Development and characterisation of ready to eat green banana (*Musa*) – based porridge with improved nutritional value

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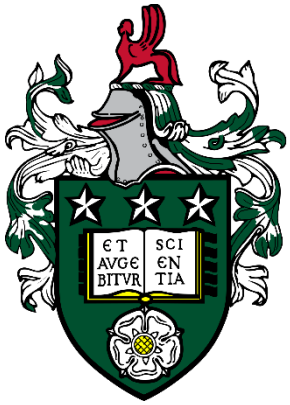

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Manuscripts in preparation for publication

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Abstract

Banana (*genus Musa)* is an important starchy fruit crop for food and nutrition security in Africa. Due to climacteric ripening, the shelf life of banana fruit is relatively short, resulting in rapid fruit deterioration during the post-harvest period. One strategy to prevent wasting of banana is to process green banana fruit into long-life products that are safe, nutritious, and acceptable to consumers.

The aim of the research was to develop a ready to eat (RTE) porridge from green banana using simple processing methods. Firstly, green banana flour (GBF) was prepared from fresh green banana by peeling, slicing, oven drying, milling, and sieving. GBF was cooked into porridge and then oven dried to make a RTE powder. The dried RTE porridge was packaged with three different materials (glass jars, paper, and plastic bags), then stored for 90 days in different environmental conditions (+4 ^{o}C: 65% relative humidity (RH), +18 ^{o}C: 58% RH, 30 ^{o}C: 20% RH). Total bacterial and coliform counts of freshly prepared GBF- RTE porridge ranged from 22 ± 0.4 to 818 ± 2.8 cfu/ml and 18 ± 0.6 to 200 ± 0.5 cfu/ml, respectively. The total plate count and coliform count were significantly ($P<0.05$) affected by packaging materials, environmental conditions, and storage time. The content of sugars, starch (total, digestible and resistant), as well as dietary fibre from green banana flour (GBF) and GBF - RTE were determined using a commercial kit. The composition of GBF was similar to that reported in the literature (60-70% starch, <1% free sugar). Cooking for 5 min rapidly and substantially increased the digestibility of starch from 18% up to 62%. Drying the porridge to RTE powder reduced digestibility from 62% down to 34.6%. Digestibility was restored to 50% by reconstituting in hot water at 85 ^{o}C. There was a slight increase of 1.9% and 6.7% in free sugar during cooking and drying, respectively. Secondly, the RTE porridge was fortified with commercially available plant – based protein because of its low protein

content (40 mg/g). For this reason, it was decided to fortify the porridge with protein using plant-based ingredients available on the market.

The porridge was fortified with 5 different plant-based powdered ingredients (hemp protein isolate powder, pea protein isolate powder, soya protein isolate powder, moringa *oleifera* leaf powder and tomato fibre powder), and casein powder was used as control. The formulation aimed for each portion of porridge to meet 25% of the recommended daily allowance for protein. Total protein of the ingredients (casein (control), pea, soy, hemp, moringa, tomato fibre and RTE banana) were 1000, 780, 900, 500, 387, 148 and 40 (mg protein/g), respectively, and the in vitro digestibility were 78, 63, 53, 81, 46, 53, and 100 (%), respectively. In vitro protein digestibility corrected amino acid score (in vitro PDCAAS) were also calculated based on the limiting amino acids. Soy protein isolate powder had the highest PDCAAS value when compared to other plant-based ingredients and could be the most promising fortificant to complement RTE banana porridge protein.

In conclusion, a microbiologically safe RTE porridge was developed from GBF using simple processing methods. The RTE porridge was enriched with protein sources. This study provides a promising approach to reduce banana wastage and improve food security in banana producing regions.

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List of Abbreviations

ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
CFU	Colony forming unit
CHD	Coronary heart disease
CVD	Cardiovascular disease
DS	Digestible starch
EIU	Economist intelligence unit
FAO	Food and agriculture organization
FSA	Food standards agency
GBF	Green banana flour
GFSI	Global food security index
ICMSF	International commission on microbiological specifications for foods
ICP-OES	Inductively coupled plasma optical emission spectroscopy
MC	Moisture content
PDCAAS	Protein digestibility-corrected amino acid score
RDA	Recommended dietary allowance
RDS	Rapidly digestible starch
RH	Relative humidity
RS	Resistant starch
RTE	Ready to eat
SCFAs	Short chain fatty acids
WHO	World Health Organization
UNU	United Nations University

Chapter 1

Introduction

1.1 Challenges the world is facing due to hunger and food insecurity

The world is facing increasing concern for food security and hunger with much concern arising from Sub-Saharan Africa, including countries like Nigeria (Otekunrin, 2022). Food security is defined as a state in which ‘‘all people, at all times, have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preference for an active and healthy life’’ (Reutlinger, 1986). Whereas food insecurity is a condition where people lack access to adequate food because of limited money or food or other resources (Gundersen and Ziliak, 2015) which can lead to health problems like undernutrition, obesity, and related diseases. Hunger is a condition, in which people cannot afford to eat enough food to meet the basic requirement of life due to lack of resources. At present, the global population accounts for over 7 billion people, and feeding them all is becoming challenging to the world. In 2019, around 690 million people of the world population were suffering from hunger while about 2 billion were undernourished (not having regular access to safe, nutritious, and sufficient food). More than 1 billion malnourished people are in Asia, 675 million are living in Africa and 250 million people are in Latin America (FAO et al. 2021). In 2020, a great number of people experiencing hunger globally witnessed a surge of devastating effect of COVID-19 and the impact resulted in about 720- 811 million people globally experienced hunger, while additional 118 million people experienced hunger in 2020 compared to 2019 (FAO et al. 2021). In 2020, African countries had the highest percentage (21%) of the population that experience hunger with lower percentages in Latin America and the Caribbean (LAC, 9.1%) and Asia

(9.0%). Hunger and food insecurity have become common on the African continent (excluding the North African countries) (Otekunrin et al., 2020). Thirty-two countries in Africa fall between middle and lower food security score levels in the 2021 Global Food Security Index (GFSI). GFSI is compiled by Economist Intelligence Unit (EIU) on an annual basis since 2012 and monitor the progress of food security for over one hundred countries, considering differences in population size, regional diversity, and economic uptake. The purpose of GFSI is to determine the countries that are most and least affected in facing food insecurity (Izraelov and Silber, 2019). The results on GFSI is measured by EIU on the basis of 28 indicators which are grouped under three main headings: 1) Affordability (the ability of people to buy the food, income, exchange rate of the country). 2) Availability (factors affecting supply of food, the ease to access food etc). 3) Quality and Safety. There were one hundred and thirteen countries captured in the 2021 report (Economist Impact 2021). Seventy percent of countries that were grouped in the class of worst affected are African countries, reflecting serious food insecurity concerns in Africa. According to the report (2012–2021 GFSI scores) of the African countries, the scores from North Africa were among the best in Africa, while four countries (Malawi, Sudan, Mozambique, and Burundi) were among the worst performers (Economist Impact 2021). Table 1.1: Global Food security Index (GFSI) score in 10 years (2012 – 2021)

Table 1.1: Global Food security Index (GFSI) score in 10 years (2012 – 2021)

		GFSI sore (0.0-100.0)									
Rank G/A	African country	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
54/1	Algeria	53.2	51.3	57.4	58.2	62.9	63.5	63.3	63.7	61.6	63.9
55/2	Tunisia	60.0	57.7	58.4	59.2	59.8	63.2	62.2	61.8	60.2	62.7
57/3	Morocco	54.1	54.8	55.7	58.4	57.8	58.2	62.0	59.2	62.1	62.5
62/4	Egypt	58.9	58.5	59.5	62.4	59.8	58.0	57.0	61.3	59.8	60.8
70/5	South Africa	55.8	56.3	56.8	59.5	63.4	60.0	61.6	59.4	58.0	57.8
74/6	Botswana	53.7	53.8	53.4	53.4	54.0	53.7	54.7	56.1	56.1	55.5
76/7	Mali	46.6	48.7	49.9	50.4	48.5	49.9	52.7	53.1	52.7	54.5
82/8	Ghana	48.8	50.4	50.5	51.9	50.7	52.8	53.5	52.9	52.8	52.0
85/9	Burkina Faso	40.4	42.7	43.1	44.1	43.9	46.3	48.1	49.3	46.8	48.1
86/10	Cote d'Ivoire	43.8	44.3	43.8	47.1	44.4	46.8	49.7	50.3	50.4	48.0
86/10	Tanzania	34.7	36.3	40.0	38.8	44.5	45.6	43.1	45.3	47.7	48.0
88/12	Niger	40.4	40.8	40.4	43.3	46.4	44.8	48.3	49.8	49.9	47.6
89/13	Senegal	41.4	42.6	45.9	48.1	48.7	46.7	48.5	48.1	45.5	47.4
90/14	Kenya	38.3	40.5	43.4	43.6	43.2	45.9	45.3	48.6	46.7	46.8
92/15	Cameroon	44.2	41.4	42.1	46.4	45.4	45.2	46.4	44.4	43.9	45.5
93/16	Benin	39.9	40.3	41.8	45.2	45.8	46.4	45.4	45.4	46.1	45.2
94/17	Togo	39.0	39.6	42.0	43.8	38.4	45.4	44.3	46.2	45.7	44.2
95/18	Uganda	40.3	42.3	46.7	47.9	46.5	46.3	40.8	43.7	43.2	43.9
96/19	Guinea	34.7	36.0	39.8	41.6	38.5	40.0	40.4	40.6	42.8	43.0
97/20	Nigeria	39.0	41.1	39.5	40.9	42.4	41.9	40.5	42.6	41.2	41.3
98/21	Angola	40.1	40.9	38.5	40.2	38.4	38.1	39.1	40.6	41.7	41.1
99/22	Chad	33.2	32.3	35.9	39.0	39.2	39.9	40.2	43.8	41.7	40.6
100/23	Madagascar	36.7	38.3	38.6	38.7	39.0	36.8	35.9	35.1	38.0	40.4
101/24	Rwanda	43.6	39.9	42.5	44.2	42.6	37.4	38.8	43.7	45.2	40.3
103/25	Congo Dem. Rep	32.3	34.8	34.6	35.1	34.3	35.4	32.8	38.0	38.1	39.1
104/26	Sierra Leone	33.8	35.2	41.7	43.1	40.7	38.0	33.9	36.6	39.8	38.1
105/27	Zambia	38.2	41.4	40.5	40.1	42.3	38.0	41.9	41.1	38.9	38.0
108/28	Ethiopia	33.7	35.4	41.6	42.1	41.9	44.5	41.3	41.5	36.7	37.6
109/29	Malawi	39.5	33.9	35.3	36.9	36.3	35.4	39.5	40.0	39.1	37.3
110/30	Sudan	34.7	33.4	36.0	36.8	39.1	40.7	38.9	39.3	36.4	37.1
111/31	Mozambique	37.7	43.0	42.5	41.6	39.8	36.3	37.3	41.7	37.2	35.9
113/32	Burundi	39.2	38.7	38,8	41.4	42.7	41.5	31.1	37.2	38.0	34.7

Source: Otekunrin (2022). Compilation from GFSI Scores (2012 – 2021) Economist Group 2021, Rank G/A= Global/Africa rank

Table 1.1 shows that Nigeria was ranked 97th and 20th in the 2021 GFSI in the world and in Africa, respectively. The change in the last decade is only marginal during the analysed decade in contrast with other countries (e.g. Kenya and Tanzania).

However, 2021 Global Hunger Index (GHI) report revealed that Nigeria's GHI score was 28.3 (serious category), ranking 103rd among 116 ranked countries. The percentage of undernourished population rose from 8.9% in 2000 to 14.6% in 2021 indicating that the country Nigeria is not on the path of achieving Sustainable Development Goal (SDG 2 – zero hunger) target by 2030 (von Grebmer et al. 2021).

From the result in Table 1.1, it can be deduced that Nigeria is suffering from food insecurity and with a large and rapidly rising population number. A number of factors could cause this food insecurity in Nigeria.

Poor governance in the country and conflict: For over fifteen years, Nigeria is facing problems resulting from poor governance, resulting that Nigeria population can no longer be established, due to opening of four land borders, leading to entering of immigrants without proper visa . According to the United Nations, conflict was the main driver of continuing food insecurity emergencies in 2020. The debilitating conflicts in Nigeria has forced many people to flee out from their homes. Farmers in the southern part of Nigeria were unable to plant food crops on their farmlands because a part of Nigeria called Fulani , a part of Hausa tribe has decided to be moving round the entire country with their cows and sheep in the form of looking for grass to eat, thereby destroying cultivated crops. This had contributed to decreasing of farming in Nigeria some years now, in which agricultural production has declined drastically. Furthermore, farmers cannot go to their farms and harvest their food crops without encountering people with weapons like guns, which these people (Fulani's) use to attack the farmers when the farmers challenge them for spoiling their food crops with

their cows. This has increased food insecurity in Nigeria and during conflict; many local markets were shut down entirely, resulting to food not being accessible for people.

Low agricultural productivity and low processing infrastructure: Nigeria has fertile land for cultivation with lack of processing infrastructure that has led Nigeria into low productivity (Phillip et al., 2009). Nigeria suffers from high food insecurity. One potential solution is to develop food products from native crops, such as banana.

1.2 Banana fruit

Bananas (*Musa*) are among the most popular fruits consumed in the world (Ghag and Ganapathi, 2017) and an important starchy fruit crop for food and nutrition security in Africa, especially Nigeria. It is produced abundantly about 2.73 million tonnes per a year supplying energy (1,422.56 KJ per serve) (Bezerra et al., 2013) in the form of starch to the indigenous population and offers an important trade and business commodity in Nigeria. Due to climacteric ripening, the shelf life of banana fruit is relatively short, resulting in rapid fruit deterioration during the post-harvest period. Therefore, it would be of advantage to utilise banana prior to ripening, in the green stage, when the content of starch is highest.

1.2.1 The origin of banana fruit

Bananas (*Musa*) are fruits that are consumed as carbohydrate food. Banana is a common name covering many varieties. All the varieties of banana fruit are derived from *Musa acuminata* and *Musa balbisiiana* that originate from Southeast Asia precisely India and Malaysia, which later came to Africa by the missionaries. The most common varieties that are cultivated are *Musa acuminata* known as dessert banana and *Musa paradisiaca,* the hybrid of *Musa acuminata* and *Musa balbisiana* commonly

known as plantain (Stover and Simmonds, 1987). According to Zhang et al. (2005) the dessert banana originate from Indonesia – Malaysia, whereas plantain comes from Central belt of Africa including Nigeria.

1.2.2 Role of banana in Nigeria's national security

National security is defined as the capability of a nation to protect its internal values (basic needs like food) against external threats (hunger). National security is one of the greatest serious trials for the nation of a given country, especially for its own firmness, sustainability, and wealth (Nguyen et al., 2021). It is the duty of the government of that country to ensure adequate level of national security and each country has its own national security (management) system and strategy to solve their problems (Jokubauskas, 2017).

Nigeria and other sub-Sahara Africa countries depend on agriculture for food, raw materials for industries, and serve as means of livelihood and general wellbeing of their populace. Banana is widely consumed and is the fourth food crop after rice, wheat, and corn by the United Nations, Food and Agriculture Organization and 2nd as fruit in the world. Bananas are produced in large quantities in the tropics, and subtropical parts of the world and mainly in the developing counties of the world where you have adequate supply of sunshine, level humidity (Ihekoronye and Ngoddy, 1985). Bananas are fruit with high nutritional value (Verma et al., 2017). Banana is among the major staple food in Nigeria and other nations which provide several nutrients to the body. It constitutes a rich energy source, also rich in minerals, dietary fibre, vitamins A, B and C, which are needed for healthy growth (Honfo et al., 2011).

Apart from their nutritional importance, marketing of banana serve as a source of employment thereby providing income to the rural and urban people in the Nigeria and

other countries in the world. When banana is exported to other countries, it will thereby generate revenue to the Nigeria government. In view of the importance of banana and plantain, their marketing become relevant to meet the demands of consumers locally and internationally, and enhance income of both marketers and producers. Banana cultivated in West Africa only account for 2.3% of worldwide production. This could indicate that production is in the hands of small-scale farmers and majority of their production is consumed (Olumba and Onunka, 2020). Cote d'lvoire ranks the highest producer of bananas with about 320,000 metric tons in West Africa. In West Africa especially Nigeria, who produces 2.73 million tonnes of banana per a year, and every other producing counties is facing some problems including pests, diseases, and environmental factors especially under smallholder management. However, there is room for progress in obtaining bigger yield of these crops. Banana and plantain production enterprises in West Africa have great prospects in the area of employment generation, contributions to national income and gross domestic product, poverty alleviation, economic and industrial growth, and rural development. Recommendations are drawn for the provision of market information outfits to disseminate information timely to banana and plantain marketers, price stability, and the need for intensive research on the growth requirements for the sustained production of the crops (Olumba and Onunka, 2020). Banana performs the dual role of providing food for the households, as well as being an additional source of cash income to the farmers (Tshiunza et al., 2001).

1.2.3 Diversity of banana fruit in Nigeria

There are different varieties of banana fruit that can be grown and consumed in Nigeria (Figure 1.1). The coloured banana types have high content of carotene (21.0 µg/g FW) and β- carotene (9.14 µg/g FW) which were found in pulp of red banana (Lokesh et

al., 2014) . In several developing countries, the deficiency of vitamin A is common which can be reduced by encouraging the growing and consumption of local, affordable, and well-accepted sources of provitamin A carotenoids foods. These foods include red bananas, dry grits (red banana pulp + whole/skimmed milk/starch). These foods can retain up to 70% carotenoids, with beta – carotene being stable after post-climacteric ripening (Lokesh et al., 2014).

Figure 1.1: Banana fruit diversity in Nigeria

The consumption of these food carotenoids regularly will help to reduce problem of bad eyesight of some people in developing countries. Increasing the diversity of banana fruit grown in the producing areas in the world could be used to solve the

problems of malnutrition. Unfortunately, these bananas are not available in the UK, and could not be used in the research.

1.2.4 Threats to banana cultivation

Many varieties of banana were developed from two common seeded *Musa* species *(M. acuminate (Musa* AA) *and M. balbisiana* (*Musa* BB) by hybridization resulting in seedless clones (Purseglove, 1972; Simmonds, 1962).

Among the seedless varieties, the most commonly commercial one is the Cavendish varieties because of its high resistant to diseases like Fusarium Wilt of Race1 strains (*Fusarium oxysporum* f.sp. *cubense* (Foc)) and ability to standard the change of weather. Now it has been found that a fungal disease called Fusarium wilt, a recently emerged strain of *Fusarium oxysporum* known as Tropical Race 4 (TR4) can infect the roots and vascular system of Cavendish banana. Infection will result in the plant not being able to transport water and nutrients and will course the plant to dead. This is a soil borne pathogen (TR4) that courses the plant to wilt and die (Drenth and Kema, 2021). Subsequent studies showed that the TR4 strain is extremely dangerous toward many banana cultivars, including Cavendish cultivars grown in large-scale monoculture plantations for export markets and many banana varieties are important for food security and domestic consumption. There are no readily available solutions to manage this disease. Moreover, this global threat connects export trade, strongly dependent on the susceptible Cavendish cultivars, to local production systems wherein ranges of banana varieties contributing to food security are also impacted. Figure 1.2. shows a cross section of banana stem infected with Fusarium fungal disease. Again the Cavendish varieties have susceptibility to a disease called Black Sigatoka (Jeger et al., 1995) and caused by fungus *Pseudocercospora fijiensis* that attacks the plants' leaves, causing cell death that affects photosynthesis, and leads to

a reduction in fruit production and quality. If Black Sigatoka is left uncontrolled, banana yields can decline by 35 to 50 percent.

Figure 1. 2: Cross section of banana stem infected with Fusarium fungal disease (photo by Gert Kema, CCBY).

Figure 1.3: Bananas in Costa Rica affected by Black Sigatoka (photo by Gert Kema, CCBY).

In banana cultivation, the entire reliance on a large and genetically uniform monoculture (Cavendish) is a risky approach that is likely to disappoint the public (researchers, farmers and the consumers), because it is prone to failure. To reduce the vulnerability to diseases, we need more genetic diversity in our cultivated bananas.

1.2.5 Role of green banana as food

In Africa, especially Nigeria, green bananas are consumed as staple carbohydrate sources during the farming season mainly (September), importantly contributing to food security. Green banana fruit is process by boiling, roasting, turn into flour after peeling, drying, and milling. Green bananas in Nigeria are also used to manage diseases such as diabetes (FAO, 2007) as it will not release quick glucose into the blood stream.

Banana is selected for this work because is a fruit that grows well, consumed, and wasted in Nigeria. It is sold in many typical Nigerian markets (Figure 1.4). A lot of the green banana has already changed colour from green to brown, which may be due to improper handling, leading to softening of the banana and may result in the banana being wasted and deposited improperly. This could be a contributing factor that about one –fifth of total banana production is wasted and disposed improperly in the production areas of the country. Thus, this project was conceived due to the aforementioned problem, uses and benefits of banana.

