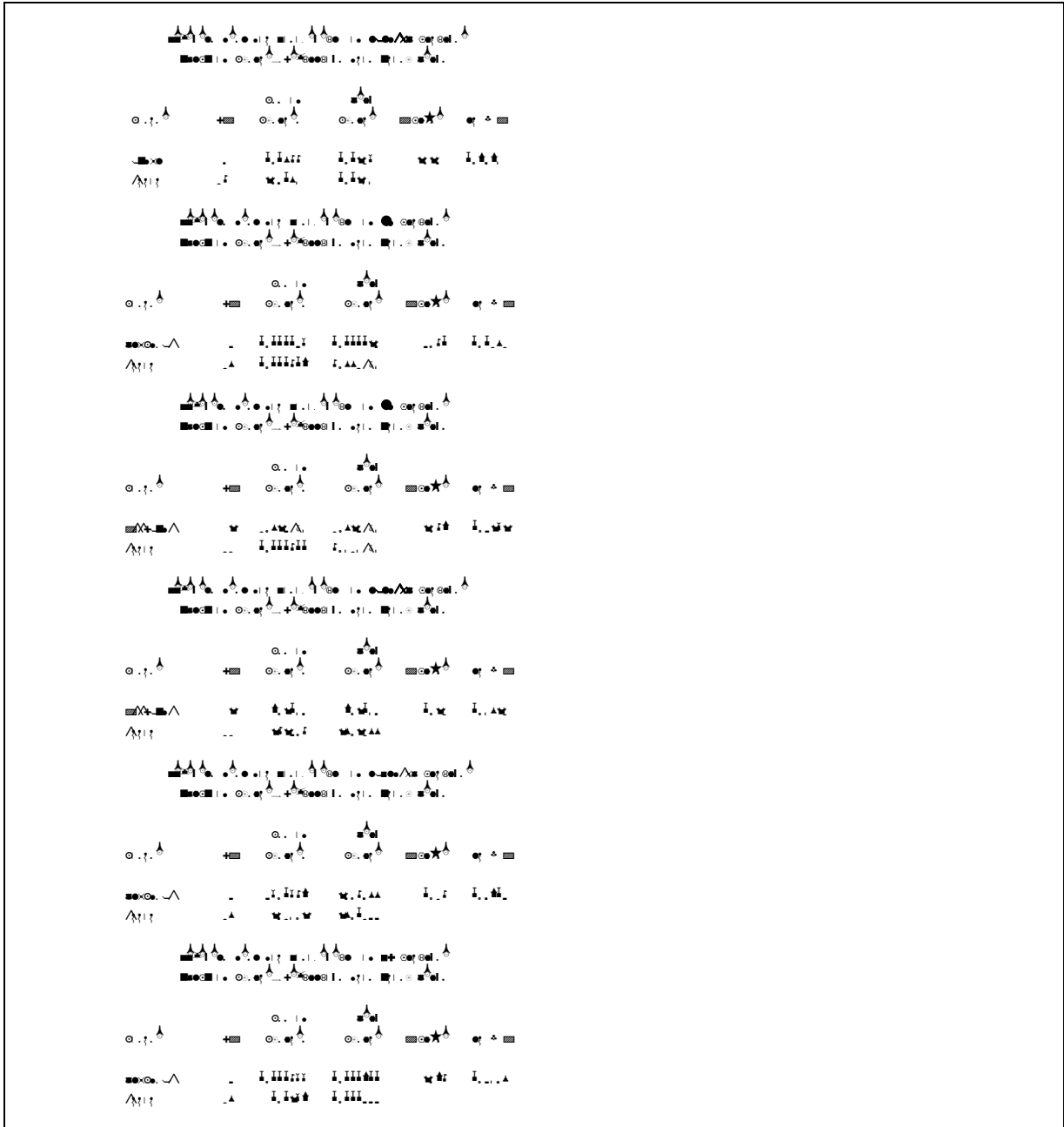


b) Test statistics





APPENDIX F: Example of Levene's test for homogeneity of data



APPENDIX H: Mean squares of ANOVA table for the effect of feed composition, moisture and rate on nutritional components