A) Green banana mixed with plantain

B) Ripening banana being transported

Figure 1.4: Banana market in Nigeria showing A) green banana mixed with plantain and B) ripening banana being transported**.**

Green banana can be consumed in many Nigeria dishes, subjected to processing like boiling, roasting, frying, and making into flour by peeling, drying, and milling into flour. See Figure 1.5

The boiled green bananas is eaten with red oil (oil from red palm fruit, after processing), salt, vegetables, and onions. The roasted banana is eaten in a similar way as boiled banana, plus cooked beans, and fish in addition. Green banana flour could be incorporated into another flour to bake or used directly to make porridge. A ready to eat (RTE) banana porridge is currently not available in the Nigerian market. It could be developed and used as breakfast.

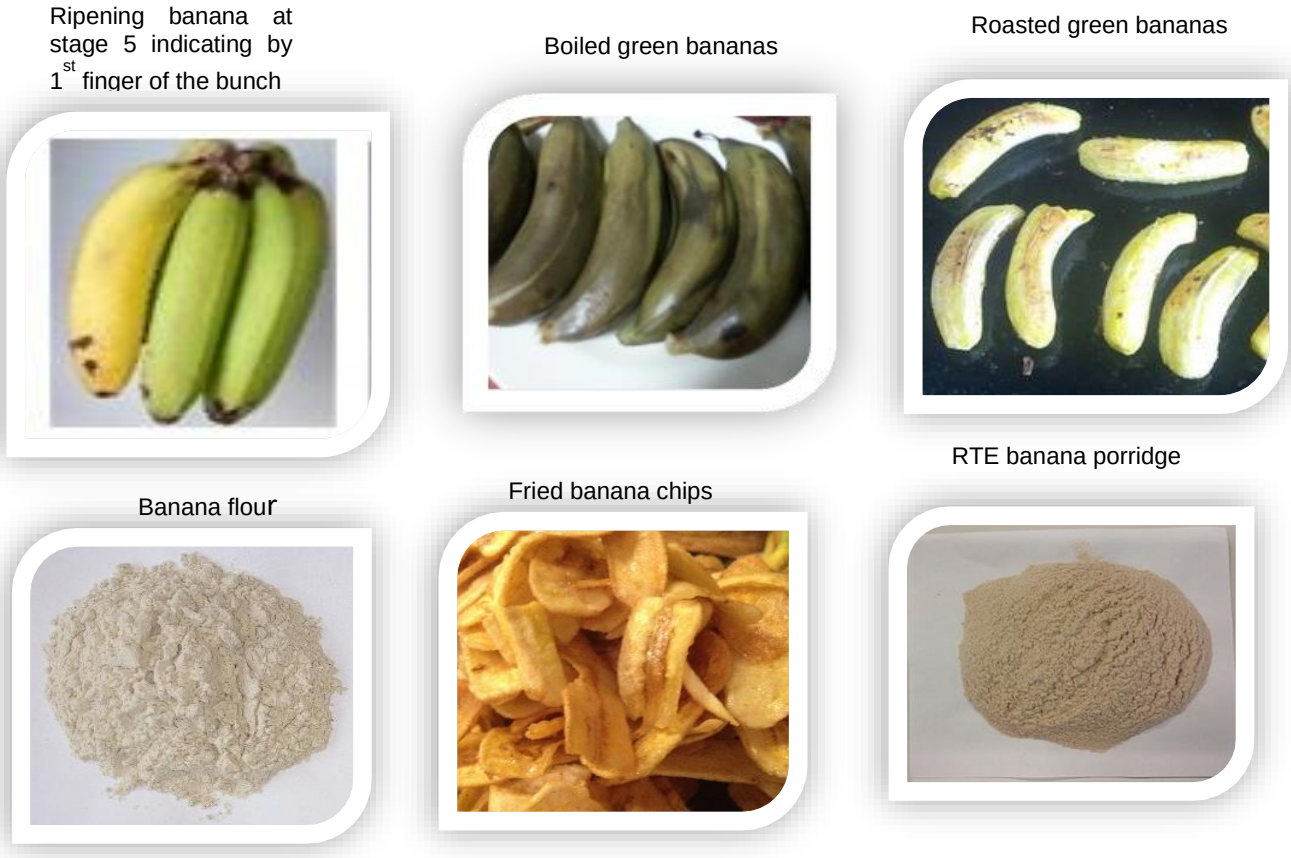

Figure 1.5: Current diet to products made from bananas, popular in Nigeria**.**

RTE banana is not yet available on the market (product of this Thesis).

1.3 Nutritional role of fruit intake

According to Lai et al. (2015), fruit intake is associated with a reduced risk of cardiovascular disease (CVD) and coronary heart disease (CHD) mortality, with a 6–7 % reduction in risk for each 80 g/day portion consumed. Specifically, women in the highest intake group of grapes and citrus experienced a significant reduction in risk of CVD and stroke respectively compared with non-consumers. These findings support promoted guidelines encouraging fruit consumption for health in women, but do not provide strong evidence to suggest that fruit type is as important. Until further knowledge is obtained from intervention studies, consumption of a wide variety of

different types of fruit is recommended. In 1990, the World Health Organisation recommended a 400 g of fruit and vegetable intake per day in people's diet, so as to prevent a number of diseases, such as cardiovascular disease and certain forms of cancer in UK. The benefit of eating fruits and vegetables is attributed to antioxidants, vitamins, and phytochemicals. The Food Standards Agency (FSA) has established its message that a minimum of five portions of fruit and vegetables a day contributes towards a healthy diet (FSA, 2010). Recent evaluations of diet quality in the UK reveal that two thirds of people still do not consume the recommended five portions of fruit and vegetables per day. Fruit and vegetables contain high concentrations of bioactive compounds including antioxidants, which may be beneficial to health. One of the precise health benefits that is gained from the consumption of antioxidant-rich foods is the reduction in the risk of cardiovascular diseases and some other diseases (Nishida et al., 2004). Again, there has been a visually evident increase in the number of beverages that use their antioxidant capacity as a marketing tool in recent years.

1.3.1 Nutritional composition of green banana fruit

Bananas are more nutritious than wheat and maize. Green banana fruit is rich in carbohydrates (starch, fibre, and sugar), vitamins (A & C), minerals (potassium and magnesium), and phytochemicals (Vatanasuchart et al., 2012; Lai et al., 2015). Starch accounts for about 70-80% on dry weight basis, which can be compared to corn and potato (Zhang et al., 2005a). When fully ripe the starch can gradually reduce to as low as 1%. These nutrients contribute to the proper functioning of the body when banana is consumed. The proximate composition of green and ripe banana fruit is shown in Table 1.2.

Table 1.2: Proximate composition of unripe and ripe banana fruit (g per 100 grams wet weight).

	Banana fruit content (g/100) dry weight.	
Nutrient	Unripe banana	Ripe banana
Water content	73.66	81.68
Starch	24.09	15.96
Crude protein content	0.35	0.35
Ash content	0.71	0.33
Crude fibre	1.19	1.43
Fat content	-	0.25

***From Ogbonna et al. (2016)**

1.3.2 Carbohydrate content of banana

Bananas are carbohydrate-rich foods. Carbohydrates can be broadly divided into two groups according to the size, sugar, and polysaccharides. Sugars in banana include glucose, fructose, and sucrose (1.7g/100 g, 1.7g/100 g and 8.2g/100 g), respectively and are generally rapidly absorbed in the small intestine of human beings (Yap et al., 2017). Polysaccharides are polymers of sugars and can be sub-divided according to their nutritional properties into two groups: starch and non-starch polysaccharides (NSP) (Englyst et al., 2007, Cesarino et al., 2016). Banana fruit when unripe contains about 70-80% starch. This starch is mainly resistant to digestion by α-amylase and glucoamylase, the two enzymes responsible for starch hydrolysis in humans. An in *vivo* experimental result showed that 75-84% of starch from green banana reached the terminal ileum in humans (Englyst and Cummings, 1986; Faisant et al., 1995). During ripening, starch is hydrolysed by plant amylases and glucosidases to glucose. Glucose is used as a substrate for synthesis of fructose and sucrose. These sugars increase the appeal of banana for consumption. Banana is commonly given to weaning children or to elderly people with low eating capacity due to its softness,

sliminess, high calorie content, sweetness, and its nutritive value. The non-starch polysaccharide (NSP) content of banana is estimated at 3.12% (Englyst and Cummings, 1986). NSP consists of mostly soluble polysaccharides including pectin and mannan, and insoluble polysaccharides including cellulose and xyloglucan. During ripening, the cell wall is partially degraded resulting in softening of the ripe fruit, again increasing appeal.

1.4 Starch composition and structure

Starch is the main carbohydrate of most plant foods. Starch is composed of two glucose polymers, amylose, and amylopectin (Wang and Copeland, 2013). Amylose is made up of 200-300 glucose units while amylopectin has up to 1,000,000 glucose units. Amylose polymers are straight chains of starch joined by α-1, 4 glycosidic bonds while amylopectin has a backbone of α-1, 4 linked glucose branched with α-1, 6 linked glucose linkages (Butterworth et al., 2011) (Appendix 1). Amylose to amylopectin ratio in generally varies between 10:90 to 20:80 (Eggleston et al., 1992). In green banana the ratio is 20:80 (Ling et al., 1982). By comparison, starch from normal maize, rice, wheat, and potato have similar amylose and amylopectin ratios, ranging from 20:80 to 30-70 (Pérez and Bertoft, 2010). Starch in plants is organised into semi-crystalline structures within organelles called ‘starch granules.’ The internal structure of a typical starch granule consists of starch (amylose and amylopectin) laid down in an altering amorphous and semi-crystalline concentric pattern (shell/ring). The diameter of the outer granule shell is between 100-400 μm (Pérez and Bertoft, 2010). The structural characteristics of amylopectin is the main determinant of the degree and type of crystallinity present in a raw starch (Cyras et al., 2006). In starch granules, three structural types (A, B, and C) (Katz, 1937) have been described. Type-A is found in cereals (e.g. maize, rice, wheat, millet, sorghum), Type-B is associated tubers

(potatoes, starches with high amylose content). Type-C is found in legumes. Type-C exhibits fixtures of type-A and type-B (Pérez and Bertoft, 2010). Banana is said to show type-B structure (Faisant et al., 1995) and still type-C. Type- A is easily digested by pancreatic enzyme than Type–B, which shows resistance to enzyme digestion. Heating starch in water loses the crystalline structure of starch and this phenomenon is called gelatinization (Cummings and Stephen, 2007).

Table 1.3: Amylose content of banana and plantain starch

Amylose content of banana and plantain starch in %		
Source of starch	Content	Authors
Banana starch (in general) *	16	Kayisu and Hood (1981)
Cavendish	19.5	Ling et al. (1982)
Cavendish	17	Garcia and Lajolo(1988)
Banana starch (in general) *	24.41-36.87	Mustaffa, (2013)
Valery	40.7	Waliszewski et al. (2003
Banana starch (in general) *green	31.41-41.66	Bi et al. (2017)
Plantains	10 -11	Zhang et al.(2005
Cereal starch	20-25	Zhang et al.(2005)

*****No clear information on the variety used

Banana starch from Cavendish variety was found to be highly irregular in shape and sizes (Ling et al., 1982). In Valery banana, the starch has irregular shape with elongated granules (7-25 μm in width and 20-50 μm in length, and spheroid (15-40 μm in diameter). The surface of starch granule of unripe banana appears to be smooth, while the surface of ripe banana have linear striations (Kayisu et al., 1981). The size of the granules ranges from 6-80μm but majority falls in between 20-60 μm. In addition,

starch granules from banana do not appeared differently from other starches when viewed under polarized light since layers of starch do appear around the hilum (Zhang et al., 2005a). The hilum is the point in starch granule around which the layers of starch are deposited. Plantain starch granule sizes range from 7.8-61.3 μm, with average of 26μm.The amylose content of banana and plantain is show in table 2. The compositional content of amylose varies as shown below is might be due to differences in variety, ripening stages used.

1.4.1 Starch classified according to their digestibility

1.4.1.1 Rapidly digestible starch (RDS)

Rapidly digestible starch (RDS) is defined as a type of starch that is rapidly converted to glucose within 20 minutes of digestion (Englyst et al., 1992). It can be found mainly in cooked starchy foods such as freshly-cooked bread and potatoes. When RDS is high in a food, it means that consumption of the food will quickly increase the blood glucose level and increase in insulin response. This sharp increase in blood glucose is not desirable for type 2 diabetics who have trouble regulating blood glucose (Englyst et al., 1999). Glycaemic index (a ranking index of carbohydrate in foods according to how it affects blood glucose level) is significantly correlated with RDS (Cesarino et al., 2016)

1.4.1.2 Slowly digestible starch (SDS)

Slowly digestible starch is a type of starch that takes a longer time to digest. SDS gives rise to blood glucose 20-120 min after consumption (Englyst et al., 1992). SDS is found in most raw, milled cereal foods, where the starch granules are physically available but are not gelatinised.

1.4.1.3 Resistant starch (RS)

Resistant starch resists enzymatic digestion in the mouth and the small intestine and finds itself in the large intestine where it is fermented by the gut microflora to produce short chain fatty acid (SCFAs).The ratio of amylose to amylopectin influences resistance of starch, as digestion of high-amylose starch is slow. Resistant starch (RS) is classified into five groups (RS1- RS5).

1.4.1.3.1 Physically inaccessible starch (RS1)

Starch that is physically inaccessible to enzymatic digestion due to the presence of intact cell walls in grains, seeds, fruits, or tubers. Hence starch is unreachable by digestive enzymes. There is no degradation of cell wall components in the gastrointestinal tract due to the fact that humans do not produce cell wall degrading enzymes (glycosyl-hydrolases, oxidoreductases, lyases, and esterases) Zhang and Hamaker, 2012). Hence, starch can remain inaccessible until it reaches the colon. Milling, homogenisation, or other types of processing which disrupt cellular structure can render RS1 accessible to enzymes (Raigond et al., 2014).

1.4.1.3.2 Native starch (RS2)

This type of starch is found in raw foods such as green banana and raw potatoes. Starch granules are crystalline nature and poorly susceptible to hydrolysis (Hernández et al., 2008). Native starch (RS2) has a compact structure that makes it inaccessible for the digestive enzymes. RS1 and RS2 digestion are slow and incomplete in the small intestine, which is good for diabetics. The size of starch granules affect the extent of enzyme hydrolysis with smaller starch granules being more highly digested. There is also a relationship between degree of starch crystallinity and the extent of enzymatic hydrolysis. Starch with B-type crystallinity (e.g. potato) is more resistant than starch with A–type crystallinity (e.g. cereals). In B-type of starch, the enzymatic digestion is

only at the surface while A-type allows some penetration of the enzyme into the granule. It was observed that starch with B-type of crystallinity like potato starch had double helices forming large amylopectin blacklegs incorporated in hard crystalline layers of granules. These blocklets may be responsible for the resistance of the potato granules (Gallant and Bouchet, 1997). It can be seen that starch resistant is dependent upon many factors like shape of the granules, size of granules, amylose content, and starch crystallinity. Banana starch has a mixed type crystallinity, expected to be resistant.

1.4.1.3.3 Modified starch (RS3)

RS3 is physical modified starch or retrograded starch. Retrograded starch formed during cooling of gelled starch so it is found in cooked foods that have been stored at low temperature. RS3 is stable and used as ingredient in many foods. Cooked and cooled potatoes and cornflakes are good examples of foods that contain RS3 (Waliszewski et al., 2003)

1.4.1.3.4 Chemically modified starch (RS4)

This group is called chemically modified starch. Chemical modifications include esterification, cross-linking and oxidation. These modifications are done to increase starch functionality, such as viscosity, but will also decrease digestibility

1.4.1.1.5 Amylose-lipid starch (RS5)

RS5 is a type of RS formed by amylose-lipid complexes. These complexes can be formed from high amylose starches in the presence of fatty acids. The complexes show increased resistance to digestion.

1.4.1.4 Importance of resistant starch

RS has prebiotic properties in the large intestine. The microbiota will ferment RS to produce short chain fatty acids (SCFAs), especially butyrate as primary energy source for colon cells. Lack of SCFAs (butyrate), will lead to endogenous starvation of enterocytes, which may cause ulcerative colitis and other inflammatory conditions.

RS is also good to the health due to its low glycaemic index which can be used to control obesity, diabetes, reduce risk of cardiovascular diseases. RS increases fullness of the stomach by producing a viscous gel which delay gastric emptying and reduce the activities of digestive enzymes (Raigond et al., 2014).

1.4.2 Summary of the carbohydrate composition of banana fruit as a plant food.

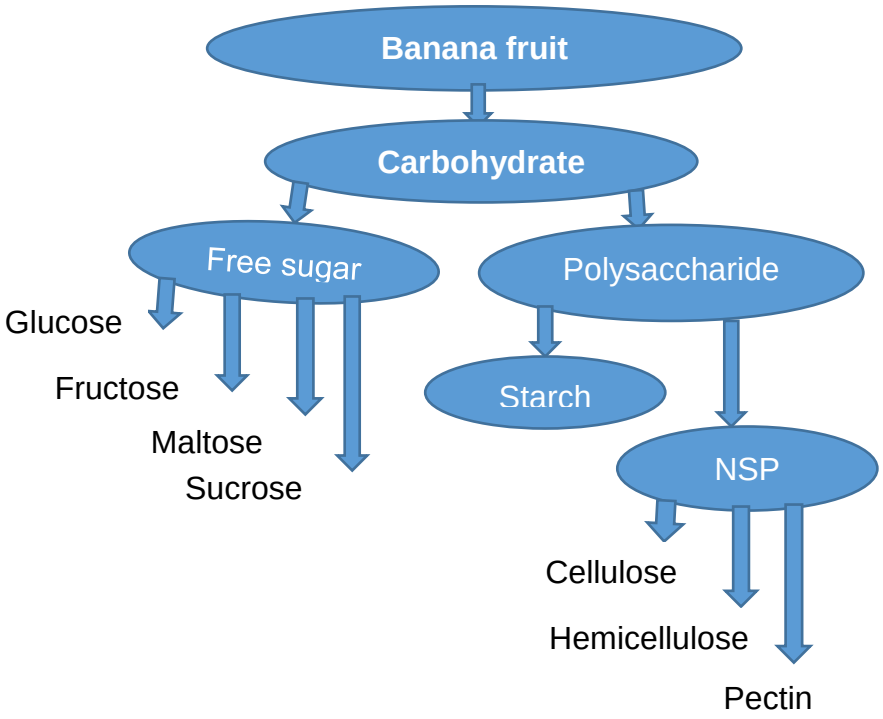

Figure 1.6: Composition of banana fruit as plant food

1.5 Starch digestion in vivo

Starch digestion starts from the mouth, where the salivary gland produces saliva

containing α-amylase. Amylase catalyses the endo-hydrolysis of α-1-4-glycosidic linkage of starch, thereby releasing maltose, maltotriose and dextrin molecules as main products (Hanhineva et al., 2010). The quantity of starch hydrolysed in the mouth will be small due to short duration of process in the mouth before swallowing. The chewed starch that forms the bolus is swallowed down into the stomach, where there is no or little digestion of starch. The acidic nature of the stomach caused by hydrochloric acid will likely inactivate the α-amylase enzyme. The chime produced in the stomach enters the duodenum, where pancreatic amylase will hydrolyse starch further. Maltase is a brush-border enzyme which will complete the digestion of starch to glucose (Table 1. 4). Glucose is then absorbed into epithelial cells of the intestine, passing into the portal vein.

Table 1.4: Starch digestive enzymes in gastrointestinal tract of humans

Position in human body	Source of fluid	Name of fluid	Enzyme in fluid	Substrate	Product
Mouth	Salivary gland	Saliva	Amylase	Starch	Maltose, maltotriose
Duodenum	Pancreas	Pancreatic juice	Amylase	Carbohydrate	Glucose
Small intestine	Intestine wall	n/a	Maltase	Maltose	Glucose

1.5.1 Factors affecting the enzymatic digestion of starch

Some factors can affect the enzymatic digestion of starch. These factors are

- **Nature of starch**.

  There are three types of starch. The first is the rapidly digestible starch (RDS). This is the starch that enzyme digest at the first 20 minutes of digestion. The second is called slowly digestible starch (SDS), starch digested as from 20 minutes to 120 minutes and the third is called resistant starch (RS). This is the starch that is not digestible after two hours of digestion.

- **Morphological characteristics of starches**

The difference in size and shape of starch granules is linked to the biological origin of the starch (Singh et al., 2006). It has been shown that small granules showed a higher hydrolysis rate than large or size granules. The large granule fraction showed a lower hydrolysis rate than and small granule fractions. The lower susceptibility of the large granule starches to enzymatic hydrolysis has been suggested to be due to their smaller contact surface area, which may decrease the extent of enzyme binding and ultimately result in less hydrolysis than small granules (Tester et al., 2006).

Starch Gelatinization and Retrogradation. Starch gelatinization was defined

As the melting of starch crystallites as determined by X-ray diffraction, in which the complete destruction of crystallite integrity was observed as a function of moisture content and temperature (Zobel et al.,1988).The starch retrogradation is a process that a gelatinized solution is cooled for a long time, it changes into gel (thicken) and rearranges itself again to a crystalline structure (granule). Consequently, the starch molecules take minimum volumes (granules) not Only in solution, but also in plants (Tako et al., 2014).

- **Protein and lipid interaction**

When there is presence of protein and lipids that are not granules occurring over the starch granules, this could reduce surface accessibility by blocking the adsorption sites and therefore influences enzyme binding (Oates, 1997).

- **Enzyme inhibitors**

These are substances found in the material that hinders or delays

the reaching out of the digestive enzymes to the starch resulting in delayed hydrolysis of starch, and consequently reduce the amount of glucose.

- **Food processing**

  By processing food, the food is changed from one form to the other, for instance, processing methods such as dehulling, soaking and germination may result in an enhancement of digestibility due to the loss of phytic acid, tannins and polyphenols that hinders the digestives enzymes.

1.6 Protein

Proteins are nitrogen-containing substances formed by amino acids which are joined together by peptide bonds. Proteins function as the main structural components of muscle and other tissues in the body and they are used to form hormones, enzymes, and transport proteins such as haemoglobin. Protein is broken down into amino acids by a number of protein digestive enzymes before the amino acids can be absorbed and utilised in the body. There are twenty amino acid needed for human growth and metabolism. Nine out of these twenty amino acid are called essential (or indispensable) amino acids, meaning that our body cannot synthesize these nine amino acids. Therefore, the diet needs to provide these amino acids. The other eleven amino acids are called non-essential (or dispensable) amino acids. The body can synthesize these amino acids as long as precursors and energy are available. However, the lack of any of these amino acids will result to inability of tissue to grow, maintained and repaired (Hoffman and Falvo, 2004).

1.6.1 Sources of protein

There are two main protein sources. Animal source include eggs, meat, milk, fish and poultry, plant sources include cereals (e.g. wheat, soy, oat), tubers (e.g. potato,

cassava), legumes (soy, pea, beans, groundnut, hemp), fruits and vegetables (e.g. tomato, moringa, spinach). Protein from animal source is often called complete protein because it contains all of the essential amino acids. While protein from plant sources are often called incomplete protein due to lack of one or two of the essential amino acids.

Table 1.5: FAO/WHO/UNU amino acid requirements based on amino acid requirements of preschool - aged children.

Amino acid	Requirement
	mg/g crude protein
Isoleucine	28
Leucine	66
Lysine	58
Total sulphur amino acids (methionine + cysteine)	25
Total aromatic amino acids (phenylanine + tyrosine)	63
Threonine	34
Tryptophan	11
Valine	35
Total	320

From FAO/WHO/UNU Expert Consultation 1985

1.6.2 Anti-nutritional factors

Some components found in plants protein sources can hinder the digestion, absorption, and utilization of the proteins and these are commonly referred to as anti-nutritional factors. These include strong enzyme inhibitors such as trypsin inhibitors and some less effective/specific inhibitors such polyphenols.

1.7 Processed foods

Processed foods can be defined as raw agricultural foods (green banana, raw potato, mango, fruits, and vegetables etc) that are subjected to any form of activities like washing, cleaning, milling, cutting, chopping, heating, pasteurizing, blanching, cooking, canning, freezing, curing, dehydrating, milling, mixing, packaging, or other

procedures that alter the food from its natural state. Processing may also involve the addition of preservatives, flavours, nutrients, and other food additives or substances approved for use in food products, such as salt, sugars, and fats (Dwyer et al., 2012).

1.7.1 Minimally processed foods

Minimally processed foods are defined as foods that are slightly altered for the purpose of preservation that do not significantly alter the nutritional content of the food (Alzamora et al., 2016). A minimally processed food retains most of its inherent physical, chemical, sensory and nutritional properties. Many minimally processed foods are as nutritious as the food in its unprocessed form (McGuire, 2011). This is achievable by the application of traditional technologies in other to preserve the nutritional properties of the food and ensure microbial safety over a suitable shelf life. Minimal processing can be applied on green banana fruit to make green banana flour that can then be used in a number of ways.

1.7.2 Role of processed foods

Both fresh and processed foods make up vital parts of the food supply. They tend to have longer shelf-lives than fresh foods, and thereby contribute signficantly to both food security (ensuring that sufficient food is available) and nutrition security (ensuring that food quality meets human nutrient needs).

1.7.3 Importance of processing banana fruit

Banana fruit can be processed into a wide range of products. These include banana juice, banana flour, banana chips, banana crisps, banana sauce, fermented banana yogurt and banana wine (Ranjha et al., 2022b). By processing banana fruits, the shelf life of the banana fruit is extended from a relatively short period to more durable period of time. This processing could be used as a tool to reduce wasting of banana fruit as

many consumable products will be produced, resulting in reducing hunger. Processing banana fruit into banana flours (raw or RTE) products can be used as an added ingredient to other flours, in other to increase fibre or mineral content of the final products, as banana fruit is rich in minerals, vitamins , carbohydrate and fibre (Ranjha et al., 2022a). However, banana flour cannot be used to enrich a product for protein enhancement, because banana flour has low protein content around 3.2% (Suntharalingam and Ravindran, 1993). Bananas brown rapidly when their tissues are cut or bruised. The brown colour is developed due to the enzymatic oxidation of phenols to quinones by polyphenol oxidase (PPO) in the presence of oxygen. Subsequently, these quinones condense and react non - enzymatically with other substances such as phenolic compounds and amino acids to produce complex brown polymers known as tyrosinase, o-diphenol oxidase, or catechol oxidase (McEvily et al., 1992). Heating produce a high efficient inactivation of polyphenol oxidase and peroxidase enzymes, but it promotes non-enzymatic reactions that slight modify the product colour depending on banana maturity (Cano and Lizarraga, 1994). This could be called caramelization, this gives the brown colour of banana product during processing like frying.

1.8 Food packaging

Packaging is the science, art and technology of enclosing or protecting products for distribution, storage, sale, and use, so as enable the products to be transferred from the manufacturing plants to the customers and consumers (Potter and Hotchkiss, 1995). Food packaging is the act of putting food products, in a food packaging material, to protect the food from neighbouring environment, and retain the food quality until the food is consumed (Petersen et al., 1999). According to Ojha et al. (2015) the purpose of food packaging is to maintaining food safety, wholesomeness, and quality of food.

Food packaging materials are materials used in packing food products. There are different types of packing materials, which include glass, cartons, plastic, foils, paper, and aluminium. There are some reasons why packaging materials should be used in the first place, namely;

- Protect the product/ finished good from environmental contaminants
- For product distribution
- For prolonging the shelf-life of the product during storage
- For labelling
- For advertisement purposes before the consumers

However, the selection of any packaging material could be based on the following below reasons, so as to avoid changing of the packing material as a result of the comments from the consumers.

- Easy to open and use
- Easy to write on and provide information about its content
- Attractive to consumers
- Economical /cheap
- Easy for recycling, being biodegradable, ready to provide the needed barrier and mechanical properties.

In all these packaging materials (glass, paper and plastic), there are some advantages and disadvantages of packing materials (Raheem, 2013).

Table 1.6: Advantages and disadvantages of packaging materials

Advantages	Disadvantages
Serves as protection for the product	Cost money to produce
Advertises the company or product with its design	Increase the cost of production
Makes shipping /transportation easier	Take time to design
Helps you to differential products	Reducing packaging affect perception
Makes product attractive	Difficulty of recycling

Raheem (2013)

1.9 Nigeria and UK regulation on safety of food consumption

Food safety is defined as the promise that food will not course harm when consumed by a consumer when eaten or prepared in agreement to its proposed use (FAO/WHO 1997), as an important part of food and nutrition security. In the whole world of about 600 million people, 1 in every 10 people, fall sick due to consumption of contaminated foods and 420,000 people die every year. Food poisoning takes over 200,000 lives annually in Nigeria due to food contaminated through improper farming, processing, preservation, and services Premium Times. Improper farming techniques and food processing methods, products adulteration as well as contamination picked up during processing are possible sources for food-borne diseases. The amount of chemicals used for preservation, medium of sale and tampering with expiry dates are also possible sources. In addition, the methods of cooking, and the sanitary conditions of the cooking environment (Onyeneho and Hedberg, 2013) as well as the personal hygiene of food handlers are all possible sources of food-borne diseases in Nigeria. In developing countries, food security could pose serious challenges to the regulation of food quality because food scarcity creates a serious problem of availability versus

quality. However, unsafe and poor quality food products have its negative impacts on the economy and public well-being, thereby justifying government intervention. Providing information about quality through product certification and labelling would be a natural way for a regulator to intervene. Nigeria, as a member of the United Nations (UN) and signatory to all Conventions and Declarations on Health, Agriculture, Environment and Trade, is committed to meeting international food safety requirements. Improving food safety and protecting the health of Nigeria's citizens would be enhanced via the enactment of food legislation. While the regulation, control, monitoring and evaluation of food safety in Nigeria was described in the NPFSIS 2014 policy document, legislation is needed to implement this policy (Okoruwa and Onuigbo-Chatta, 2021).

1.9.1 Organisms relevant to banana products

There are three different groups of organisms that are associated with spoilage of banana foods: bacteria, yeast and moulds. According to Ajayi (2016), gram positive (+v) bacteria (*Bacillus cereus*, *Bacillus globisporus*, *Bacillus circulans* and *enterococcus spp*). and gram negative bacteria (*E. coli*, *Klebsiella*, *Salmonella typhi*) can be found in banana. These two groups of bacteria need different agars for their detections. Microbial spoilage of banana may occur due to gram negative microbes or fungi causing fruits to undergo undesirable changes, while moulds include *Aspergillus flavus* and *Aspergillus niger*.

1.10 Aim and objectives

The aim of this work was to utilise green banana fruit at stage 1 of ripening by processing it into a RTE porridge with stable shelf-life, and to enhance its naturally low protein content with through fortification with commercially available plant-based protein ingredients.

Objectives

Chapter 2:

- To process green banana fruit into green banana flour (GBF) using simple processing methods
- To develop a RTE banana porridge
- To evaluate the microbiological quality of the RTE banana porridge during storage for 90 days in three different packaging materials
- To determine the effect of processing of GBF on carbohydrate digestion.

Chapter 3:

- To determine the in vitro protein digestibility and protein quality of the 5 plant-based protein ingredients
- To increase the protein content of the RTE banana porridge by fortification with 5 plant-based protein ingredients individually
- To determine the effect of fortification on carbohydrate digestion
- To select the best fortificant to be used in fortifying RTE banana porridge.

Chapter 4:

- To discuss the potential of the research on food and nutrition security in Nigeria

Chapter 2

Development and characterisation of a ready to eat porridge made from green banana: microbial safety, carbohydrate composition and digestibility

Highlights

- A ready to eat (RTE) porridge has been developed from green banana using simple processing methods.
- Microbial analysis of the freshly prepared RTE porridge showed low load of total and coliform bacteria ranging from 22 ± 0.4 to 818 ± 2.8 cfu/ml and 18 ± 0.6 to 200 ± 0.5 cfu/ml, respectively. The counts remained below the safety limit throughout storage indicating that the developed product is likely to be safe for human consumption when stored up to 90 days**.**
- Green banana flour (GBF) showed low content of sugar (1.8%), as well as low carbohydrate digestibility (18%) attributed to intact starch granules surrounded by cell wall material – typical features of resistant starches type RS1 and RS2.
- Cooking the GBF in water to a porridge substantially increased digestibility of starch to 62% and was associated with the formation of a starch-cell wall polysaccharide hydrated gel structure.
- Drying of the porridge reduced digestibility of starch to 34.6% likely due to retrogradation of starch (RS3).
- Reconstituting the porridge with hot water at 85^{o}C and 100^{o}C restored digestibility to 50 and 55% of the fresh porridge, respectively.

2.1 Introduction

Healthy carbohydrate-rich foods are essential for good health and consumers are seeking minimally processed starchy plant-based foods that fit into dietary guideline recommendations (USDA, 2015). Banana (genus *Musa)* is an important starchy fruit crop for food and nutrition security in Africa. It is produced abundantly all year-round supplying energy in the form of starch to the indigenous population, particularly during the hungry season. It is also an important trade commodity, contributing to income for producing communities.

Due to climacteric ripening, the shelf life of banana fruit is relatively short, resulting in rapid fruit deterioration in less than 2 weeks after maturity. This can occur pre-harvest or during the post-harvest period (Yap et al., 2017). Bananas are often harvested green for storage and distribution. People do not generally consume raw green banana fruit due to its hardness and high astringency caused by presence of phenolic compounds including tannins (Sarawong et al., 2014). Green banana fruit is traditionally eaten in Africa by roasting or boiling but it can also be processed industrially by drying, frying, or refining into commercial products such dehydrated banana chips, fried banana chips, banana flour and banana starch (Mohapatra et al., 2011). It can also be mixed with other flours to produce bread (Juarez-Garcia et al., 2006). Processing changes the physical and chemical properties of green banana, and improves palatability (Suntharalingam and Ravindran, 1993). Processing can also alter nutrient content of banana products (Yarkwan and Uvir, 2015).

Recently, a number of cereal-based RTE porridges have appeared on the market. Porridge is mainly used for infant feeding but can also be consumed as a refreshing food for other age groups (Onyango et al., 2020). The RTE porridge based on green

banana could be used as a way to utilise green bananas and create a new product for the general market.

Carbohydrates are the main components of GBF, comprising 1.8% free sugar and 78.6% starch, out of which 25.99% is digestible while 48.9% is resistant. Fibre content is 7.2%, without resistant starch (RS) and can be as high as 56.24% with RS and fructans (Menezes et al., 2011). These values are consistent with those of Zhang et al. (2005b). Starch in GBF is present in starch granules with elongated oval shapes of different sizes ranging from 20 – 60 µm (Zhang and Whistler, 2002). Nutritionally, starch can be categorised into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on the speed of enzymatic digestion in *v*itro (Englyst et al., 1992). The RDS is measured after 20 min while SDS is measured after 120 min of digestion. RS is the undigested starch remaining after 240 min (McCleary et al., 2020).

Previous research has shown that starch in GBF has low (16%) digestibility when raw (Faisant et al., 1995). The digestibility of banana starch was tested in vivo using 6 healthy participants who were fed first with 100 g of ham, and 15 g of eggs, together with 30 g GBF mixed with 200 mL of yoghurt and 20 g sugar and 10 g sunflower oil. All were served with 200 mL orange juice, tea, or coffee. Ileum samples by intubation technique were collected 30 minutes continuously for 14 hours. Samples collected were observed using scanning and transmission electron microscopy. Results showed 84% of the ingested starch reached the end of ileum. Microscopic observations showed that raw GBF contained irregularly shaped dense starch granules with smooth surfaces. Some starch granules had visible holes indicating where the enzymes had digested the starch. Cell walls closely associated with starch granules could have hindered enzyme access to starch, suggesting that encapsulation could be partly

responsible for the low digestibility of starch in GBF (type 1 RS), together with the intrinsic resistance of banana starch granules (type 2 RS). Assuming that only 16% of the starch was digested in the small intestine, it will lead to little or no increase in the glucose blood level after consumption. By cooking GBF, the digestibility of starch increases (Kataria and Chauhan, 1988). When starch granules are heated in excess water, the granules will absorb water and swell. Amylose is solubilized and eventually the starch granules will breakdown releasing starch polymers into solution, forming a thick paste. These changes are collectively referred to as starch gelatinization and result in the loss of the characteristic birefringence and semi-crystalline structure of intact granules. Gelatinized starch is much more readily accessible and digested by digestive enzymes (Zhang et al., 2005). Cooked products can further be processed for preservation in other to extend the shelf life. The cooked food is generally cooled, dried, and stored. During these processes, amylose, and amylopectin chains re-associate to form a more orderly structure (Wang et al., 2015). This phenomenon, known as retrogradation, can reduce digestibility, affect texture and the acceptability of the food (e.g. staling in bread) (Miles et al., 1985).

2.1.1 Aim and objectives of this study

The aim of this work is to produce a safe and nutritious RTE porridge from green bananas.

2.1.2 The objectives are as follows:

1) To develop an RTE porridge from green bananas using simple processing techniques.
2) To assess the microbial safety of freshly produced and stored RTE porridge.

3) To evaluate the carbohydrate content and digestibility at each step of processing.

2.2 Materials and Methods

2.2.1 Plant material

Green banana (*Musa* sp cavendish) at stage 1 of ripening (figure 1) were purchased from Leeds City Kirkgate market, Leeds, UK.

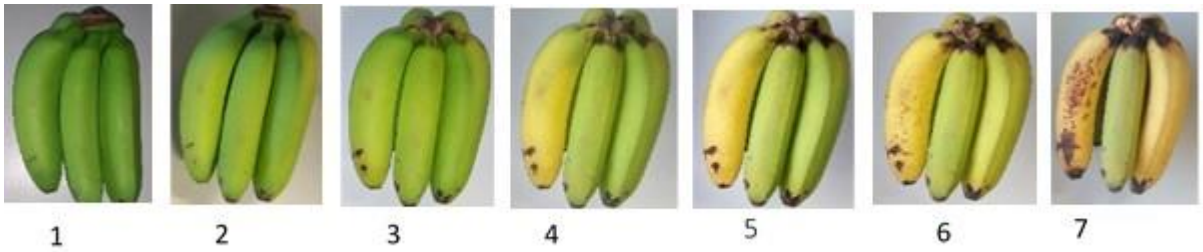

Figure 2.1: Bananas at 7 stages of ripening as indicated by the first finger in each bunch.

2.2.2 GBF production

The GBF was prepared according to the method of Vatanasuchart et al. (2012) with some modifications. Seven fingers of green bananas were washed with potable tap water at 20 ^{o}C, cleaned with a clean hand towel and weighed (636 g). The fruit was peeled with a stainless steel knife, weighed (403 g), cut into 3 mm thick slices without using any chemical to reduce enzymatic browning, immediately dried into chips in an air hot Memmert laboratory oven at 50 °C for 8 hours, with air velocity of 1.0 m/s. After drying, the banana chips were milled with a blender (Kenwood Xtract) and sieved (355 µm mesh sieve). The flour was weighed (103 g) and stored in a cellophane bag and kept in a freezer (-20 °C) for product development and other further analysis. The yield (103 g) was said to be one sixth of the total weight of the banana fruit used. This was

because water content of banana used was 300 g and peel, head and tail removed amount to 233 g. All these amount to reduction in the yield.

2.2.2.1 Reference GBF sample

The GBF was bought from the market as a commercial product, to be used as a reference sample to the produced lab GBF with a brand name, natural evolution.

2.2.3 RTE porridge development

A flow chart showing development of the RTE porridge is in figure 2.

2.2.3.1 Porridge making

Porridge was prepared according to the method of Loypimai and Moongngarm (2015) with some modifications. GBF (2000 mg) was added to preheated potable water (85 °C) at 20% (w/v) and mixed by stirring with wooden spoon continuously, on an electric heating mantle set at 85 °C. The cooking continued for up to 20 min to give a yield of 12 g porridge.

2.2.3.2 Cooking time of the porridge

Cooking time variations were achieved by using a fixed amount of GBF 20%, w/v of preheated water (85 °C) and varying cooking time (5, 7.5, 10, 12.5, 15, and 20 min) to determine the optimum cooking time for carbohydrate digestion.

2.2.3.3 Drying of the flesh porridge into RTE porridge

Cooked banana porridge was poured into a stainless steel tray lined with aluminium foil and oven-dried at 50 ^{o}C for 8 hours, then the temperature was allowed to cool down to room temperature and milled with a Kenwood xtract blender, type BL 23 with speed, 2 plus pulse for 10 min with a yield of 11.8 g of RTE porridge. The milled dried sample was packaged in a cellophane bag and kept in freezer (- 20 ^{o}C) for future use as RTE porridge.

2.2.3.4 RTE porridge reconstitution

The RTE porridge was reconstituted by using 20% w/v of dried porridge and preheated boiled water at 85 or 100 ^{o}C. The mixture was covered for 5 or 10 min after which it was opened and stirred continuously for few minutes without any further cooking, giving a yield of 11.9 g of RTE porridge.