S.O.V	DF	DM	FAT	CP	CF	CHO	PD	TP	CT
A	4	10.046*	11.148*	140.810*	128.423*	895.728*	54.222*	1966.721*	0.0410*
B	2	13.486*	0.016*	0.034 ^{ns}	0.069*	6.045*	104.875*	5.263*	0.0088*
C	1	4.949*	0.001 ^{ns}	0.079 ^{ns}	0.001 ^{ns}	16.202*	43.085*	4.092*	0.0043*
Rep	2	0.145	0.001	0.007	0.004	0.468	0.157	0.016	0.0001
A*B	8	13.421*	0.029*	0.210*	0.082*	11.468*	0.609*	0.402*	0.0005*
A*C	4	6.373*	0.042*	0.152*	0.051*	7.900*	0.624*	0.323*	0.0003*
B*C	2	0.662*	0.009*	0.192*	0.016*	0.418 ^{ns}	0.659*	0.025 ^{ns}	0.0001*
A*B*C	8	7.308*	0.021*	0.209*	0.074*	5.224*	0.421*	0.091*	0.0003*
Error	58	0.558	0.001	0.024	0.002	0.580	0.110	0.032	0.0002
R²		0.891	0.976	0.997	0.997	0.991	0.987	0.999	0.999
C.V		7.968	7.075	1.683	1.841	1.202	3.681	0.997	1.956

Key: A= Feed composition; B= Rate of water addition to the extruder; C= Feed rate; S.O.V= Source of Variations; DF= Degree of Freedom; DM= Dry Matter; CP= Crude Protein; CF= Crude Fibre; CHO= Carbohydrates; PD= Protein Digestibility; TP= Total Phenolics; CT= Condensed Tannins; C.V= Coefficient of Variations; R²= Coefficient of Determination; *= Significant at p<0.05; ns= Not significant at p<0.05.

APPENDIX I: Mean squares of ANOVA table for the effect of feed composition, moisture and rate on extent alterations to proximate components during extrusion cooking