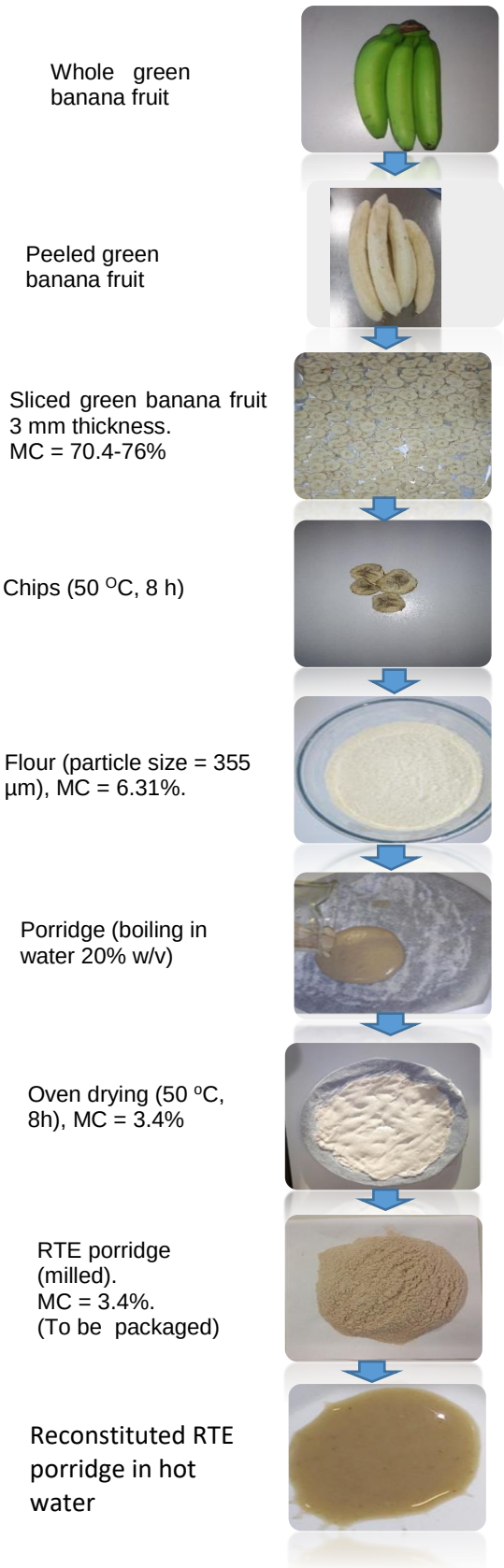

Figure 2.2: Flow chart for the production and reconstitution of ready to eat (RTE) porridge made from green bananas. MC = moisture content

2.2.3.5 Packaging and storage of RTE porridge

The RTE porridge (80 g) was packaged in three different packaging materials (glass, plastic bag, and paper) each time and stored for 90 days under three different temperature and relative humidity (RH) conditions (+4 ^{o}C: 65% R.H, +18 ^{o}C: 58 % R.H, 30 ^{o}C). The glass packaging was a 100 mL lab borosilicate bottle with cap (Getty). The plastic was made from polythene with bottom gusseted base that made it possible to stand-alone. During the period of analysis (0, 30, 60 and 90 days) samples were collected as stated below. Sample 0.5 g of RTE porridge was measured out from the glass packaged RTE stored at 4 ^{o}C, 18 ^{o}C, and 30 ^{o}C in 3 triplicates. This was repeated for paper and polythene accordingly.

2.2.4 Microbial safety analysis

Aerobic total plate count and coliform count bacteria were analysed using AOAC 1997 method 990.12 and 983.25, respectively. Briefly, 0.5 g of RTE porridge powder was homogenised in 50 ml of milliQ water in a 50 mL tube and incubated for 30 min in a shaking water bath set at 37 °C. Serial dilution up to 10^{-5} was made and plated onto sterile agar plates. Twenty-five molten nutrient agar (25 ml) for total plate count or MacConcay agar for coliform count were poured into sterile labelled plates, and allowed to set. Then, 1 ml of each serial dilutions (10 mg/ml) pipetted into the same corresponding labelled petri dishes. Plates were incubated for 48 hrs at 37 °C and colonies counted. Further confirmatory test for *E.coli* was done with eosin methylene blue agar. A sterile loop was used to collect individual colonies from MacConkay agar plate, and streak the new poured set eosin plate and incubated as described before. At the end of the incubation period, viable cells were counted and expressed as colony forming unit millilitre of the sample (cfu/ml) according to (Muhammad et al., 2016). Colour is used to identify the presence of *E.coli*.

CFU/ml = No of colonies /Vol (ml)*Total dilution factor

Formula for calculation: CFU/ml = ∑c/v (n1 + 0.1* n2) d

∑C = Sum of colonies (mesophilic bacteria per/ml) counted on the dishes from 2 successive dilutions (one of which must contain at least 15 colonies). V = Volume of inoculum added to each dishes in mL.

n1 = number of dishes of dilution 1

n2 = number of dishes of dilution 2

d = dilution factor of the 1st dilution

2.2.5 Morphology

2.2.5.1 Scanning electron microscopy

To assess the morphology of the samples, scanning electron micrographs of banana flour using scanning electron microscopy (SEM) (Quanta –200, FEI Ltd., Holland) according to method described by Bi et al. (2017) with some modifications. About 50 mg of the flour was sprinkled on double-sided sticky tape, coated with gold-palladium, and then tapped to release excess flour. The prepared sample was placed inside the specimen chamber on top of the specimen stage, then the cover plate was closed, and the acceleration voltage was set at 5 kV, and the micrographs of banana flour was obtained.

2.2.5.2 Cytochemical staining of starch and cellulose

About 20 mg of banana flour was mixed with distilled water in a small beaker. Clean pipette was used to collect some of the mixtures of the sample. One drop of suspension of the sample was placed on a clean thermo scientific microscope slides (75x25x1.0mm) coated with polysine (VWR). One drop of iodine (1.005 g/mL, Sigma-

Aldrich) was added to the slide and cover slips placed upon them. Excess solution was removed with tissue.

Another set of slides were labelled with 1 drop of Calcofluor white (0.25 (w/v) fluorescent Brightener 28, Fluka) followed by 1 drop of 10% NaOH. The two were mixed on the slide before placing the cover slip. All the slides were taken for observation under light and UV microscope (fluorescence microscope, Olympus BH-2).

2.2.6 Chemical composition

2.2.6.1 Proximate composition

Moisture, fat, and ash contents of raw samples were determined following the AOAC methods with codes 925.45, 920.39 and 923.03, respectively and protein with inductively coupled plasma optical emission spectroscopy (ICP-OES), where N was analysed and then converted to protein by multiplying with 6.25.

2.2.6.1.1 Carbohydrate determination and digestion

2.2.6.1.2 Sugar determination using DNS assay

The detection of sugar was performed by the DNS assay according to (Miller, 1959; Fei et al., 2014) with some modifications. Briefly, 5 g of DNS (Sigma-Aldrich) was dissolved in 250 mL of preheated (80°C) distilled water and cooled to room temperature. Then 100 mL of 2 N NaOH plus 150 g of potassium sodium tartrate-4-hydrate (Sigma-Aldrich) were added to the solution and mixed together using magnetic stirrer and volume made up to 500 mL with distilled water as DNS solution. Serial dilutions of 0.1- 2 mg/mL were made from a stock solution of glucose or maltose standard of concentration 2 mg/mL. One mL of DNS solution was added into separate test tubes containing 2 mL of sample and standards. The mixture was shaken using

vortex and placed in a boiling water bath (Grant SBB 14) for 15 min to facilitate reaction of the reducing sugar with DNS reagent. After cooling, 9 mL of distilled water was added to the solution in the tube and 200 µL of each solution was pipetted into a 96 well plate. Absorbance was read at 540 nm using a multimode plate reader (SPARK, TECAN). A blank was made by replacing the sample/standard with distilled water. Absorbance of blank was subtracted from the sample and standard readings. Standard curve was used to calculate reducing sugar values in the samples.

2.2.6.1.3 Free sugar determination

Free sugars were extracted from GBF by mixing 250 mg of GBF with 5 mL of water, vortexing for 5 min, and centrifuging for 10 min at 20^{o}C for 4000 rpm .The supernatant was analysed for reducing sugar using the DNS assay.

2.2.6.1.4 Starch determination

The RDS, SDS and RS were determined enzymatically using a commercial resistant starch assay kit (rapid format) from Megazyme (Bray, Ireland) according to the updated AOAC 2009 Method 2002.02 and AACC Method 32-40 by McCleary and Monaghan (2002) with some modifications. The solutions for the RDS and SDS were terminated by using 20.0 mL of 50 mM acetic acid while the solution for RS determination was terminated by using 4.0 mL of 95% v/v ethanol. The two enzymes used in the kit were pancreatic α-amylase (PAA) and amylo- glucosidase (AMG) as used by (Englyst et al., 1992) except that both enzymes have been purified, standardised, and stabilised. Digestion was performed using 0.4 units of PAA and 0.17 units of AMG with stirring at pH 6, 37 ^{o}C for 4 h, simulating *in vivo* conditions in the human small intestine. 100 mg of sample was added to 3.5 mL of 50 mM sodium maleate buffer at pH 6 in a tube and mixed by vortexing for 5 secs at room temperature. The tube placed in a shaking water bath (200 strokes/min) set at 37 °C

for 5 min to equilibrate, and 0.4 units of PAA and 0.17 units of AMG were added. The digestion mixture was incubated for 20 min, 120 min and 240 min to measure RDS, SDS and RS. The reaction was terminated by adding 20 mL of 50 mM acetic acid, mixed vigorously by vortexing, centrifuging at 4,000 rpm for 10 min for RDS and SDS. The supernatants collected contained the soluble products of digestion at different times. Then, 0.33 U of AMG was added to each of the supernatant and incubated at 50 ^{o}C for 30 min to hydrolyse soluble digestion products to glucose**.**

The reducing sugar in the supernatants was analysed using DNS colorimetric assay. The pellet from starch digestion containing non-digested RS was mixed with 2 mL of 1.7 NaOH and stirred continuously for 20 min in ice water bath placed over a magnetic stirrer. Then, 8 mL of 1.0 M sodium acetate buffer (pH 3.8) was added, followed by 0.33 U of AMG. The mixture was incubated at 50 °C for 30 min with intermittent shaking. The digestion mixture was transferred into a 100 mL flask and topped up with water, then centrifuged as previously described. The supernatant was analysed for reducing sugar content using the DNS assay.

The following calculations were performed:

- Total Starch = TS ($DS_{240\ min}$ – FS + RS)
- Available carbohydrate = $DS_{240\ min}$ + FS
- Total carbohydrate = Total starch + FS Where $Ds_{20\ =}$ RDS, $Ds_{120\ =}$ SDS and starch that did not digest after 240 min of digestion is RS).

2.2.6.1.4 Total fibre

Total fibre was determined by weighing out the residue from the resistant starch determination method. The residue was filtered through an A4 filter paper (Whatman). The filter paper was dried, cooled in a desiccator and weighed. Fibre was calculated

by subtracting the weight of the filter paper after drying the residue. The difference in weight is the amount of the total fibre. The fibre value did not include RS.

2.2.7 Statistical analysis

All digestions and analyses were done in triplicate, except morphological results. Data are means with standard deviations. A statistical package (SPSS version 12.0 for windows) was used for data analysis including one-way analysis of variance (ANOVA) at 95% confidence levels.

2.3 Results

2.3.1 Microbial analysis of RTE porridge

The microbial quality results of RTE porridge are shown in Table 2.1. The total bacteria count of the fresh RTE porridge was 22 ± 0.4 (cfu/ml) for sample packed with glass and stored for 90 days at +4 ^{o}C : 65% RH and 818 ± 28.2 (cfu/ml) for sample packed with paper and stored for 90 days at +4 ^{o}C: 65% RH.

The coliform count results ranged from 18 ± 0.6 (cfu/ml) for sample stored in glass at +4 ^{o}C: 65% R.H for 60 days to 200 ± 0.5 (cfu/ml) for sample stored in paper at 30 ^{o}C: 20% R.H for 90 days.

Microbial loads increased significantly with temperature and storage duration, likely due to presence of moisture in the samples, which encourage microbial growth. The moisture content of RTE banana porridge was 3.4% before storage and increased to 4.8%, 5% and 14.2% when measured at 30, 60 and 90 days, respectively. Paper has the highest permeability to air moisture, and shows the highest microbial counts. This result is similar to those of Bharani et al., (2016) where microbial load of GBF increased upon storage as storage length increased. Also environmental conditions (temperature and RH) had an effect on the microbial load of this experiment. Storage

of GBF at a temperature of + 4 ^{o}C with relative humidity of 65% gave the least microbial results while storing at a temperature of + 30 ^{o}C with relative humidity of 20% gave the highest microbial results. The higher temperature of 30 ^{o}C is closer to the optimum temperature of the mesophilic microorganisms. Hemery et al. (2020) found similar results on the influence of storage conditions and packaging of fortified wheat flour on microbial load. According to the International Commission for Microbiological Specification for Food (ICMSF, 1996) total plate count for RTE foods should be between 0 – 1000 (cfu/ml) to be acceptable for human consumption, while 10,000 – 100,000 (cfu/ml) is a tolerable level, but anything above 1,000,000 (cfu/ml) is not acceptable. Based on this, all RTE porridges were within the acceptable limit for human consumption, even those stored at the highest temperatures (30 ^{o}C; 20% R.H).

In addition, gram-negative bacteria, which includes coliforms, should not be more than 10,000 cfu/g in RTE foods according to ICMSF (1996) coliform counts ranging between 100 and 1,000 cfu/g is considered to be at the borderline whiles values less 100 cfu/g are satisfactory (www.foodstandards.gov.au).

Based on the above, all the stored and analysed RTE banana porridges are within the acceptable limit. The results showed that RTE banana porridges had no microbial contamination under any conditions up to 30 days of storage. The contamination of gram positive and gram negative bacteria could be attributed to opening of the packaging materials during collection of samples for microbial test and storing for another 30 days. It could also result from permeability of moist air into the packaging materials, which could promote microbial growth. The opening of the packaging material could be stopped by packaging in individual small packs that could be used at once and again, the adopted approach is more representative of the handling by the consumers

Most importantly, *E.coli* was not found in any of the samples of RTE banana porridge tested by colour confirmation with Eosin agar. This agar forms dark purple colour colonies but if *E. coli* is present, it will give a bright purple colour. According to Gilbert et al. (2000), the presence of coliforms in food no matter how small, is an indication of contamination because of poor hygienic practices. The zero contamination from 0 day to 30 days has shown that RTE banana porridge was produced in hygienic conditions, but that opening of the package at 30 days could have introduced contamination. Future experiments should include more samples that should be stored for longer periods without opening at 30 days. During the analysis, it was observed that the moisture content of the GBF was 6.31 g and when it was process into RTE porridge the moisture content was 3.48 g and when stored for 30, 60 and 90 days the moisture content increased as the day progress. The first 30 days, the moisture content rose from 3.48 g to 4.89 g, by 60 days, the moisture content increased from 4.89 g to 4.95 g and by 90 days, the moisture content then rose from 4.95 g to 14.02 g. These results indicate that the higher the number of days, the more the samples absorbs moisture, irrespective of the packaging materials. According to past research, it will be assumed that paper would be more permeable to air than glass and polythene. While polythene would be more permeable to air than glass. The results generated showed that it was only at 90 days that the moisture content of the RTE porridge could be above 13%.

Table 2.1:Total plate and coliform count of RTE banana porridge stored for up to 90 days in three types of packaging materials at three environmental conditions

	Storage time (days)	Plate count (cfu/ml)			Coliform count (cfu/ml)		
		Glass	Paper	Polythene	Glass	Paper	Polythene
	0 day	0	0	0	0	0	0
+4^{o}C : 65% R.H	30 days	0	0	0	0	0	0
	60 days	22±0..4^{a}	45 ±1.5^{a}	27±0.5^{a}	18 ±0.6^{a}	33±0.5^{a}	30±0.5^{a}
	90 days	29±0.8^{b}	61±3.8^{b}	36±0.8^{b}	39 ±1.2^{b}	69±1.4^{b}	57±1.1^{b}
	0 day	0	0	0	0	0	0
+18^{o}C : 58% R.H	30 days	0	0	0	0	0	0
	60 days	190±5.8^{a}	215±10.4^{a}	210±10.1^{a}	106±3.4^{a}	116 ±5.6^{a}	110 ±5.6^{a}
	90 days	390 ±19.4^{b}	610 ±29.4^{b}	460±19.2^{b}	150 ±7.3^{b}	180 ±15.2^{b}	160±8.3^{b}
	0 day	0	0	0	0	0	0
+ 30^{o}C : 20% R.H	30 days	0	0	0	0	0	0
	60 days	110±2.9^{a}	200 ±15.6^{a}	172 ±6.8^{a}	45±1^{a}	55 ±2.5^{a}	50 ±6.6^{a}
	90 days	200 ±8.1^{a}	818 ±28.2^{a}	396±19.3^{a}	130±0.8^{b}	200±0.5^{b}	190 ±1^{-2}

Data are mean with SD of n =3.Different letters within a column indicate significant differences ($p<0.05$) determined following one-way ANOVA. CFU = Colony forming units. R.H = Relative humidity. ICMSF (1996) thresholds for bacterial count in ready to eat foods: acceptable range = (0 – 1000), tolerable range = (10, 000 – 100, 000), not acceptable is from 10^6 and above

2.3.2 Morphology assessment

2.3.2.1 Scanning electron microscopy of green banana flour

Scanning electron microscopy images were used to gather information on the size and shape of starch granules, their surface integrity and the presence of fibre or other structural features (Kumar et al., 2019). The results showed the existence smooth intact starch granules with irregular shapes mainly elongated and oval with varying sizes in raw banana fruit and GBF (Figure 2.3). This observation is in accordance with (Kayisu et al., 1981). Sizes of the elongated starch granules were approximately 19–61 µm in length and 7 – 25 µm in width for raw green banana fruit (A), experimental raw banana flour (C) and green banana flour purchased from the market (D). The smooth starch granular surface suggest that there was some materials covering the granule (such as protein and fibre) as in the case of chickpea and pea flour (Aguilera et al., 2009). There is presence of broken cell wall, visible in (F) resulting from milling and could result in good accessibility of the enzymes to the starch granules. Image E shows the dried milled flour.

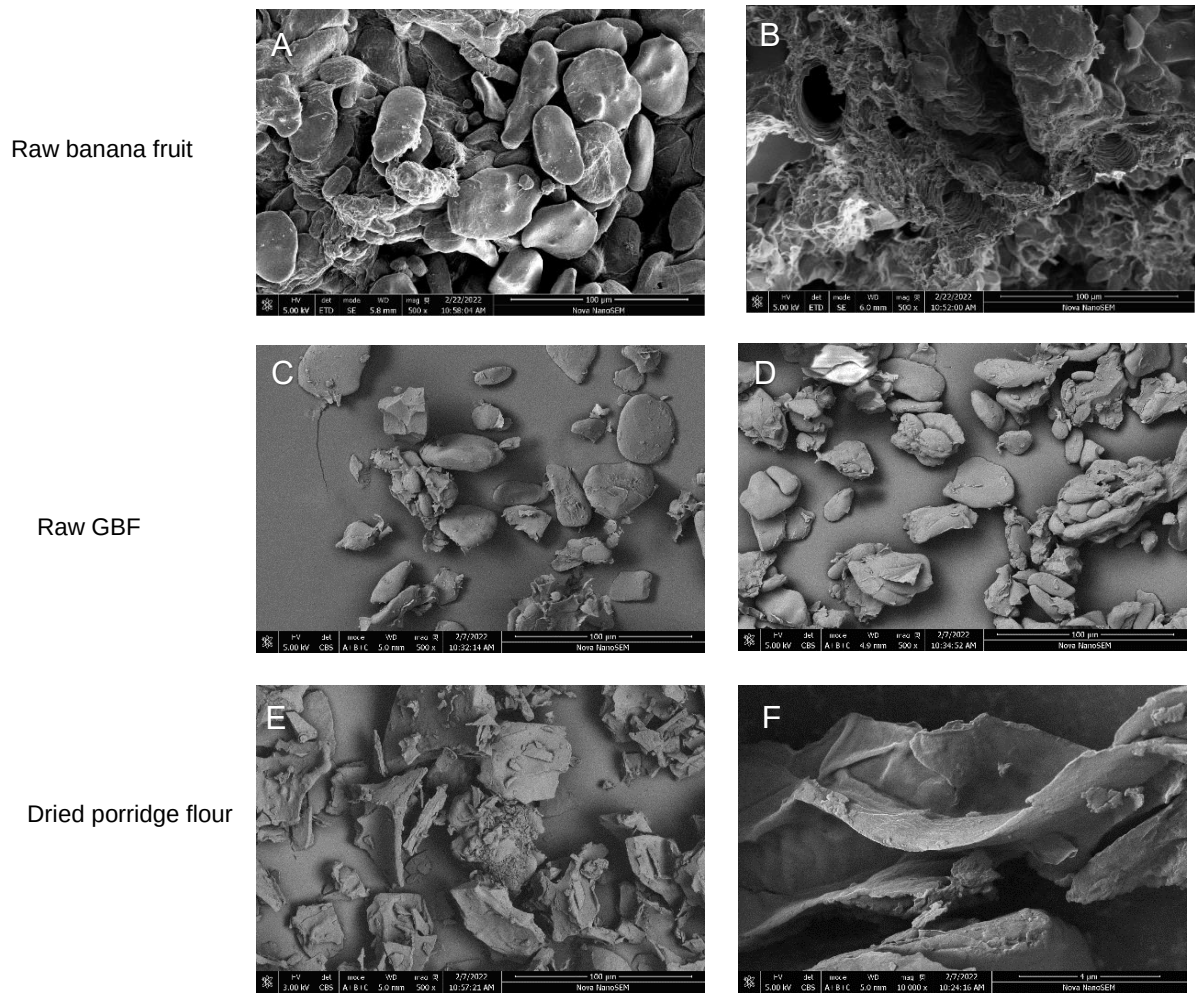

Figure 2.3: Scanning electron microscopy images of raw green banana fruit (A and B), raw GBF produced (C), raw GBF purchased from market (D), RTE dried porridge (E), and (F) is a close up on a broken cell wall found in GBF. Micrographs shows the presence of intact smooth starch granules for raw green banana fruit (A) and raw flours (C and D). E shows dried flour. Scale bar (A-E) = 100µm; F = 4µm.