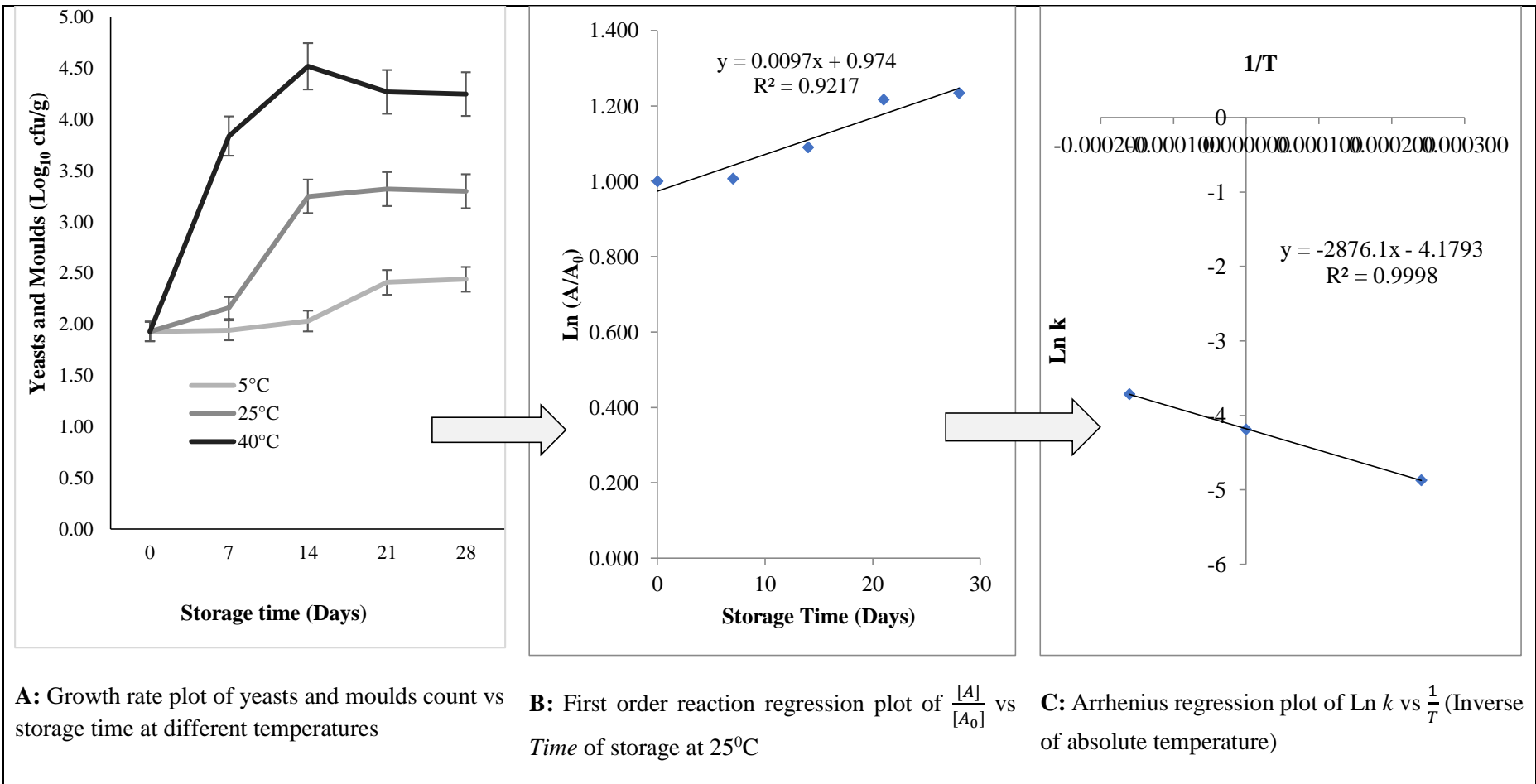
S.O.V	DF	Protein	Fat	Fibre	Carbohydrates
A	4	4.143 ^{***}	0.212 ^{***}	24.730 ^{***}	41.476 ^{***}
B	2	0.035 ^{ns}	0.015 ^{***}	0.070 ^{***}	6.040 ^{***}
C	1	0.079 ^{ns}	0.001 ^{ns}	0.001 ^{ns}	16.239 ^{***}
Rep	2	0.006	0.001	0.002	0.466
A*B	8	0.210 ^{***}	0.029 ^{***}	0.082 ^{***}	11.466 ^{***}
A*C	4	0.154 ^{***}	0.042 ^{***}	0.051 ^{***}	7.899 ^{***}
B*C	2	0.192 ^{***}	0.009 ^{***}	0.016 ^{**}	0.418 ^{ns}
A*B*C	8	0.209 ^{***}	0.021 ^{***}	0.074 ^{***}	5.231 ^{***}
Error	58	0.025	0.001	0.002	0.580
R²		0.937	0.989	0.999	0.915
C.V		3.082	1.878	3.187	4.553

Key: A= Feed composition; B= Rate of water addition to the extruder; C= Feed rate; S.O.V= Source of Variations; DF= Degree of Freedom; ; C.V= Coefficient of Variations; R²= Coefficient of Determination; *= Significant at p<0.05; ns= Not significant at p<0.05.

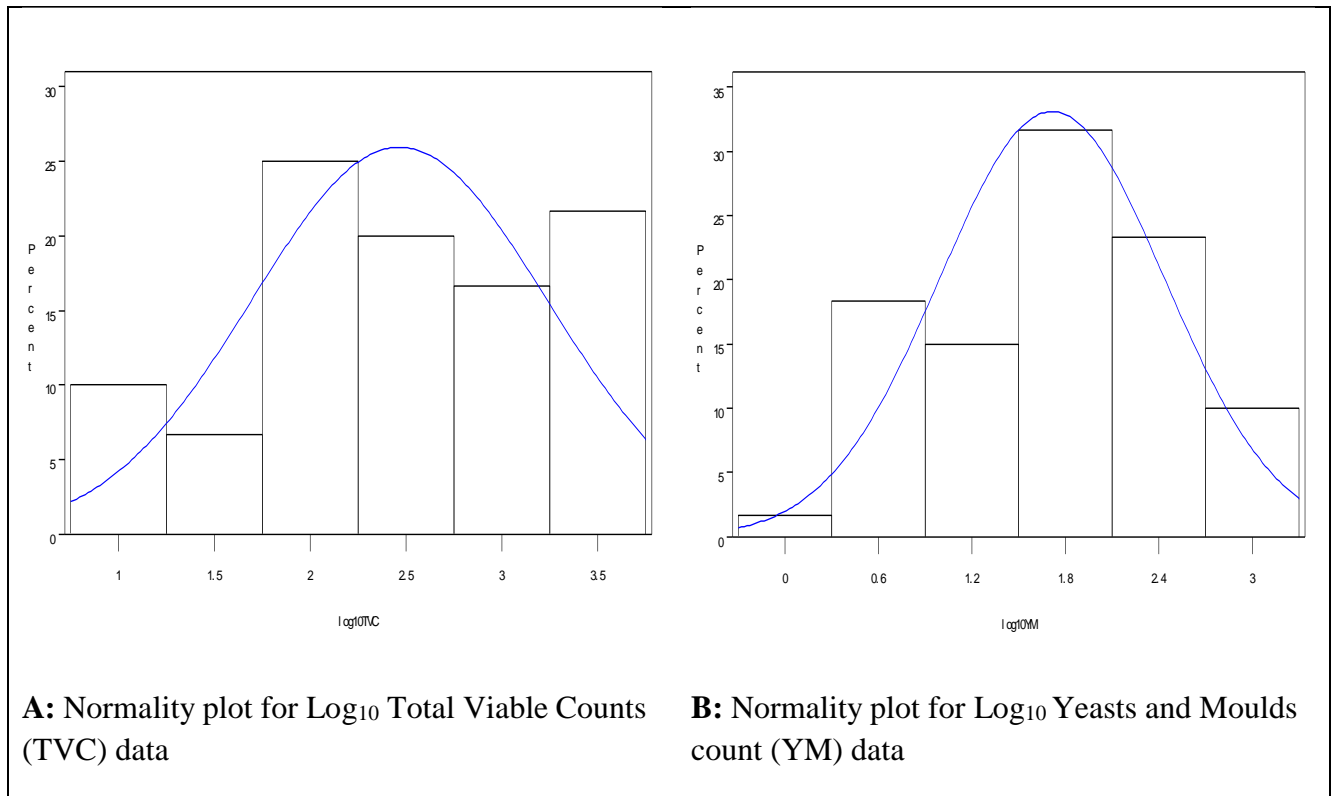
APPENDIX J: Phenotypic characteristics of isolated fungi

Fungi	Genus	Colony morphology	Microscopic characteristics
Moulds	<i>Cladosporium</i> spp.	<ul style="list-style-type: none"> • light green to greyish velvety surface; • Grey to black back surface 	<ul style="list-style-type: none"> • Blastoconidia: Conidiophores are branched and look like tree • Conidia are unicellular and ellipsoidal
	<i>Alternaria</i> spp.	<ul style="list-style-type: none"> • Dark greenish-black surface with grey periphery • Black on reverse side 	<ul style="list-style-type: none"> • Large brown club-shaped macroconidia • Macroconidia appear in chains • Septa with distinct conical narrowing or at the apical end
	<i>Paecilomyces</i> spp.	<ul style="list-style-type: none"> • Both surface and reverse are white with some yellowish-brown patches especially at the centre 	<ul style="list-style-type: none"> • Elliptical/ cylindrical microconidia
	<i>Syncephalastrum</i> spp.	<ul style="list-style-type: none"> • Colonies are very large • White with to greyish centre • Reverse pale or yellowish brown. 	<ul style="list-style-type: none"> • Sporangiohores bear rod-shaped (cylindrical) sporangioles, each containing a row of spherical spores.
	<i>Cunninghamella</i> spp.	<ul style="list-style-type: none"> • Very large colonies • Cotton like white surface • Yellowish reverse side 	<ul style="list-style-type: none"> • Sporangioles are borne on spicules (spikes) from vesicles • Vesicles are borne terminally or irregularly on the sporangiophores.
	<i>Aspergillus</i> spp.	<ul style="list-style-type: none"> • Bluish-green with sulfur-yellow areas on the surface 	<ul style="list-style-type: none"> • Conidiophore is not septate • Phialide and metula are formed on the apex and radially arranged • Conidia are formed on phialide
	<i>Trichothecium</i> spp.	<ul style="list-style-type: none"> • White to pink surface • Reverse side same as the surface 	<ul style="list-style-type: none"> • Single transverse septum, • V- shaped chains • Two-celled conidia.
Yeasts	<i>Candida</i> spp.	<ul style="list-style-type: none"> • White, rough surface and serrated edge colonies with a crater 	<ul style="list-style-type: none"> • Cells are oval in shape with multipolar budding
	<i>Saccharomyces</i> spp.	<ul style="list-style-type: none"> • Colonies are cream white with a crater and smooth surface and edge. 	<ul style="list-style-type: none"> • Cells are round cylindrical with multipolar budding
	<i>Zygosacchomyces</i> spp.	<ul style="list-style-type: none"> • Colonies are cream white 	<ul style="list-style-type: none"> • Cells bud with ascospores
	<i>Rhodotorula</i> spp.	<ul style="list-style-type: none"> • Colonies have a crater and orange in colour with a smooth surface and edge. 	<ul style="list-style-type: none"> • Cells are oval with polar budding