2.3.2.1 Light and UV microscopy of banana ripening at stage 4

According to Rongkaumpan et al. (2019), ripe banana tissue showed elongated and intact cells even at the two apical ends and contained several starch granules when stained with iodine. The intactness of the cell was confirmed when green banana was stained with calcofluor white. These findings were in agreement with the microscopic

observation of fresh banana fruit that revealed the presence of elongated cells containing abundant starch granules stained with iodine (Figure 2.4 4). According to Vinoy et al. (2016), cell walls could prevent the starch granules from being digestible by the amylase digestive enzymes by protecting the starch granules and forming a barrier to the enzyme penetration.

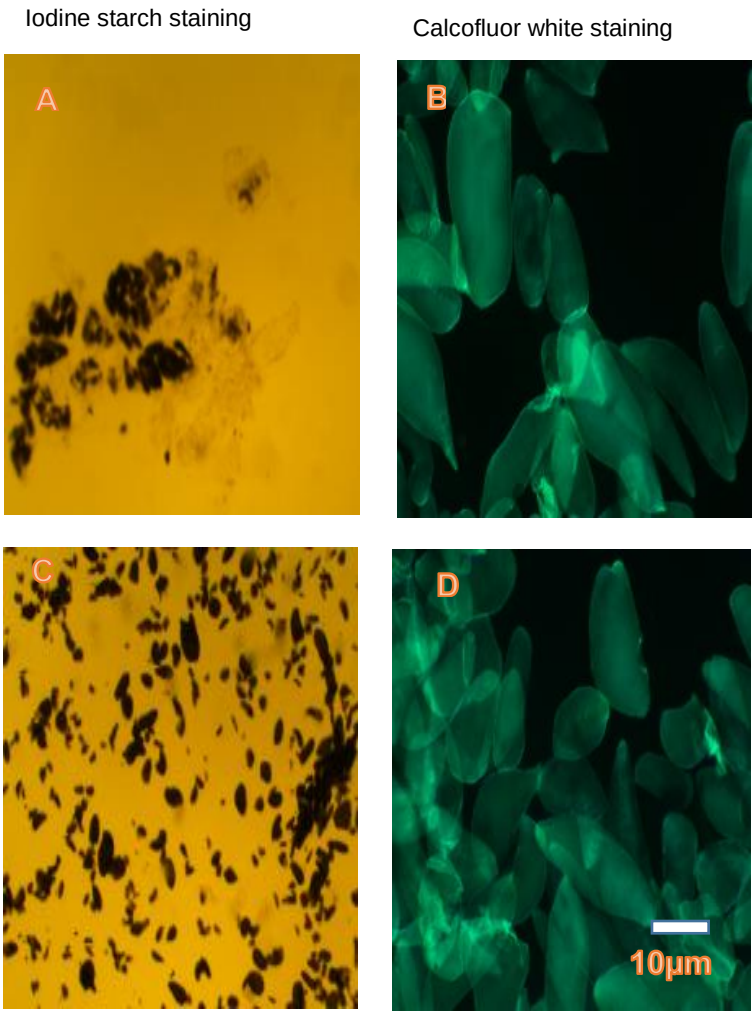

Figure 2.4: UV and light microscopy images of freshly crushed banana fruit at stage 4 of banana ripening. Cellular contents stained with iodine (A and C) and calcofluor white (B and D). Scale bar=10µm**.**

Microscopic observations revealed that milled raw GBF contains intact starch granules stained with iodine, most of which are free (Figure 5). Some maybe surrounded by cell

wall material (stained with calcofluor white). Intact granules are typical of RS1, while starch granules surrounded by cell wall material characterises RS2. Upon cooking, it can be observed that the starch granules have gelatinised. It is hypothesised that the cooked porridge forms a starch – cell polymer gel during cooking (Figure 5C and 5D) with some remaining intact starch granules. This could be proved experimentally by the use of use of Differential Scanning Calorimetry (DSC), which measures the heat absorbed by the starch granule as a function of temperature. Thermal properties of starch-based systems provide information about the gelatinization process, The analysis of the thermal properties of starch is based on the heat absorption or loss that occurs as a result of phase changes (such as melting or crystallization) (Wang et al., 2015). By gelatinising, alpha amylases could have a quicker access to the starch molecules compared to raw. This will improve digestibility of starch.

Upon drying, the starch appears to shrink in size with the appearance on angular structures (possibly clustered, dried starch). It is expected that retrogradation occurred during this processing step, which may affect digestibility of starch. Reconstitution did not restore the fully swollen appearance of the starch granules. However, retrogradation of starch is not fully reversed which initially involves rapid recrystallization of amylose molecules and the long-term development of gel structure and crystallinity of processed starch are involved in the staling of bread and cakes, are considered to be due to retrogradation of amylopectin (Fadda et al., 2014)

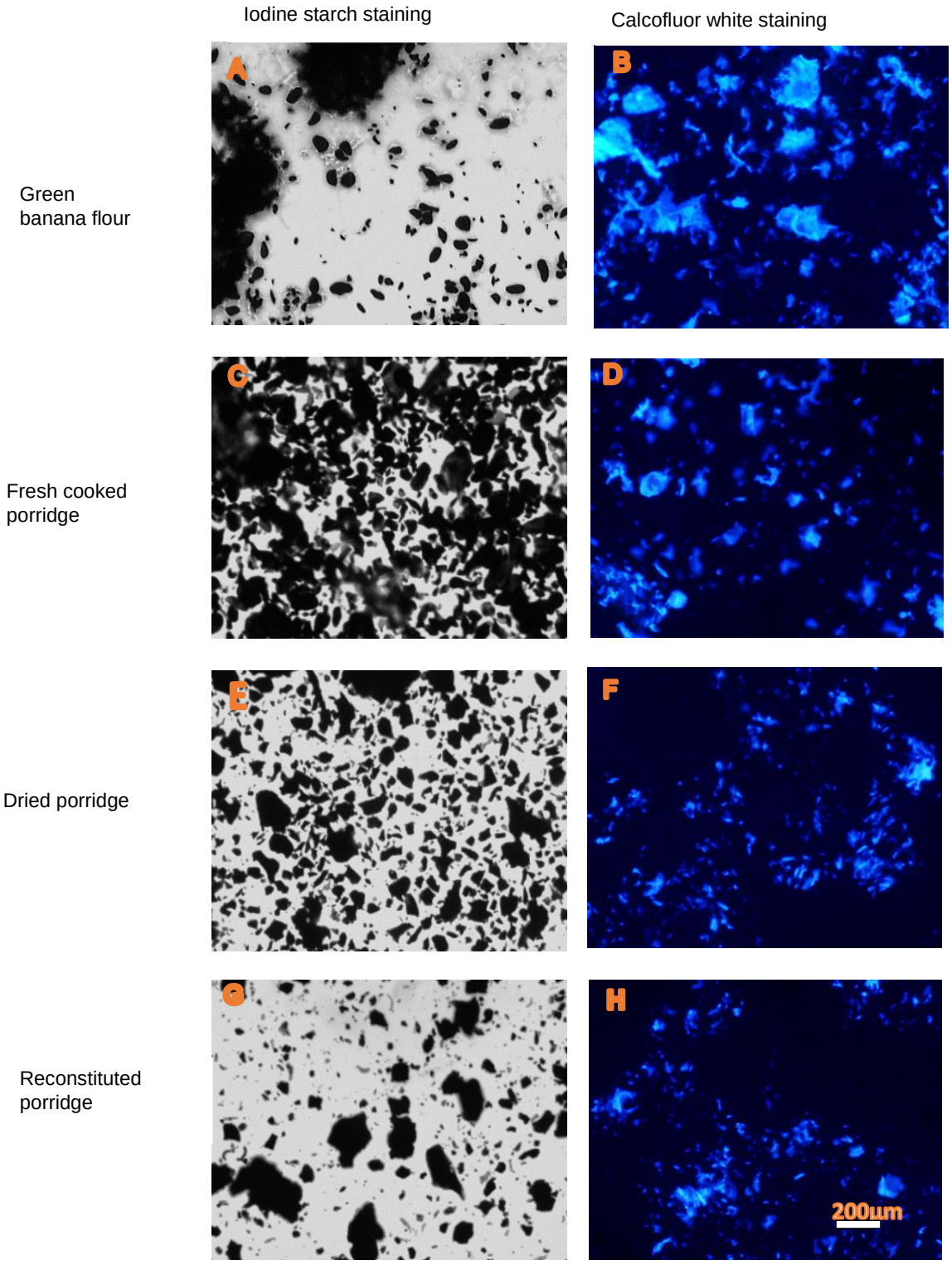

Figure 2.5: Light and UV microscopy images of four different products produced during the developmental stages of RTE banana porridge. Cellular contents were stained with iodine (A, C, E and G) showing starch in all, where shows intact starch granules, B swollen granules with gel formation, E with shrunk granules. Cell walls were stained with calcofluor white (B, D, F and (H). Magnification is X 4. Scale bar = 200µL

2.3.3 Proximate composition

The proximate composition of the experimental GBF is shown in Table 2.2. Moisture content of the raw flour was found to be 6.31%. According to Oi et al. (2013), any flour used for industrial purposes must have its moisture content below 13%. This value is the lowest surviving limit for microorganisms. Potter and Hotchkiss (1995) said that all processed, milled products that are less than 13% moisture content will be stable during storage and will be free from moisture deterioration. This could imply that GBF produced in this study could be stable during storage, although it will depend on storage conditions. Ash content was found to be 3.13 g/100 g, mineral content results include iron, phosphorus, potassium, magnesium, manganese, and zinc (0.69, 85.9, 1040.05, 99.4, 1.73, 0.57 mg/100 g respectively).

The protein content of GBF was found to be 4.23 g/100 g, which is higher than the value of 3.60 g/100 g obtained in previous research (Menezes et al., 2011). The method used by Menezes et al. (2011) is similar to the method used in this work. The simillarity is that both methods require oven drying at 50 ^{o}C for 8 h. The difference could be as a result of different varieties of green banana fruits and different methods of carbohydrate calculations. The protein content was lower when compared to flours from cereals. For example, flour from different varieties of rice showed protein content ranging from 7.5 g/100 – 9.16 g/100 g (Muhammad et al., 2012). The protein content of maize flour from normal flour and from high protein maize flour from IITA was found to be 8.96 g/ 100 g and 11.76 g/100 g, respectively (Edema et al., 2005). These results showed that GBF has the least protein content when compared with rice and maize flours. This implies that it may need fortification with another source of protein to satisfy nutritional needs of children and adults.

The fat content of GBF is 0.007 g/100 g. This shows that green banana fruit has a low fat content. This low fat content could help in preventing or lowering rancidity that might occur during storage of the flour.

Total carbohydrate content of the flour was 69.15 g/100 g. This value is lower than the results (80.20 – 86.45) % found in flours made from different varieties of green banana (Daramola and Osanyinlusi, 2006), which were calculated by difference. If carbohydrate content was calculated for the GBF prepared in this research, total carbohydrate content would be 85.61 g/100 g. This indicates that carbohydrate calculated by difference may over-estimate carbohydrate content.

In this work, the updated method of McCleary et al. (2020) (AOAC Method 2002.02. AACC Method 32-40.01) which employs incubation conditions of 4 hours and pancreatic amylase closely simulate human digestion, was used.

Free sugar content was low at 1.84 g/100 g, an indication of stage of maturity of the fruit at the time of processing (do Nascimento et al., 2006). Total starch (TS) was 67.28 g/100 g, lower than the TS value of 73.4 g /100 g reported by Juarez-Garcia et al. (2006). TS value from the current study is in line with the TS reported by (Zhang et al.(2005b) which ranges from 60-80 g/100 g, and similar to those suggested by Megazyme (RS and DS of 46.9 g/100 g and 16.6 g/100 g respectively), and Menezes et al. (2011) (RS of 48.99 g/100 g). The variation could be to variability in the raw material or processing methods.

GBF has available carbohydrate of 14.00 g/100 g and total carbohydrate of 69.15 g/100 g. The dietary fibre (DF) content is 14.50 g/100 g. The DF of the GBF is almost the same as the value of 14.52 g/100 g found in a previous study (Juarez-Garcia et al., 2006). When different varieties of banana flours were determined for fibre, the

results ranged between 6.28 g/100– 15.5 g/100 g (da Mota et al., 2000). Insoluble fibre could be the main fibre found in dietary fibre of GBF and how much it contains could vary in different varieties of GBF. The Insoluble dietary fibre could be influenced by ripeness and the analytical techniques used for analysis.

Table 2.2: Proximate composition of green banana flour (GBF).

Compound	Amount ±SD (g/100 g)
Moisture	6.31± 0.13
Protein	4.23 ± 0.05
Ash	3.13 ± 0.06
Fat	0.007± 0.01
Total carbohydrate	69.15± 0.05
Free sugar	1.84±0.1
Total starch	67.28 ± 6.4
Digestible starch	12.11 ± 1.45
Resistant starch (RS)	55.11 ± 5.41
Dietary Fibre (DF)	14.50 ± 0.8

Fibre excludes resistant starch. Data expressed as mean in g/100 g with standard deviation (SD, n=3).

2.3.4 Effect of processing of GBF into RTE porridge on carbohydrate digestibility

The carbohydrate content of the samples were analysed in raw GBF, freshly cooked porridge, dried RTE porridge and reconstituted porridge (Table 2.3). Results showed significant difference in total starch, DS, RS, sugar, available carbohydrate, total carbohydrate, and fibre ($p< 0.05$). The raw GBF had low DS at 20 min digestion (RDS = 5.8 g/100 g), 120 min (SDS = 7.03 g/100 g) and at 240 min (12.11 g/100 g,) with RS content of 55.11 g/100 g. The digestibility during the raw stage could be due to inaccessibility of the starch owing to its crystalline state and to a lesser extent, the entrapment of starch in cell wall material. Furthermore, small size of starch granules are more easily digestible than large starch granules due to larger contact surface

area. As GBF has big starch granules, this again could be a contributing factor for low digestibility. During cooking into porridge for 5 minutes, the digestibility value of the samples digested for 20 min,120 min and 240 min were 11.59 g/100 g; 51.10 g/100 g and 62.69 g/100 g; respectively. The resistant starch decreased from 55.11 g/100 g to 16.06 g/100 g.

The significant difference in TS during cooking is surprising, with a slight increased values measured in fresh sample compared to the others. This could be due to the analytical methods, which rely on enzymes to digest the starch. Other methods such as acid hydrolysis could be more effective at totally hydrolysing starch, which should have made the TS not to be different from other stages of processing. The method used also reflected in the higher fibre content in RTE porridge, which may not have occurred if acid hydrolysis was used.

The RTE porridge showed a decrease in DS (34.57 g/100 g) for 240 min and increase in RS (31.07 g/100 g) compared to fresh porridge. This could be due to the occurrence of retrogradation, which is re-ordering of the molecular structure of the starch (Matignon and Tecante, 2017). This type of resistant starch is known as RS3. Following reconstitution, the DS is increased from 34.57 g/100 g to 50.07 g/100 g and RS decreased from 31.07 to 17.6 g/100 g. These values are higher than that of the dried product, indicating that retrogradation can be partly reverted by reconstituting at 85^{o}C. The DS for reconstitution at 240 min (50.07 g/100 g) is lower than DS for fresh porridge at 240 min (62.69 g/100 g). The overall results showed clearly, how processing affects starch digestibility.

The free sugar ranges from 1.84 g/100 g in GBF to 7.03 g/100 g in reconstituted samples. This could be due to degradation of starch to free sugar during cooking,

and/or the presence of endogenous enzymes in GBF, which are active during cooking (especially at early stages).

Table 2.3: The effect of processing stages on carbohydrate content of green banana flour (GBF) and porridge at various stages of processing (g/100 g dry weight).

	DS_{20}	DS_{120}	DS_{240}	RS	TS	Free Sugar	AV.CHO	TOT.CHO	Fibre
Raw GBF	5.8±0.1.0^{d}	7.03±0.03^{d}	12.11±1.4^{d}	55.11±5.4^{a}	67.22±6.4^{b}	1.84±0.1^{d}	14.00±1.4^{d}	69.06±6.4^{a}	14.50±0.8^{a}
Freshly cooked porridge	11.59±0.37^{a}	51.10±0.0.6^{a}	62.69±2.6^{a}	16.06±5.2^{d}	78.75±3.4^{a}	1.99±0.3^{c}	64.68±2.6^{a}	80.74±3.4^{a}	15.80±0.7^{a}
Dried porridge	8.07±1.5^{c}	26.5± 0.15^{c}	34.57±1.1^{c}	31.07±1.6^{b}	65.64±1.1^{b}	7.03±0.3^{a}	41.6±1.1^{c}	72.67±1.1^{a}	17.27±0.9^{b}
Reconstituted porridge	10.02±1.2^{b}	40.05±1.0^{b}	50.07±1.8^{b}	17.60±1.2^{c}	67.67±1.8^{b}	6.77±0.2^{b}	56.84±1.8^{b}	74.44±2.7^{a}	14.90±0.4^{a}

Data are mean with SD of n= 9. Different letters within a column indicate significant differences ($p<0.05$), determined by Tukey test following one-way ANOVA. DS (20 – 240) min. = Digestible starch at 20 min, 120 min. and 240 min. RS = Resistant starch. TS = Total starch. AV.CHO = Available carbohydrate (DS + Sugar). TOT.CHO = Total carbohydrate (TS + Sugar). DS = Digestible Starch.

The effect of cooking time on DS and RS of fresh porridge was also analysed (Figure 6). Results were calculated as percentage of TS. Starch digestion increased rapidly at 5 minutes cooking time, increasing DS from 18% to 65.9% with a concomitant decrease in RS from 81% - 34.1% significantly ($p< 0.05$). Progressively as cooking time increases, the digestibility increases up to 10 minutes with 81.1% DS and 17.7% RS. During the period of cooking from 7.5 minutes to 15 minutes there were not significant ($P < 0.05$) changes in starch digestibility.

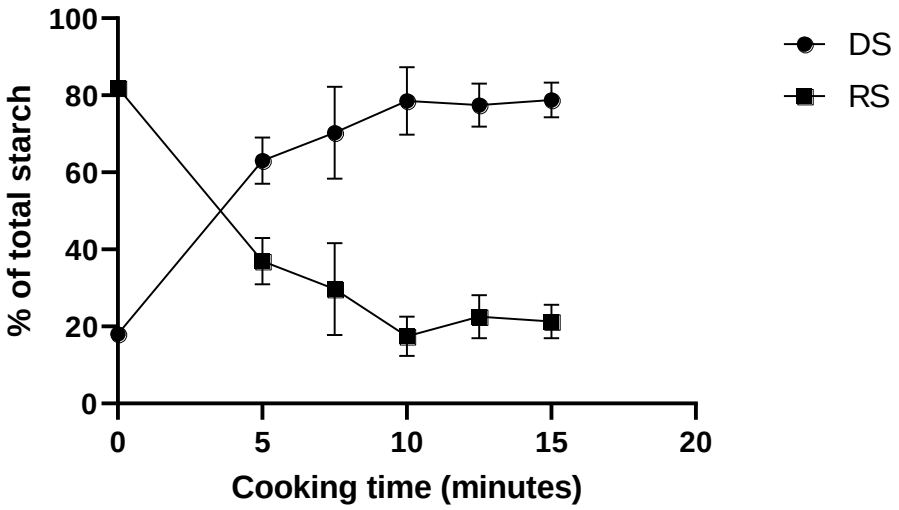

Figure 2.6: Effect of cooking time on digestibility of unripe banana flour. Data are mean with SD of n = 6. Digestible starch and resistant starch expressed as percentage of (TS) at every time point as measured by DNS. Digestible starch = DS. Resistant starch = RS

The effect of two temperatures of reconstitution at 85^{o}C and 100^{o}C for 5 minutes was also evaluated (Figure 2.7).There was a level of significant difference ($p\leq 0.05$) reduction in the digestibility of -30% and -25% of RTEs reconstituted in water at 85 ^{o}C and 100 ^{o}C, respectively compared to fresh porridge ($p<0.05$). However, the difference between the two temperatures was not statistically significant.