APPENDIX K: Shelf-life analysis procedure in graphical form



APPENDIX L: Example of normality plots for microbial load data



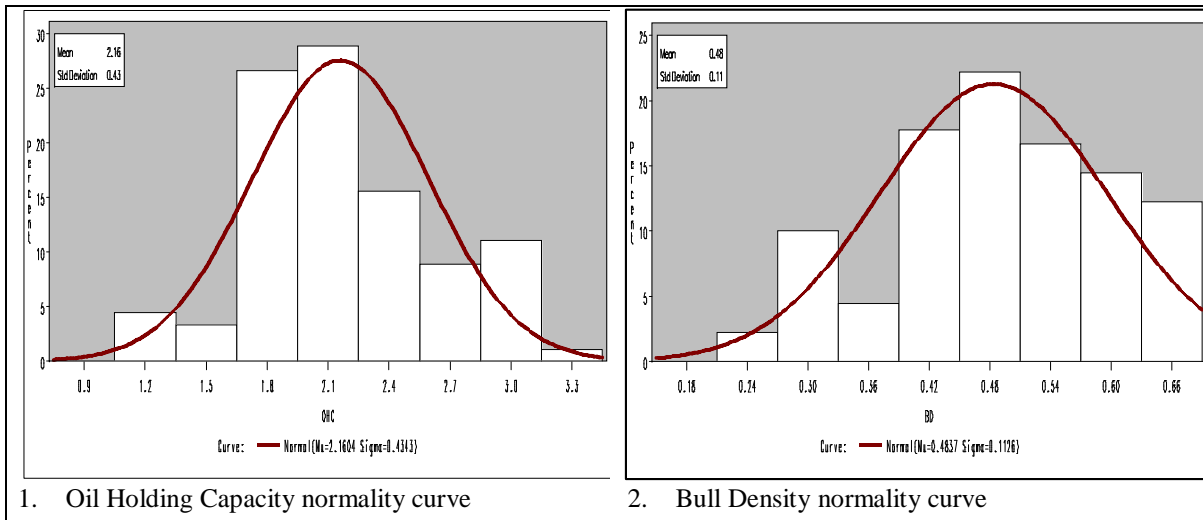
APPENDIX M: ANOVA mean squares table for microbial data

S.O.V	DF	Log ₁₀ TVC	Log ₁₀ TCC	Log ₁₀ YM
A	4	2.267***	4.040***	3.436***
B	2	5.668***	4.092***	0.275***
C	1	0.049 ^{ns}	7.059***	0.389***
Rep	1	0.027	0.101	0.006
A*B	8	0.855***	0.603***	0.702***
A*C	4	0.511***	0.913***	0.249***
B*C	2	0.040 ^{ns}	1.439***	0.009 ^{ns}
A*B*C	8	0.627***	0.717***	1.045***
Error	29	0.013	0.094	0.040
R ²		0.989	0.947	0.963
C.V		4.623	23.398	11.633

Key: S.O.V= Source of Variations: A= Feed Composition; B= Rate of Added Water to the Extruder; C= Feed Rate; R²= Coefficient of Determination; C.V= Coefficient of Variation; DF= Degree of Freedom; TVC= Total Viable count; TCC= Total Coliform Count; YM= Yeasts and Moulds; ns= Not Significant at p≤0.05; ***= Significant at p<0.05.

APPENDIX N: Example of normality test outputs for the physical properties data

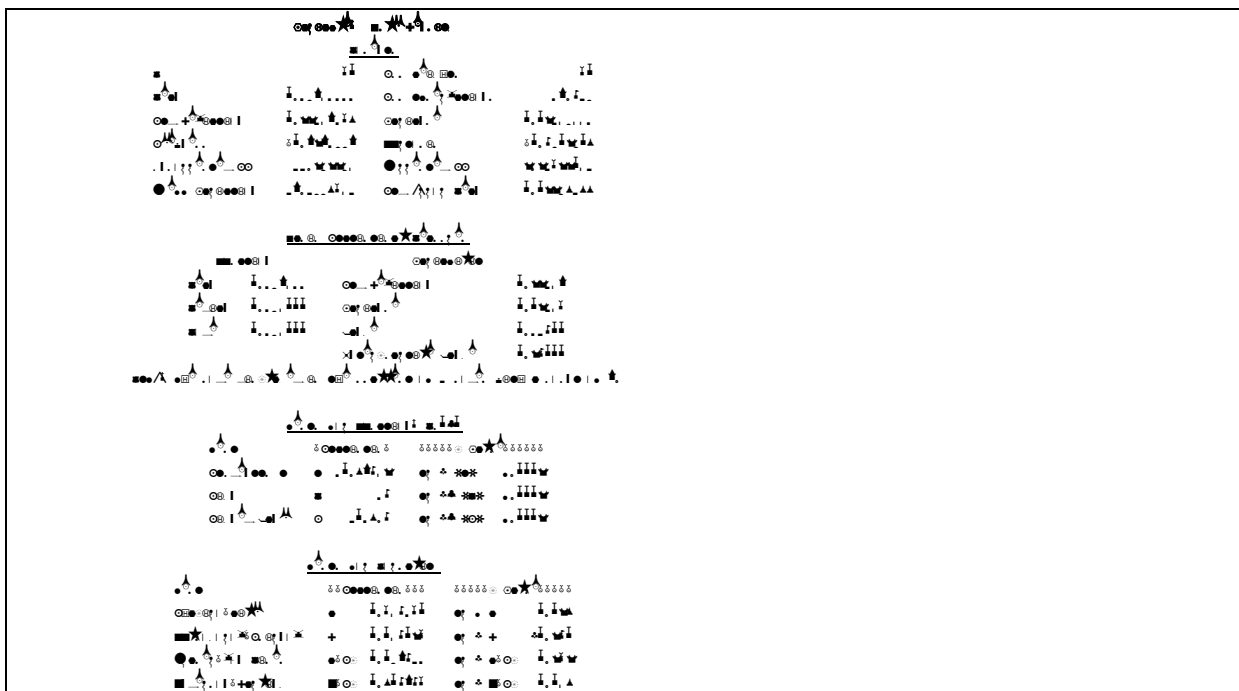
A. Example of the normality plots of physical properties



1. Oil Holding Capacity normality curve

2. Bull Density normality curve

B. Example of test statistics for normality



APPENDIX O: Mean square values of Analysis of Variance (ANOVA) for physical properties