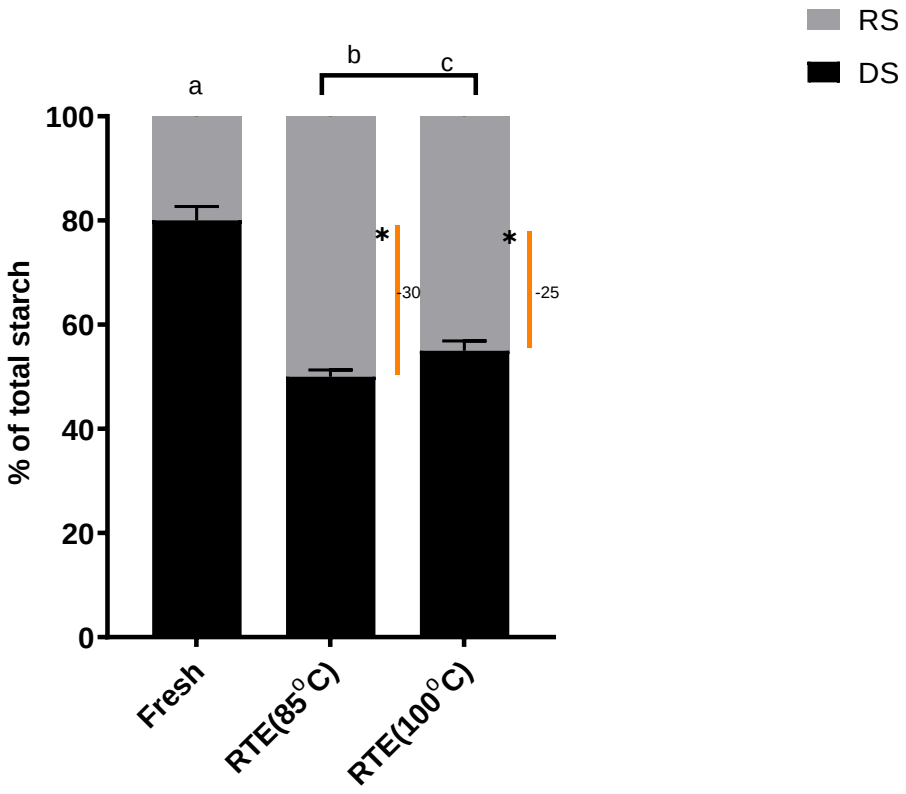

Figure 2.7: Effect of reconstitution at 85 ºC and 100 ºC for 5mins on starch digestibility.

2.4 Discussion

RTE banana porridge has been developed using simple processing methods without chemical additives. Firstly, GBF was produced and shown to have low moisture and good microbial safety characteristics. This is in line with Khoozani et al. (2019) who investigated the effects of different drying conditions to make GBF and found that no matter the type of drying method used for green banana flour production, a low moisture content can be achieved. The low moisture content is an indication of good stability during shelf life. The microbial load of GBF and RTE porridges were low during storage, which indicates that it could be a good product for further development and commercialisation. Other microbial tests need to be done, including challenge tests, and a wider set of microbes analysed. Furthermore, from the appearance of the products, slight deterioration in terms of colour occurred. This indicates that oxidative enzymes such as

polyphenol oxidase were not active in GBF. More analyses of the organoleptic properties, including sensory trials, are needed to understand its stability and acceptance during processing and storage.

Regarding its nutritional properties, GBF has low free sugar and high RS content appear to be desirable from a glycemic point of view (Brand-Miller et al., 2009). RS ends up in the large intestine where it could bring potential health benefits to colonic health due to fermentation in the large intestine by gut microflora resulting in production of short chain fatty acids, mainly butyrate. Butyrate is the main energy source for colonocytes (Wong et al., 2006). Epidemiological and experimental evidence suggests that non-digestible carbohydrates (NDC) including RS are protective against colorectal cancer. Butyrate can bind to G-protein to regulate inflammation and other cancer related processes (Malcomson et al., 2015). However, GBF is unlikely to be eaten in its raw state.

Wang and Copeland (2013) found that starch changes during gelatinization and retrogradation are very important because they are key determinants to their functional properties for food processing, during digestion and industrial application. These properties determine the acceptability of the product, nutritional quality of the food and shelf life.

Cooking GBF to porridge massively increased the digestibility to nearly 62% with high levels of SDS. This can be explained by the rapid gelatinisation of free starch granules found in the GBF. This proposal could be confirmed by conducting human studies where healthy human beings will be fed with cooked RTE porridge and their blood sample will be collected to find the effect of the cooked RTE porridge after consumption on their blood glucose level. Rapid Visco Analyzer, amylograph or Viscograph could be used to analyse changes in viscosity as a

result of granule swelling and the solubilisation (leaching) during gelatinization process (Schirmer et al., 2015). At 10 minutes of cooking, GBF appeared to be fully cooked and the starch paste became clear. This rapid cooking can be beneficial in some circumstances where time or energy availability is low. In addition, the fast digestion can be beneficial to individuals who need energy quickly. The 20% RS and 14.5% fibre contents are still relatively high compared to some refined carbohydrate products, and likely to still bring benefits to gut health. It is expected that processing GBF into porridge will increase digestibility.

The low protein content of GBF is of concern. The protein content of the developed RTE banana porridge is low 4.88 g/100 g, hence the need for enrichment or fortification to increase the protein content.

2.5 Conclusions and future work

Development of a safe RTE porridge from green bananas using simple processing techniques has been achieved. The product appeared to be safe from a bacterial point of view even after 3 months of storage. Further microbial tests, such as a challenge test, is required to verify the safety of the product. Furthermore, as GBF is low in sugar and could be quite astringent, the organoleptic and sensorial properties of the product need to be tested, both instrumental techniques to complement the sensory studies(rheology, DSC, flavour using gas and Liquid Chromatography with Mass Spectrometry) and using human sensory trials (Schirmer et al., 2015. A sensory trial was planned originally, but had to be abandoned due to covid19 restrictions on human interactions.

This chapter clearly illustrates that the digestibility of starch is affected significantly by processing. The carbohydrate digestibility was measured using a

commercial kit which reflects gastrointestinal conditions (e.g. 4 hours of digestion with pancreatic amylase). Other in vitro methods, such as the infogest simulated digestion method could be used as more realistic methods of digestion (Minekus et al., 2014). Furthermore, *in vivo* methods that measure carbohydrate availability (e.g. glycemic response) in combination with measure of hormones such as insulin could corroborate the in vitro results. Hafiz et al. (2022) worked on the effect of processing type (whole, puree, pasta, and mashed potato as control) on postprandial glycemic response in chickpea intake in vivo. They found that Chickpeas are among the lowest glycaemic index carbohydrate foods causing prolong digestion and enhanced satiety responses not minding the processing methods used. Experiments which measure the microbiome response to the product would also be interesting to further understand the role of RS in health pathways.

The protein content of the GBF was found to be low, and the RTE porridge is unlikely to satisfy even 10% of the protein requirements of a child. This could be a risk if the product is consumed by children or other groups in need of protein. In the next chapter, experiments were carried out to enrich the RTE porridge with commercial available protein sources and to understand the impact of processing on protein digestibility.

Chapter 3

Fortification of ready to eat (RTE) green banana porridge with five different plant-based protein sources

Highlights

- A ready to eat (RTE) green banana porridge has been fortified with five selected plant-based ingredients (moringa *oleifera* leaf powder, hemp protein isolate powder, soya protein isolate powder, tomato fibre powder and pea protein isolate powder).
- The increased protein content aimed to meet around 25% of the protein requirements for children and adults based on standard portion sizes of 30 g and 100 g (dw), respectively.
- The in vitro Protein Digestibility Corrected Amino Acid Score *(in vitro* PDCAAS) values for the ingredients (moringa *oleifera* leaf powder, hemp protein isolate powder, soya protein isolate powder, tomato fibre powder and pea protein isolate powder) were 0.38, 0.52, 0.55, 0.01, and 0.48, respectively, while RTE banana porridge and casein (control) have 0.004 and 1, respectively. The amino acid score values for moringa *oleifera* leaf powder, hemp protein isolate powder, soya protein isolate powder, tomato fibre powder and pea protein isolate powder were 0.828, 0.64, 1, 0.024 and 0.76, respectively, RTE banana had 0.039 and casein protein powder (control) had1.

- Carbohydrate digestion was not significantly affected, apart from the addition of tomato fibre powder, which reduced starch digestibility by 50%.
- Overall, soy protein powder was found to be the most promising fortificant ingredient for green banana porridge, based on colour and protein content and quality. In addition, fortification with moringa *oleifera* increased the iron content of RTE banana porridge by 8%.

3.1 Introduction

Results from the previous chapter have shown that a highly digestible and safe RTE banana porridge can be developed from GBF. It is however known that GBF is a poor source of protein (4.23 g/100 g) with low biological value (Ndife et al., 2011).

One way of addressing the low content and biological value of protein in GBF is by fortifying RTE porridge with external protein sources like commercially available plant-based ingredients. Fortification is defined as the process of adding one or more required components not already in the food or only present in the food in small quantities and needed to increase the nutritional value of the developed food product (Lorusso et al., 2017). Fortification of RTE banana porridge with external protein sources could improve not only the protein content but also the content of other micronutrients like Fe, K, Mg, Mn, P, and Zn (Mehlomakulu and Emmambux, 2020).

The recommended daily allowance (RDA) of a child 1-3 years, adult female and adult male are 18, 45 and 56 gram of protein per kilogram of body weight per day,

respectively. These values represent intakes that satisfy the needs of 95% of the population.

Studies have shown that fortification of fresh porridges with commercially available vegetable proteins increase the nutritional content of the porridges. Alamu et al. (2016) worked on producing porridge using maize meal, fortified with soy flour and local groundnut paste individually and compared it with the powdered milk fortified porridge. They found that soy-fortified porridges provide comparable ash, crude fibre, and fat contents to powdered milk fortified porridge but with higher protein than powdered milk-fortified porridge. Soy flour raised the protein and ash content of the porridge by 90% and 63% respectively, the groundnut paste raised the protein and ash content by 88% and 41% and the powdered milk by 87% and 65% respectively.

Another study showed the effect of varying proportions (0, 0.5, 1.0, 1.5, 2.0 and 2.5%) of *moringa oleifera* leaf powder on the physicochemical and nutritional properties of stiff dough “amala” prepared from plantain (*musa paradisca*) flour. They found that addition of the moringa powder to the plantain flour significantly increased protein content from 3.5 to 10.3%, ash and fat content from 1.7 – 2.9 and 1.8 to 2.3% respectively. Also Ca, Mg, K , Na and Fe contents of amala increased with the addition of *moringa oleifera* powder. Again, water absorption capacity, bulk density, swelling power and pasting properties of the fortified plantain flour decreases with increasing concentration of *Moringa oleifera* leaf powder (Karim et al., 2015). Adunni and Olaposi (2010), fortified banana flour with partially defatted soy flour by substitution method, increasing the protein content by a range of 6.9 to 11.5%. Teterycz et al. (2021) and Terra et al. (1983) worked on the effect of hemp *(Cannabis sativa L*.) seed powder addition on the physicochemical properties, cooking quality, texture parameters and sensory

properties of durum wheat pasta. Samples of wheat flour were fortified with 5 – 40% of commercially available hemp powder or 2.5 – 10% of hemp cake (hemp cake made by pressing oil out from hemp seed). The addition/fortification with hemp powder or hemp cake resulted in an increase in protein, total dietary fibre, and ash in the fortified pasta samples. Pasta fortified with 30 – 40% hemp protein powder contained 19.5 – 20.8% protein (dry weight basis) and 17 – 21.4% of total dietary fibre and compared well with the control (wheat) which contained 14.6% protein and 4.4% total dietary fibre.

In addition to the quantity of protein, the protein quality should be considered. According to (Hughes et al., 2011), protein quality is the ability of a protein to provide adequate amounts of essential amino acids for human requirements. In 1991, Protein Digestibility-Corrected Amino Acid Score (PDCAAS) was established but was developed in 1989 by a Joint FAO/WHO Expert Consultation on Protein Quality Evaluation (FAO/WHO, 1991) to compare the essential amino acid content of a test protein in mg/g protein to a reference protein in mg/g protein for a given age group. This is in other to generate a ratio known as the amino acid score or the chemical score in which the essential amino acid with the lowest ratio is called the most limiting amino acid (Hertzler et al., 2020). In 1991, the PDCAAS was adopted by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) (Schaafsma, 2000) as a method that can be used to measure protein quality of a food. Therefore, protein quality is based on the profiles of essential amino acids profile and their protein digestibility (Katz et al., 2019).

Protein digestibility could be affected by some factors like inaccessibility due to entrapment, antinutritional factors, amino acid sequence of the proteins, and protein folding and crosslinking (Joye, 2019).

The aim of this study is to fortify RTE banana porridge with commercial plant-based ingredients in other to increase the protein content.

3.1.1 The objectives are as follows

1) To add individual fortificants (soya protein isolate powder, pea protein isolate powder, hemp protein isolate powder, moringa leaf powder and tomato fibre powder) to RTE green banana porridge, to achieve a protein content of ~14% on a dry weight basis, which would satisfy 25% of the protein requirements of children and adults based on portions of 30 and 100 g, respectively.
2) To assess the protein quality of the fortificants through determination of in vitro PDCAAS.
3) To assess the effect of fortification on mineral content.
4) To determine the effect of fortification on carbohydrate digestion.

The hypothesis is that the fortified RTE banana porridge can meet 25% of the protein requirement for children and adults at standard portion sizes.

3.2 Materials and methods

3.2.1 Plant-based ingredients

Following market online research, a few commercially available protein sources were identified (Figure 3.1). Five fortificants, namely moringa *oleifera* leaf powder, soya protein powder, tomato fibre powder, pea protein powder, and hemp protein powder were selected based on their composition, colours, and aromas. The RTE banana porridge was produced in chapter 1 of this work. Soy powder, pea powder and hemp powder were purchased from Buywholefoodsonline.co.uk. Moringa *oleifera* powder and tomato fibre powder were purchased from Morrison

(Leeds, UK). RTE banana porridge and the five identified fortificants are shown in figure 3.1 and their composition in Table 3.1

Figure 3.1: Glass containers with ten g of samples. 1 = RTE banana porridge; 2 = Moringa powder; 3 = Soya powder; 4 = Tomato fibre; 5 = Pea powder; 6 = Hemp powder

Table 3.1: Proximate composition (g/100g) of the selected plant-based ingredients used to fortify ready to eat (RTE) green banana porridge in this study

	Proximate composition (g/100 g)					
Compounds	RTE	TF	Moringa	Hemp	Pea	Soy
Protein	4.88	14.84	38.7	50.0	78	90.0
Fat	0.007	6.01	6.1	11	5.4	2.4
Carbohydrate	69.15	-	26.1	24.0	4.8	1.2
Sugar	1.84	0.78	3.8	5.0	Trace	0.3
Fibre	14.50	86.15	27.1	19	Trace	2.0

Data according to products labels

3.2.2 Methods

3.2.2.1 Fortification of RTE banana porridge

Food-to-food fortification method used was adapted from Alamu et al. (2016) in order to improve the protein content of RTE banana porridge using forticants with

some modifications. Different proportions of RTE porridge were mixed with the forticants to achieve roughly 14 grams of protein per 100 grams of mixture, as shown in Table 3.2. In the case of tomato fibre which has low protein content, a larger amount was needed and the 14% target was not achieved.

The method used for reconstitution of porridge sample was adapted from Loypimai and Moongngarm (2015) with some modifications. RTE banana flour + fortificant flour (100 g) was reconstituted by using the same 20% w/v of RTE banana porridge in the previous chapter. This level was used because studies have shown that 20% w/v is good to form a good paste. The RTE banana porridge and the fortificants were weighed, mixed and 500 ml of boiled MillQ water was poured on the mixed fortified sample, covered for 5 minutes, and stirred and served without further cooking. The control porridge, 100g of RTE banana porridge was mixed with 500ml of hot water (ratio 1:5) covered for 5 minutes, stirred, and served without further cooking. Fortification calculations were shown in appendix 4.

Table 3.2: Fortification of RTE banana porridge with fortificants

	Quantity of RTE used (g)	Quantity of fortificants used (g)	Quantity of fortified mixture to be used (g)	Protein content (g per 100g) of fortified mixture
RTE only	100	-	100	4.88
RTE+ Moringa	76.44	23.56	100	12.85
RTE+ Hemp	81.76	18.24	100	13.11
RTE+ Soya	89.9	10.1	100	13.48
RTE+ Tomato fibre	38.55	61.45	100	11.00
RTE+ Pea	88.31	11.69	100	13.43

Data for quantity of RTE to be used with fortificant, and protein content of fortified mixture

3.2.2.2 Protein determination

The method used for protein determination was inductively coupled plasma optical emission spectroscopy (ICP-OES), where N was analysed and then convert to protein by multiplying with 6.25. The protocol used was adapted from Phan-Thien et al. (2012) with some modifications. About 600 mg of RTE banana porridge, fortified RTE banana porridge with % addition moringa and fortified RTE banana porridge with % addition of Tomato fibre were weighed in a glass test tube separately, and 3 ml of 69% HNO_3 (Hiperpur; Panreac, Spain) and 2 ml of deionized water (Milli-Q; Merck, Spain) were added to each tube. The mixtures were digested by microwave treatment (Milestone; Ultra wave, Italy) at 240 ◦C and 40 bars for 40 min at 1,500 W. Once digested, they were brought to a final volume of 50 ml with Milli-Q water. Carbon and N were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES).

3.2.2.3 Protein quality by *in vitro* PDCAAS

The method used for determination of protein quality was Protein Digestibility Corrected Amino Acid Score (PDCAAS), which was adapted by Megazyme as the chosen method for the measurement of the protein value in human

nutrition (Schaafsma, 2000). The method is based on comparison of the concentration of the first limiting essential amino acid in the test protein (fortificants) with the concentration of amino acid in a reference protein (casein). The amino acid of the samples (fortificants) used in this work were derived from the Literature and shown in Table 3.5. The protein quality of the protein sources were determined as follows:

$$\text{PDCAAS (\%)} = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times \text{in vitro digestibility}$$

Table 3.3: Amino acid profile of the selected fortificants and casein protein powder.

Amino acid	Green Banana flour(gAA/ 100 g protein)	Moringa leaf powder (gAA/ 100 g protein)	Soy Protein Powder (g AA/ 100 g protein)	Tomato fibre(gAA/100g protein)	Pea protein powder (g AA/100 g protein)	Hemp protein powder (gAA /100 g protein	Casein protein powder (gAA /100g protein
			Essential Amino acid				
Valine	1.186	5.5	5.1	32	4.9	4.98	7.6
Methionine	0.098	1.5	1.28	9	0.9	1.39	2.9
Phenylalanie	1.464	5.9	5.19	26	5.7	4.57	5.5
Isoleucine	0.506	4.4	4.89	23	4.4	3.99	5.9
Lysine	11.91	4.8	6.3	14	6.7	4.16	8.5
Leucine	1.859	7.7	8.15	38	7.6	6.63	10.2
Tryptophan	-	-	1.36	-	0.9	-	1.4
Histidine	13.66	2.4	2.6	7	2.4	2.81	3.1
Threonine	6.004	4.1	3.73	24	3.8	3.67	4.6
			Non Essential Amino acid				
Aspartic Acid	13.319	9.3	12.37	123	11.9	9.41	7.5
Glutamic acid	16.037	15.0	19.42	385	16.4	16.14	22.7
Cysteine	0.00	1.6	1.31	7	1.0	0.17	0.4
Serine	11.659	4.2	5.09	25	5.4	5.18	6.5
Arginine	13.054	5.8	8.44	23	8.4	9.19	3.7
Alanine	3.787	6.2	4.25	38	5.4	4.50	3.2
Tyrosine	1.283	3.6	3.88	-	4.0	3.67	5.9
Glycine	4.924	4.9	4.20	37	4.0	3.99	1.9
Proline	0.959	5.1	5.24	23	4.4	4.53	10.5
Total AA	100.75	92.0	102.8	834	98.2	90.79	112
TEAA/TAA(%)	36.687	36.3	38.6	173	37.3	37.12	49.7
Reference	(Rachman et al., 2021)	(Mostafa et al., 2021)	(Hughes et al., 2011)	(Knoblich et al., 2005)	(Liu et al., 2019)	(Wang et al., 2008)	(Liu et al., 2019)

The limiting amino acid amount in RTE banana porridge is 0.98 mg of methionine in 1 gram of RTE banana porridge, divide by 25 mg of methionine in 1 g of reference sample (FAO/WHO 1991 standard) , multiply by the digestibility of RTE banana (0.1) as a fraction. This is done to all the essential amino acid in the test protein so that amino acid scores will be generated and the least value of the entire amino acid sore in a test protein is selected as limiting amino acid score.

Therefore, protein value of the fortificant can be gotten by the product of amino acids score and the corresponding in vitro digestibility score of the test protein.

3.2.2.4 Optimization of casein digestion

Digestion of casein powder was performed enzymatically by using multiple enzymes (pepsin, trypsin, and chymotrypsin) method with different quantities of casein powder ranging from 20 mg – 100 mg. This was done to find the quantity of casein that will give the highest yield of digestible casein resulting in having the highest digestibility. The rest of the experiment was carried out as described in the in vitro protein digestibility determined enzymatically according to Plank (2017) as adapted by Megazyme. Casein quantities were added to 50 mL tube each and was mixed with 19 mL of HCl (0.06 N) and incubated for 30 mins then 1 mL of pepsin was added, mixed, and incubated for another 60 mins. The pH adjusted to 7.4 by putting 2 mL of 1.0 M Tris buffer, pH 7.4, after which 200µl of trypsin and chymotrypsin was added mixed and digested for another four hours. At the end of digestion the undigested protein was remove though precipitation, by adding 1 mL of 40% TCA solution and incubate for overnight at +4$^{OC.}$ sludge was formed at the bottom of the tubes. The supernatant was collected containing all amines present in the sample and spectrophotometric method was used for evaluating the primary amine concentration corrected for dilution and weight in the original sample after the in vitro digestion of protein

3.2.2.5 Protein digestibility of the protein sources

In vitro protein digestibility was determined enzymatically according to Plank (2017) with some modifications Fortificants (50 mg) each, and casein (50 mg) were added into 50 mL tubes separately and 19 mL of HCl (0.06 N) was added to each tubes and mixed by vortexing and incubating for 30 minutes at 37 ^{o}C in

a water at 200 rpm . The tubes were removed, uncap and 1mL of pepsin solution was added to sample in a tube, vortex and incubate for 60 minutes at 37 ^{o}C in a water bath at 200 rpm. After 1 hour of pepsin incubation, the tubes were removed from the water bath , and pH adjusted to 7.4 with addition of 2 mL of 1.0 M Tris buffer, pH 7.4 , cap the tubes and mix well by vortexing. Then, 200 µL of Trypsin /Chymotrypsin mixture was added to each sample of the protein sources mixed well by vortexing and incubate for 4 hours at 37 ^{o}C in water bath at 200 rpm. At the end of trypsin /chymotrypsin incubation, samples were removed from the water bath and place in boiling water bath for 10 minutes. This was to stop protein digestion. After 10 minutes of boiling, samples were removed from the boiling water, mixed by vortexing, and allowed to cool to room temperature for 20 minutes. Then 4 mL of each solution from each tube was transferred into a new 15 mL tubes separately and 1 mL of 40% TCA solution was added to each tubes, cap the tubes, vortex and incubate at 4 ^{o}C overnight at least for 16 hours. The remaining solutions after removing 4 mL from each tubes were stored in the freezer at – 80 ^{o}C for future analysis. At the end of 16 hours incubation, 1.75 mL of incubated samples each (avoiding precipitate) was pipetted into a 2 mL centrifuged tubes and centrifuged for 10 minutes at 15,000 x g at room temperature. A 10- fold dilution in acetate buffer (50 mM, pH 5.5) was made for blank (mixtures of all the reagents) and L-glycine while casein as control reference protein powder and fortificants were made into 40- fold dilution. All the diluted supernatants of the sample solutions including the sample blanks, calibration samples (L-glycine) and the casein control sample (L-glycine) were subjected to colorimetric determination of amines. This was achieved by pipetting 0.100 mL of sample solution, blank, standard solutions of glycine from ST 11 – ST 0 each in 96-well plate. Ninhydrin regent (2%) of about 0.050 mL was added

to each of the diluted solutions and place a lid on the plate and cover with foil and place on pre-heated water at 70 ^{o}C for 35 minutes at 100 rpm. At the end of 35 minutes the plate was removed from the water bath but foil was not removed and it and it was allowed to cool for 10 minutes. After 10 minutes, the foil was removed and 0.150 mL of 50% v/v of reagent alcohol was added and mixed, then sent to TECAN to read absorbance of the solution at 570 nm against the L-glycine standard (ST 0). The protein digestibility was calculated using the formula below.

Digestibility = Formula: $C2 = \frac{C1*V*D*1.25}{\text{Sample}}$

Where C1 = concentration of primary amines in the diluted sample (mM), convert C1 in mM to mg/mL:

V = Total digestion volume, D = dilution factors. Then 1.25 = dilution with TCA:

C2 = corrected primary amine concentration (mg/mL).

3.2.2.6 Mineral determination

Minerals (F, K, Mg, Mn, P, and Zn) were analysed by inductively coupled plasma mass spectroscopy (ICP-MS) using the analysis parameters from Otero-Romaní et al. (2009). Analysis was performed on a PerkinElmer Optima 4600 DV ICP analyser (Waltham, United States). The running parameters were set as follow: plasma flow 15 L/min, auxiliary flow 0.2 L/min, nebulize flow 0.8 L/min, power 1,300 W, reading distance 15 mm, reading position radial (K) and axial (Mg, Mn, Zn, Fe, and P), integration time 5–10 s, and number of replicates 3

3.2.2.7 Carbohydrate determination

The carbohydrate content properties of fortified RTE banana and reconstituted porridges were examined using enzymatic determination method of resistant starch assay procedure (rapid format) from Megazyme. Measurement of the released reducing sugar after the in vitro digestion was determined by DNS

method. A 100 mg of mixed fortified and non-fortified RTE banana samples (RTE porridge and reconstituted porridge) were suspended in 3.5 mL of sodium maleate buffer (pH 6.0) and hydrolysed with 0.4 U of pancreatic α – amylase and 0.17 U of amyl glucosidase. The mixture was incubated for 4 hours at 37 °C in a shaking water bath. Supernatants were collected and top up to 100 marks on measuring flask as digestible solution, resistant starch called pellet was broken down by adding 2 mL of 1.7 M NaOH and stirred for 20 minutes under ice and then 8 mL of 1.0 M sodium acetate was added with 0.33 U of AMG . Then incubated at 50°C for 30 minutes with intermittent shaking. The supernatant collected from each tube was topped up to 100 mL with millQ water and determination using DNS method as describe in chapter one was carried out.

The result of when fortified RTE banana and reconstituted porridges are compare can be represented equational as shown below.

The digestibility of carbohydrate can be obtained by finding the quotient of (digestible over total) starch and multiple by 100 over one. To obtain digestibility this formula can be used

$$\text{Digestibility} = DS/TS * 100$$

Where Digestible starch (DS) and Total starch (TS

3.2.2.8 Ash and fat content determination

The ash and fat content were determined following the AOAC method with codes 923.03 and 920.39, respectively.

3.2.2.9 Total fibre

Total fibre was determined as same as in previous chapter in section 2.2.7.2.4.

3.2.3 Statistical analysis

Experiments were carried out in triplicate; data are means of triplicate measurements evaluated using one-way analysis of variance (ANOVA) and post hoc test bonferroni, statistical significance was determined at $p<0.05$.

3.3 Results

3.3.1 Visual appearance of the fortified porridges and reconstitution of the mixed porridge samples

Figure 3.2 and 3.3 shows the different colours when different fortificants were mixed with RTE banana porridge before reconstitution and after reconstitution respectively. The porridges were visually acceptable and had not strong aroma.

Figure 3 2: Fortification/mixtures of RTE banana porridges with the different fortificants and their colours**.**

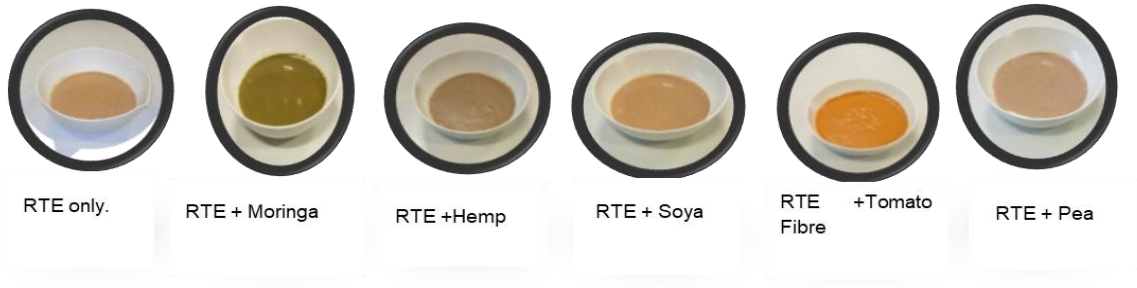

Figure 3.3: Pictures of non-mixed (RTE only) and mixed RTE banana porridges with different fortificants imparting their colours on the reconstituted mixed porridges**.**

3.3.2 Nutrient composition of fortified and non-fortified RTE banana porridges

The results in Table 3.4 showed a significant difference in the nutrient composition of RTE banana porridge fortified with moringa *oleifera* leaf powder and tomato fibre powder. These two fortificants were chosen from all other ones because they were only two that were available at the time of the experiment. The protein content of RTE banana porridge is 4.88 g /100 g but when fortified with moringa leaf, it increased to 14 g/100 g. With addition of tomato fibre powder the protein content increased to 11%. This showed that meeting the 25% target of protein contribution to protein requirement was only possible for moringa *oleifera* leaf powder and not for tomato fibre. Total carbohydrate content of RTE banana porridge was 77.67 g/100 g, while the fortified RTE banana porridge with moringa powder and with tomato fibre powder had 64.50 g/100 g and 29.93 g/100 g, respectively.

The ash content of RTE (3.13 g/100g) increased to 3.64 g/100 g as the highest ash content when RTE was fortified with % addition of moringa leaf powder and 3.25 g/100 g when tomato fibre powder was added. The fat content also differed significantly ($p< 0.05$) between the RTE, and the fortified RTE with addition of (moringa and tomato fibre). The fat content for RTE is 0.007 g/100 g, while fortified RTE with moringa *oleifera* leaf powder had 1.44 g/100 g and the highest was 3.67 g /100 g when fortified the RTE with addition of tomato fibre. The fibre content of RTE was 14.50 g / 100 g while fortified RTE with addition of moringa oleifera leaf powder was 17.47 g/100 g and fortified RTE banana porridge with addition tomato fibre was 58.53 g/100 g. There was increase in all the mineral content analysed, when fortified either with addition of moringa or tomato fibre. Iron, potassium, and magnesium have highest values when RTE banana

porridge is fortified with moringa leaf powder. While phosphorus, manganese and zinc had the highest values when RTE banana porridge is fortified with addition of tomato fibre.

Table 3.4: **Nutrient composition of RTE banana porridge and two fortified RTE banana porridge per 100 g**

	RTE	RTE banana porridge with moringa powder	RTE banana porridge with tomato fibre powder
Protein (g)	4.88±0.1^{b}	14.00±0.1^{a}	11.00±0.1^{b}
Ash (g)	3.13±0.06^{c}	3.64±0.5^{a}	3.25±0.6^{b}
Fat(g)	0.007±0.01^{c}	1.44±0.15^{b}	3.67±1.15^{a}
Total carbohydrate (g)	77.67±0.05^{a}	65.50±0.1^{b}	29.93±0.5^{c}
Fibre (g)	14.5±0.02^{c}	17.47±0.5^{b}	58.53±0.2^{a}
Iron (mg)	0.69±0.01^{c}	8.52±0.12^{a}	3.81±0.13^{b}
Phosphorus (mg)	85.91±0.01^{c}	131.43±0.05^{b}	225.72±0.02^{a}
Potassium (mg)	1040.05±0.01^{c}	1074.17±3.5^{a}	1057.01±1.2^{b}
Magnesium (mg)	99.40±0.00^{c}	197.39±1.5^{a}	158.53±0.5^{b}
Manganese (mg)	1.73±0.01^{b}	2.23±0.1^{a}	2.37±0.0^{a}
Zinc (mg)	0.57±0.01^{c}	0.86±0.0^{b}	1.62±0.0^{a}

Data are expressed as mean with standard deviation (n= 3). Different letters within a row indicate significant differences ($p < 0.05$), determined by Tukey test following one – way ANOVA. Moringa powder = Moringa oleifera leaf powder

3.3.3 Protein digestibility of the fortificants

Optimization of the digestion protocol was done first using casein as the positive control before the protein digestion of the fortificants. The results obtained for optimization of casein powder is shown in Table 3.5. The results showed that as the quantity of casein powder was increasing, the quantity of digestible protein was increasing up to a stage that the digestible quantity of casein start to decrease (641.3 mg glycine/ g sample at 60 mg). However, at 50 mg of casein, the highest amount of digestible protein (761.9 mg glycine/ g sample) was

achieved. At higher amounts, digestibility was lower. This could be as a result of having too much of the sample in the tube that were not digested.

Table 3.5: Optimization of casein digestion and percentage digestibility

Quantity of casein used (mg)	Digestible protein (mg glycine/ g sample)	% digestibility
20	466.9±0.0^{f}	47^{d}
30	532.8±0.0^{e}	53^{c}
40	669.0±0^{b}	67^{b}
50	761.9±0.0^{a}	76^{a}
60	641.3±0.0^{b}	64^{b}
70	573.3±0.0^{d}	57^{c}
500	99.9^{g}	10^{e}

Data are expressed as mean with SD (n = 3). Different letters within a column indicate significant differences ($p < 0.05$), determined by Tukey test following one – way ANOVA

Table 3.6 shows the results of digestible protein, total protein, and digestibility of all the fortificants used for the fortification of RTE banana porridges and casein powder used as control/reference protein. From the result, casein powder had the highest digestible protein (779 mg glycine /g sample) with highest total protein (1000 mg/g) and with 78% digestibility. Pea powder had 491 mg glycine /g sample for digestible protein, 780 mg/g for total protein content and 63% for percentage digestibility. RTE banana porridge had the least value 40.6 mg glycine /g sample for digestible protein and 40 mg/g for total protein having 100% digestibility.

Table 3.6: Protein digestibility of the fortificants, control and RTE banana Porridge.

	Digestible protein (mg glycine /g sample)	Total protein content (mg/g)	% digestibility
Casein powder (control)	779± 0.2[a]	1000± 0.0[a]	78[b]
Pea powder	491± 0.3[b]	780± 0.5[a]	63[c]
Soya powder	479± 0.8[c]	900±0.1[a]	53.45[d]
Hemp powder	403± 0.3[d]	500±0.5[b]	81[b]
Moringa powder	178± 4.5[e]	387±0.8c	46.12[d]
Tomato fibre powder	78.6± 8.2[f]	148±0.5[d]	53.13[e]
RTE banana porridge	40.6± 3.1[g]	40±0.4[e]	100[a]

Data are expressed as mean with SD (n = 3). Different letters within a column indicate significant differences ($p < 0.05$), determined by Tukey test following one – way ANOVA . Pea powder, soya powder and hemp powder are all protein isolates.

3.3.3.1 Protein quality of the forticants

The results from Table 3.7 showed that casein protein powder used as reference protein had the highest protein quality value of 1, followed by soy protein 0.55 and hemp protein powder with 0.52 protein quality value. The fortificant that had the least protein quality value is tomato fibre powder with 0.01. By using PDCAAS method in determining the protein value, the more the result is closer to 100% or 1, the more the protein sample is rated as high quality protein. If the result after 100 or 1 has fractions the fraction is always truncated, eg like 100.52, the result should be 100. Casein protein powder (control) , pea protein isolate powder, soya protein isolate powder, hemp protein isolate powder, moringa *oleifera* leaf powder, tomato fibre powder and RTE banana porridge have the following limited amino acid scores (1.32, 0.76, 1.04, 0.64, 0.828, 0.024 and 0.039 respectively). These results were gotten by finding the limiting amino acids in the test sample as mg of limiting amino_acid in 1 g of test protein and divide

this with mg of same amino acid in 1 g of reference protein (casein) and multiply by the digestibility of the test protein.

Table 3.7: Protein quality of the selected fortificants

Fortificant source	Limiting amino acid	Amino acid score	PDCAAS
Casein (control)	Methionine + Cysteine	1.32	1
Pea protein powder	Methionine + Cysteine	0.76	0.48
Soya protein powder	Methionine + Cysteine	1.04	0.55
Hemp protein powder	Methionine + Cysteine	0.64	0.52
Moringa leaf powder	Lysine	0.828	0.38
Tomato fibre	Lysine	0.024	0.01
RTE banana porridge	Methionine + Cysteine	0.039	0.004

PDCAAS: Protein Digestibility Corrected Amino Acid Score

3.3.3.2 Effect of fortificants on carbohydrate digestion

The comparison of carbohydrate digestion of non-fortified RTE banana porridge and fortified RTE banana porridge as dried RTE porridge and reconstituted RTE porridge with moringa and tomato fibre fortificants as shown in Table 3.8. These two ingredients were used here because of their availability at the time of experiment. The results showed that during dried stage of non-fortified RTE banana porridge, the digestible starch was 69.64g/100 g and when fortified with moringa and tomato fibre separately, the digestible starch decreased to 65.35 g/100 g with moringa and 44.83 g/100 g with tomato fibre. When reconstituted the RTE banana porridge without fortification the digestible starch increased from 69.64 to 71.78 g/100 g but when fortified the digestible starch decrease to 62.19 g/100 g after fortification with moringa and 39.91 g/100 g with tomato fibre. These results showed that carbohydrate content of the fortified RTE banana porridge is lesser than that of non-fortified RTE banana porridge in all the stages conceded. It could be said that fortification hinders digestion of starch. Fortification could

contains polyphenol that delayed the digestion of starch by delaying the reaction of the digestive enzymes alpha amylase and beta-glucosidase, two enzymes involved in starch digestion (Sun and Miao, 2020).

Table 3.8: Comparison of carbohydrate digestion content of Non- fortified RTE banana porridge and fortified RTE banana porridge during drying and reconstitution stages.

	Carbohydrate Content (g/100 g dw) n= 3.			
	Digestible starch	Resistant starch	Total starch	Sugar
Dried RTE porridge				
RTE	69.64±5.3^{b}	5.89±1.3^{b}	75.53±2.7^{b}	1.8±1.5^{c}
RTE + moringa	65.35±0.5^{c}	5.12±0.3^{b}	70.47±0.8^{c}	5.18±0.6^{a}
RTE + tomato fibre	44.83±0.5^{d}	7.31±0.8^{a}	52.14± 1.2^{e}	2.7±0.09^{c}
Reconstituted RTE porridge				
RTE	71.78±0.01^{a}	5.88 ±0.1^{b}	77.66±3.6^{c}	1.5±1.5^{c}
RTE + moringa	62.19±0.7^{c}	5.94±0.44^{b}	68.13±01^{d}	3.6±0.5^{b}
RTE + tomato	39.91±0.8^{d}	7.54± 0.4^{a}	47.45±1.4^{f}	2.2±0.9^{d}

Data are expressed as mean with SD (n= 9). Data in different letters within a column indicate significant differences ($p < 0.05$), determined by Tukey test following one – way ANOVA. Recon. RTE porridge = Reconstituted RTE porridge.

The effect of fortification on carbohydrate content of fortified RTE banana porridges after reconstitution with all the individual fortificants is shown in Table 3.9. Results showed significant difference in digestible starch (DS), total starch (TS), sugar, fibre, and resistant starch. RTE banana porridge had the highest digestible starch of 71.78g/100 g without addition of any fortificant while other porridges with fortificants had lesser digestible starch. Non-fortified RTE banana porridge had the least value of 5.88 g/100 g for resistant starch. The reconstituted porridges with moringa, hemp, soya, and pea have different values of digestible starch and these values are significantly different ($p \leq 0.05$) from that of tomato

fibre. The porridge that had the least digestible starch is the porridge fortified with tomato fibre, which is significantly different ($p \leq 0.05$) from other fortificants used. There is no significant difference in the values of resistant starch of the reconstituted porridges.

Table 3.9: Effect of fortifications on carbohydrate content of fortified RTE banana porridge after reconstitution (g /100 g) dry.

	Digestible starch	Resistant starch	Total starch	Fibre	Sugar
RTE	71.78± 0.01^{a}	5.88± 0.01^{e}	77.66±0.0^{a}	14.90±0.4^{d}	1.50±0.3^{c}
RTE+ moringa	62.19± 0.74^{b}	5.94± 0.67^{f}	68.13±1.0^{b}	17.76±1.5^{b}	3.6±0.5^{a}
RTE+ hemp	59.53± 0.97^{b}	6.67± 6.75^{d}	66.20±1.1^{b}	15.64±0.27^{c}	1.2±0.27^{c}
RTE+ soya	65.53± 2.85^{b}	10.47±3.55^{b}	76.00±1.4^{a}	13.59±0.1e	1.5±1.73^{c}
RTE+ tomato fibre	39.91± 1.1^{c}	7.54± 1.38^{c}	47.45±2.2^{c}	58.68±0.1 ^{a}	2.2±0.37^{b}
RTE + pea	64.97± 0.15^{b}	16.13±1.37^{a}	81.10±1.2^{a}	13.16±0.15^{c}	1.4±1.19^{c}

Data are expressed as mean with SD (n = 3). Different letters within a column indicate significant differences ($p < 0.05$), determined by Tukey test following one – way ANOVA.