S.O.V	DF	Hydration properties				ER	OHC	Colorimetric properties			BD	Mass Flow Properties	
		WHC	WAI	WSI	SC			L*	a*	b*		PR	SR
Ratio (A)	4	0.1184***	22.6725***	0.2730 ^{ns}	5.2383***	19.1948***	0.9233***	2678.2007***	127.3738***	48.1202***	0.0585***	5.9436***	5.9436***
Water (B)	2	0.0063 ^{ns}	0.5429 ^{ns}	0.0394 ^{ns}	1.0041**	2.1426***	0.0237 ^{ns}	79.4754***	0.7071**	2.7868 ^{ns}	0.0008 ^{ns}	1.4657***	800.6657***
Feed rate (C)	1	0.0292***	6.0218***	0.0191 ^{ns}	6.084***	1.3250***	0.7544***	0.5601 ^{ns}	1.5471***	23.3071***	0.0583***	469.1338***	4.2337***
Replication	2	0.0002	0.2111	0.0749	0.0334	0.1181	0.0259	18.8591	0.0454	0.8574	0.0001	0.0228	0.0228
A*B	8	0.0219***	4.7314***	0.7193 ^{ns}	1.3088***	0.6789***	0.0743 ^{ns}	134.0916***	2.2784***	1.8623 ^{ns}	0.0531***	1.4643***	1.4643***
A*C	4	0.0063*	0.9681**	0.0181 ^{ns}	6.9946***	0.3806***	1.4323***	110.9459***	1.6771***	8.4024***	0.0293***	0.8579***	0.8579***
B*C	2	0.0043 ^{ns}	0.0351 ^{ns}	0.0489 ^{ns}	0.5790*	0.3148***	0.6343***	30.5254 ^{ns}	1.5658***	1.2434 ^{ns}	0.0025***	0.1033 ^{ns}	0.1033 ^{ns}
A*B*C	8	0.0379***	5.1055***	0.1462 ^{ns}	1.6387***	1.0612***	0.11654**	36.1121**	0.4554**	1.4204 ^{ns}	0.0340***	0.8184***	0.8184***
Error	58	0.0023	0.2572	0.0601	0.1503	0.0402	0.0573	10.8180	0.1222	1.0077	0.0003	0.0627	0.0627
R ²		0.8839	0.9238	0.2774	0.9037	0.9769	0.8019	0.9532	0.9871	0.8300	0.9865	0.9930	0.9978
C.V		12.443	8.676	7.005	8.4495	8.2386	11.083	6.776	5.442	5.272	3.346	0.8173	1.146

Key: S.O.V= Source of Variation; DF= Degree of Freedom; WHC= Water Holding Capacity; WAI= Water Absorption Index; WSI= Water Solubility Index; ER= Expansion Ratio; OHC= Oil Holding Capacity; L*= Lightness; a*=Yellowness; b*=Blue-Greenish; BD= Bulk Density; SC= Swelling Capacity; PR= Mass flow Rate of Product from the extruder; SR= Mass flow rate of the flushed steam at the extruder exit; ns= Not Significant at p≤0.05; *= Significant at p=0.05; **= Significant at p=0.01 and ***= Significant at p= 0.001.

Example of SAS output of ER for the above ANOVA

Source	DF	Mean Square	F-Value	Pr > F
Ratio (A)	4	0.1184	19.1948	<.0001
Water (B)	2	0.0063	0.1033	0.9037
Feed rate (C)	1	0.0292	0.4731	0.4942
Replication	2	0.0002	0.0033	0.9978
A*B	8	0.0219	3.5784	0.0001
A*C	4	0.0063	0.1033	0.9037
B*C	2	0.0043	0.0707	0.9299
A*B*C	8	0.0379	6.1875	<.0001
Error	58	0.0023		

APPENDIX P: Images of extrudates and resultant flour

Blend	Extrudates	Resultant Flour
100:00:00		
70:30:00		
60:30:10		
55:30:15		
50:27:23		

APPENDIX Q: NACOSTI license

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Optimization of protein content and dietary fibre in a composite flour blend containing Rice (*Oryza sativa*), Sorghum [*Sorghum bicolor* (L.) Moench] and Bamboo (*Yushania alpina*) Shoots

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Abstract

Initiatives on tackling food insecurity among global emerging economies are being focused on enriching native staple foods with locally available nutritious underutilized crops. The objective of this study was to optimize protein content and dietary fibre in rice (*Oryza sativa*) flour using Sorghum (*Sorghum bicolor* L.) and Bamboo shoots (*Yushania alpina*). An extreme vertices design of mixture approach with 11 runs was employed in the study using MINITAB® software. The 11 blends from 11 generated runs and individual ingredient samples were analyzed for nutritional composition. Energy value and energy-to-protein ratio for the samples was calculated. Bamboo shoots flour (BSF) had highest content for all proximate components except total carbohydrates on dry weight basis. Rice had highest content of total carbohydrates at 77.71% and energy to protein ratio of 53.72 kcal/g. Sorghum had highest mean total phenolic and condensed tannins of 45.512 (mg GAE/kg) and 2.512 (mg CE/g) while rice the least with 0.042 (mg GAE/kg) and 0.102 (mg CE/g), respectively. Fresh bamboo shoots had highest level content of HCN of 117.81 mg/kg. Other dried ingredients had a mean HCN content of 2.313, 1.584 and 0.066 mg/kg for dried BSF, sorghum and rice respectively. Increasing the quantity of BSF and sorghum flour in the blends consequentially increased the protein content, dietary fibre and total minerals. Optimum blend was established to be 50:27:23 for rice, sorghum and BSF, respectively. This blend had 13.4% protein, 6.2% dietary fibre and 3.9% total minerals. Regression analysis showed that apart from dry matter, all other constituents were significantly predictable during optimization with $R^2 > 0.7530$. Cluster analysis showed that the nutritional components analyzed are in four main clusters. Cluster 1: Dry matter and protein digestibility, cluster 2: Carbohydrates, energy value and energy ratio, cluster 3: Protein, fibre and ash while cluster 4: Crude fat only. These findings of the optimum composite ratio and other blends could contribute in addressing the food insecurity for low income countries.

Keywords

Optimization, Protein, Dietary fibre, Bamboo shoots, mixture analysis

Influence of feed rate, moisture and mixture composition from composites containing Rice (*Oryza sativa*), Sorghum [*Sorghum bicolor* (L.) Moench] and Bamboo (*Yushania alpina*) shoots on physical properties of extruded flour and mass transfer

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Abstract

Raw material composition, loading rate and moisture level in extrusion processing have a direct bearing on physical properties of the product besides other extruder operational parameters. These properties greatly influence the consumer appeal, package design and shelflife of the product. This study aimed at evaluating the effect of sorghum and rice flour containing bamboo shoot flour (BSF) and other ingredients on resultant flour and product throughput. A commercial single screw extruder with a constant barrel temperature of 250°C and screw speed of 1480 rpm was used. Five different blends (100:0:0, 70:30:0, 60:30:10, 55:30:15 and 50:27:23 for rice, sorghum and BSF respectively, on dry weight basis) were extruded at three levels of water addition (15, 20 and 25 kg/h) and two feed rates (1800 and 2100 kg/h). Extrudates were analyzed for expansion ratio (ER). Extrudates were then milled to particle size of 2 mm and flour samples were analyzed for hydration properties, colour, bulk density (BD) and oil holding capacity (OHC). Product throughput was calculated using mass balance equation based on dry matter content of the raw materials and the corresponding extruded product. Mixture composition significantly affected all physico-chemical properties analyzed for the products except water solubility index (WSI). Feed rate significantly affected all physico-chemical of the product properties except WSI and colour lightness (L*). Water addition significantly affected product mass transfer, ER, BD, colour and swelling capacity (SC). Therefore, BSF can be blended with other ingredients and extrusion parameters be manipulated to give forth a product with desirable properties.

Keywords

Bamboo shoots, Extrusion, Physical properties, Mass transfer

Feed rate, water addition rate and mixture composition nexus role on alterations of nutritional properties in extrusion of composites containing rice (*Oryza sativa*), sorghum [*Sorghum bicolor*], and bamboo (*Yushania alpina*) shoots

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Abstract

Extrusion cooking impacts positively on the nutritional status of a food when compared to their raw form. The objective of this study was to determine the effect of ingredient variables: Feed Rate, Water Addition Rate, and Mixture Composition on the extent of nutritional changes to rice flour enriched with sorghum (*Sorghum bicolor*) and bamboo (*Yushania alpina*) shoots on extrusion processing. A $5 \times 3 \times 2$ factorial experimental set up for feed blending, water addition rate, and feed rate in single screw dry type extruder with a constant set barrel temperature of 250°C and screw speed of 1,480 rpm was used. All ingredient variables showed a varying effect on the extent of loss in proteins, condensed tannins, and dietary fiber while increasing carbohydrates, protein digestibility, and total phenolic content. These alterations are due to magnitude of mixing and shear impact, mass moisture, and specific mechanical energy that influence the degree of bio-reactions.

Practical applications

The current emphasis in addressing food insecurity especially in low economic countries is on enriching staple foods with locally available underutilized food crops. Rice is a staple food across the globe while sorghum, though nutritious, has remained to be one of the most underutilized cereal crops. Consumption of bamboo shoots is gaining global popularity due to being high in nutrients as well as possessing functional properties. Inasmuch as incorporating sorghum and bamboo shoots to rice will result in enrichment but also any further could cause changes to nutritional components. Hence, understanding the relationship between effect of ingredient variables and nutritional changes during extrusion will inform on novel product(s) develop-