The digestibility results of fortified porridges after reconstitution are shown in Figure 3.4 using digestibility bar chart for 100 g fortified porridge for each bar. Hundred gram of each mixture was reconstituted with 500 mL of hot water, allow to cool down for 5 minutes and hydrolysed as described before. The first one was RTE only that was not fortified, which serves as control. The second fortified porridge was RTE banana porridge fortified with moringa . It has digestible starch 62.41 g/100 g and resistant starch 5.93 g/100 g with a total starch digestibility of 91%. The 3rd is RTE porridge fortified with hemp which had digestible starch (59.53 g/100 g) for digestible starch, 6.67 g/100 g for resistant starch and with

total starch digestibility of 89%. The next porridge was RTE porridge mixed with soya. The digestible starch was 65.53 g/100 g, resistant starch 12 g/100 g and its digestibility was 85%. The next porridge was RTE porridge mixed with tomato fibre. This porridge had a total digestibility of 82% and finally the RTE porridge mixed with pea had a total digestibility of 80%. This RTE porridge mixed with pea had the least digestibility among the fortified porridges formed. It could be observed from the bar chart that the non- fortified porridge being RTE banana porridge had the highest digestible starch, resulting in having highest carbohydrate digestibility in the porridge formed.

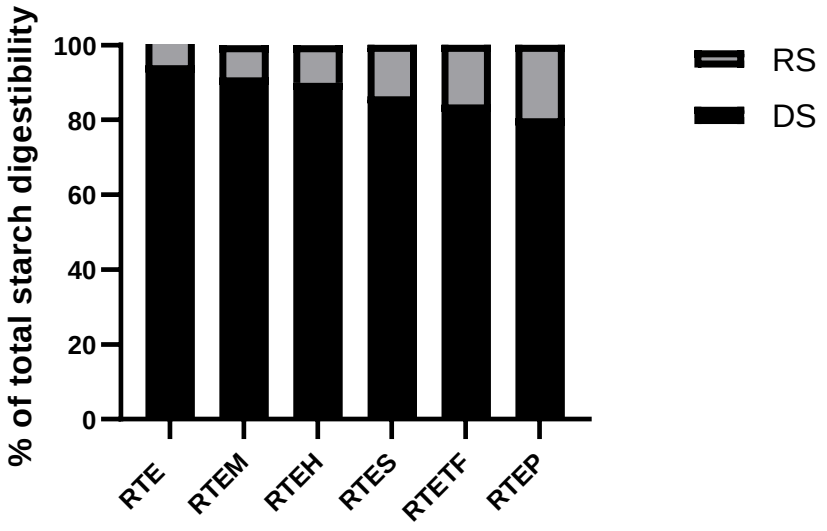

Figure 3.4: **Porridge mixed with fortificants.**

Figure 3.4: Digestibility bar chart for 100 mg reconstituted RTE banana porridge (RTE) and RTE mixed with different fortificants hydrolysed by 0.4 U of pancreatic α – amylase and 0.17 U of amyl glucosidase and incubated for 4 hours at 37°C in a water-shaking bath. Data expressed as mean with SD (n=6) % of hydrolysis of total porridge starch. RTE = Ready to eat banana porridge. RTEM = Ready to eat banana porridge mixed with moringa. RTEH = Ready to eat banana porridge

mixed with hemp. RTES = Ready to eat banana porridge mixed with soya. RTETF = Ready to eat banana porridge mixed with tomato fibre RTEP = Ready to eat banana porridge mixed with pea.

3.4 Discussion

Developed RTE banana porridge has been fortified with five different commercial plant-based ingredients. Interestingly, these 5 plant-based ingredients used are found in most developing countries where they are grown, process and eaten in different forms, and are now found online without much stress. These ingredients would not be entirely new to many people in the developing countries.

Fortification has been used to increase the protein content of the developed RTE banana porridge from 5% to 14% on dry weight (dw) basis. This result is in line with Alamu et al. (2016) who developed a maize-meal porridge and fortified with 3 different fortificants (soy flour, groundnut paste and skim powder milk as control). They found that all the 3 ingredients increased the protein content of the maize porridge. Interestingly, soy ingredient was found to increase the protein content of the maize porridge from 2.5% to 12.66%. They concluded that this increase amount to 90% increase from soy flour. According to the results of this work all the fortificants (moringa, hemp, soya, tomato and pea) used increased the protein content of RTE banana porridge from 5% to 14%, except tomato fibre fortificants that increased from 5% to 11%. Table 3.4 shows that the increased in protein content would be sufficient to meet around 25% of the protein requirements for children and adults based on standard portion sizes of 30 g and 100 grams (dw) respectively.

Having looked at the protein content of the fortified porridges, it will be good to consider the protein quality of the fortificants used.

The results of protein digestibility of the fortificants used showed a significant difference ($p < 0.05$) in digestible protein, total protein and % digestibility. Casein protein powder was optimized for digestion and had the highest digestible protein at 76% digestibility. The PDCAAS result of casein from Table 3.7 is 1. This PDCAAS of 1 is in line with other literatures that said that animal protein sources usually have a PDAAS value of 1. This is because they are regarded as complete protein with good levels of essence amino acid and good digestibility. According to Acton et al. (1982) protein digestibility of casein is 90.1% and is line with (Hsu et al., 1977). The limiting amino acids found in casein were methionine and cysteine with amino acid score of 1.32. Cysteine as a limiting amino acid in casein is in line with the findings of Liu et al. (2019) who compared amino acid availability of a dairy and vegetable protein blend compared to casein. Pea, soy, and hemp protein powders are from legumes but have significant difference in total protein content (780, 900 and 500 mg/g dw) and digestible proteins (491, 479 and 403 mg glycine/g sample) as shown in Table 3.6. However, soy protein powder had the highest total protein content among the pea, soy, and hemp protein powders. Table 3.7 showed that pea, soya, and hemp protein powders have the same limiting amino acids (methionine and cysteine). They also have different amino acids scores and different % digestibility resulting in different PDCAAS values. The PDCAAS value indicate the composition of the essential amino acids and its digestibility in the human body after consumption. However, protein digestibility shows the proportion of ingested amino acids that can be made available for utilization by the body after digestion and absorption, in general, animal proteins have a high digestibility (>95%) (Päivärinta et al., 2020). Among the five selected ingredients, soya protein powder had the highest PDCAAS value of 0.55 and 1.04 for amino acid score based on methionine and

cysteine as limiting amino acids. This means that among the five selected ingredients, soya had the highest protein value and is next in protein value relative to casein (control). This is line according to Qin et al. (2022), who found that soya protein is a high-quality protein with a PDCAAS of 1.00, which is close to some of the proteins from animal sources. Soy proteins contain well-balanced essential amino acids but it is limiting for sulphur-containing ones like methionine and cysteine. The difference in the value of PDCAAS and in amino acid score could be error coming from the values of amino acid profile gotten from literature as no two or three literature on one ingredient are the same. Soya bean protein powder is rich in essential amino acids like lysine and threonine. Due to high lysine content, soya bean is sometimes added to low protein carbohydrate foods. It also contains polyphenols such as phenolic compounds ranging between 68.94 and 73.25 mg GAE /100 g (Astawan et al., 2020). Soya bean protein powder has been found not to be allergen-friendly (Nunes et al., 2006) and this is a problem in using soya bean for fortification.

The next in protein value after soya protein powder is hemp protein powder, with a protein digestibility value of 81%, PDCAAS value of 0.52 and 0.64 value for amino acid score and with the same limiting amino acid as in soya protein powder and. According to House et al. (2010) the protein digestibility of dehulled hempseed, depending on the sources, was 90.8 to 97.5%, which is still similar to 97.6% for casein. The PDCAAS value for hemp protein, depending on the source, was 0.48 to 0.61. The PDCAAS value obtained in this work for hemp protein powder is within the range given above. Hemp powder contains all the essential amino acids needed by the body for human growth and about 65% of total protein is digestible with the protein mainly made of globulin. The digestibility of hemp protein is ranging from 83 - 92% (Teterycz et al., 2021) with a low Protein

Digestibility-Corrected Amino Acid Score (PDCASS), 46–51% for hemp seed meal. This could be the effect of low content of essential amino acids such as lysine and tryptophan (House et al., 2010). Moringa protein powder is a good source of protein that contains 16 – 19 amino acids of which, ten of them are essential amino acids. The highest occurring essential amino acids in moringa leaf powder is leucine (Mostafa et al., 2021). According to Wahyuni et al. (2021), the essential amino acids in moringa leaf protein powder are as follows: Threonine (7041.83832 µg/g), Valine (15301.47228 µg/g), Methionine (4795.69096 µg/g), Isoleucine (825.526,598 µg/g), Phenylalanine (5719.83016 µg/g), Histidine (11329.1744 µg/g), Lysine (15726.06126 µg/g), and Tryptophan (2219.54373 µg/g). The highest essential amino acid content was in Valine at 15301.47228 µg/g and the lowest was in Tryptophan 2219.54373 µg/g. Moringa *oleifera* leaf powder had 0.828 amino acid score with 0.38 PDCAAS and the limiting amino acid is lysine. This could mean that moringa *oleifera* leaf powder could have a good level of essential amino acids but with a low protein digestibility among the selected fortificants. This poor value of digestibility could explain PDCAAS low value of moringa *oleifera* leaf powder. Nutrient composition of fortified RTE banana porridge with moringa leaf powder has shown that moringa leaf powder increased the protein content of fortified RTE banana porridge up to 25% of protein requirement.

Pea protein powder had PDCAAS value of 0.48 and amino acid value of 0.76 with same limiting amino acids as soya and hemp. The PDCAAS value for pea protein powder is less than that of soya and hemp; this is an indication that soya and hemp protein powders have more levels of essential amino acid than pea protein powder and better digestibility than pea protein powder. According to Rutherfurd et al. (2015) the PDCAAS of pea protein concentrate (PPC) is 0.893. According

to Amagliani et al. (2017), the protein in pea are of two types, albumins (18 – 25%) and globulins (55 – 65%) and are digestible with good biological value. Pea protein powder contains 8.58 g /100 g Lysine and 0.50 g/100 g tryptophan than cereal grain (Shevkani et al., 2015). It also has high amino acid profile and is a good allergen-friend (Nunes et al., 2006).

Tomato fibre powder had 0.024 as amino acid score and 0.01 as the PDCAAS and lysine is the limiting amino acid. This could mean that tomato fibre powder has not gotten adequate levels of essential amino acids and the tomato fibre protein digestibility is low resulting in low PDCAAS Value. RTE banana porridge had 0.039 as the amino acid score and 0.00392 as the PDCAAS and having methionine and cysteine as limiting amino acids. Tomato fibre is the by-product of processing tomato fruit. It is made from peel that ground, dried and milled into flour (Herrera et al., 2010). Tomato fibre flour does not contain the seeds, it is only made up of dehydrated peels of tomato alone. The protein content tomato fibre is about 14% (Del Valle et al., 2005; Alamu et al., 2016)

Generally, fortification could increase the micronutrient composition of the fortified product. According to Ntila et al. (2020) who showed that addition of moringa leaf powder from concentration 0% – 3% resulted in 11% increase in protein, 67% increase in mineral content, Zn by 60%, Fe by 45%,total provitamin A by 41%. These results show that moringa leaf has the potential for increasing the nutritional properties of white maize soft porridge. Based on the results of the mineral analysis gotten from this work on moringa and tomato fibre (other forticants were not assessed for mineral content), all the minerals measured could be increased as a result of fortification. However, none except iron result meet the RDA recommendation. The RDA for iron is 8 mg per a day and

fortification with moringa could meet this recommendation. In future work, the other forticants should be analysed for mineral content.

Vitamin micronutrients were not assessed as part of this thesis, but should be considered for future work. According to Meena (2017) moringa leaf powder is rich in vitamin A, B and Lycopene is a principal component of the tomato skin but carotenes are also present in abundance. Further work needs to be done to estimate the potential of tomato fibre to meet vitamin A requirements through similar fortification strategies.

Results in Table 3. 9 showed a significant difference between non- fortified RTE banana porridge and fortified RTE banana in terms of carbohydrate digestibility. Fortification with moringa leaf led to a reduction in digestible starch content of 4.29 g/100g and increase of 3.38 g of sugar. These results indicate that moringa leaf powder contains something which is capable of reducing the carbohydrate digestion during in vitro digestion. Leone et al. (2015) states that moringa powder contain total polyphenols ranging from 2813 ± 51, 3552 ± 388, to 2545 ± 194 mg/100 g) depending on growth regions (Chad, Sahrawi Camps and Haiti) and that moringa polyphenols can inhibit α-amylase activity . A similar decrease in digestible starch results was observed when all the fortificants were used with RTE banana porridge individually (table 3.6), this means that fortificants could affect digestion by hindering the digestive enzymes, which resulted in getting lower digestible starch than in RTE porridge (Gemede and Ratta, 2014). These fortificants could contain phytochemicals like polyphenol and fibre, which could result to the inhibition or delay of the enzymes. These results could suggests that these fortificants have some components such as polyphenol and fibre, which reduce the activities of digestive enzymes like α-amylase during in vitro digestion of starch (Griffiths, 1986). RTE banana porridge with pea had the least

carbohydrate digestibility of 80%. This could imply that pea protein powder had the least effect among all the fortificants used. It could be seen from Table 3.6 that RTE banana with pea had resistant starch (RS) of about 16.13 g/100 g showing highest RS in the mixed porridges. Petropoulou et al. (2016) confirmed that the starch in pea protein flour is resistant starch and together with the effect of fibre (having the lowest digestibility indicating that pea protein powder had the least effect highest digestibility among the fortificants used and it has a greater part of soluble fibre (Lam et al., 2018). Research has found that polyphenol decreases the overall digestibility of carbohydrates in the rat intestinal tract (Moseley et al., 1979). According to Sun et al. (2019) who found that polyphenol inhibit α-amylase and α-glycosidase, hence controlling the glycaemic response of carbohydrate. Sun, et al. (2019) found that inhibition of α-amylase by polyphenols results from molecular binding interaction between the enzymes and the polyphenols and that if the food is rich in soluble polysaccharides, this may reduce the binding and inhibitory action of the polyphenol. Research has shown that postprandial blood glucose level of a diabetic person could be controlled by the inhibition of digestive enzymes (α-amylase and α-glucosidase). Finally fortificants can be used as a tool to decease rapidly digested starch and increased slowly digestible starch and hydrolysis index of the cooked-to-optimum samples (Rocchetti et al., 2020).

3.5 Conclusion

Fortification of banana porridge with four different fortificants separately have successfully increased the protein content from 5% to 15%. Fortification with tomato fibre only increased it by 11%. This fortification has resulted in producing different varieties and colourfully RTE banana porridges. Fortification reduced

digestibility of both carbohydrate and protein, likely due to the presences of other dietary compounds present in the fortificants, e.g. polyphenol. Among all the fortificants tested hemp protein powder had high digestibility of 81% and protein quality, and was selected over soy due to low allergen potential. Moringa leaf power looks a promising forticant to increase iron content, and tomato fibre to increase pro-vitamin A. Therefore, this method is good when you have many ingredients to consider and at the end be able to pick one.

Chapter 4

General discussion

4.1 Food waste in Nigeria and the significance of the new developed RTE banana product.

The population of Nigerian is over 190 million people with 13 million people roughly suffering from hunger (FAO, 2017). The global hunger index in Nigeria is now 31.1 (Sunday et al., 2022), and the cereal import dependency ratio (which is the ratio of importation of cereal foods to production of cereal foods) for Nigeria is 21.7 with a food deficit of 56 million tons (FAO, 2017). Yet each year, Nigeria loses and wastes 40% of its total food production, which equals to 31% of its total land use (Bloomberg, 2019) as food waste or food loss. Food could be defined as any substance that gives energy for exercise, growth, and all physiological processes, as well as maintaining immune system health, and the amount of food a person requires varies from person to person depending on sex, age, and activity, among other factors (Slavin and Lloyd, 2012). According to Fabi (2018), the solution to hunger is achieved through better use of the food that is already available, as much food produced for human use is lost or wasted.

However, one-third of the food produced is wasted. Food waste is not the same as food loss because their causes are different. In developing countries, “food loss” or unintentional wastage is usually high due to poor equipment, transportation, and storage conditions (United Nations, 2013). Food loss comes from the production, harvest, post-harvest, and processing phases while food waste is often caused by retailers and consumers throwing perfectly edible/spoilt foodstuffs into the trash (Gustavsson et al., 2011).

Nigerian crops are mainly carbohydrate-rich foods including rice , cassava, yam, maize , banana , plantain , millet, sorghum and others for the survival of its population. These foods are been cultivated and harvested in Nigeria having good weather for their plantation. One of the major problems Nigeria is facing is high food waste and loss. Nigeria has been identified as the highest food waste country in Africa, with Nigeria recording 40% of food waste and loss from their total yearly food production (Arigbaba, 2022). This could be one of the leading factors that is responsible for making Nigeria to face food insecurity. This could be an indicating factor that Nigeria may not be able to get to zero hungry target in 2030. Figure 3.6 shows an example of food wasted in Nigeria.

Figure 4.1: Mixture of food waste dumpsites in Nigeria (Pictures from Bloomberg, 2019)

4.1.1 Reasons for huge food loss in Nigeria

Nigeria experiences great food loss, which could be because of lack of expert production companies in the country, that should understood how and what best techniques to use in different agricultural produce. This will help in preserving and extending the shelf life of all the most perishable produce in Nigeria. The country Nigeria is lacking proper harvesting tools, lack of proper equipment, poor transportation system and poor storage facilities (Akerele et al., 2017).

4.2 The significance of the new developed RTE banana product

4.2.1 Consumer acceptability, food safety and sensory properties

Nigeria produces many varieties of banana, and these are primarily used for domestic consumption. Nigerians already consume green bananas regularly, in dishes such as roasted green bananas. Therefore, the raw material for making the RTE banana porridge is readily available and the concept of eating green bananas is already in consumer's habits.

The research in chapter 2 showed that, as far as it could be investigated, the RTE porridge was safe for consumption if appropriately processed, packaged and stored. It should be noted that the tests carried out were simple microbiological tests. Further tests using quality assurance approved laboratories are needed to demonstrate food safety for consumers.

Furthermore, the microbiological results from chapter 2 showed that once the package of porridge was opened and exposed to moisture, it could grow microbes within a month. This means that it is a rich medium for bacterial growth, owing to its rich carbohydrate and general nutrient content. The fortified porridges were not tested for microbial safety. This needs to be done in the future, as every ingredient will bring an additional safety concern.

In terms of sensory properties, the researcher was able to confirm that the porridges produced had agreeable sensory properties in terms of aroma, taste, texture and appearance. A sensory evaluation test was planned in the original research proposal of this thesis. Discussions were held regarding the best tests to evaluate the sensory properties and consumer acceptability of the porridges using human sensory panels. Discussions focused on the sensory attributes to be selected (e.g. sweetness, bitterness, astringency).

An ethics proposal was being worked on in 2019, but was in the end not submitted, firstly because the ethics committee did not accept applications during the covid lockdown, and secondly because face to face sensory trials were not allowed during the first or second national lock downs. It was therefore decided not to conduct these sensory trials.

The information from the sensory trials would have provided information on potential improvements that could be made to the porridges to improve acceptability.

4.2.2. Nutritional properties

A general motivation for the research was improving the food and nutrition security situation in Nigeria. As demonstrated in chapter 2, the RTE green banana porridge has good nutritional properties in terms of carbohydrate content. After reconstitution, >2/3 of starch could be quickly digested, while 1/3 remained undigested as resistant starch. Both these attributes are important, as the digestible starch would provide energy to the consumer, while resistant starch would provide a source of fermentable dietary fibre. The experiments also clearly showed that the digestibility could be easily modifiable using processing. For instance, reconstituting at a lower temperature reduces digestibility, and this

could be useful for consumers who want to reduce glycaemic spikes. The RTE porridge also contained good levels of non-starch polysaccharides, adding to the gut benefits of resistant starch.

A limitation of the research is that the digestibility tests were done using a static digestion experiment based on the commercial Megazyme digestible starch kit. These kinds of models are useful in research as they are quick and easy to perform, and can be used to screen different product formulations and processing conditions. However, static models fail to take in to account the dynamic nature of digestion in the intestine. More sophisticated semi- or dynamic models are available in a number of laboratories. Again, other limitation includes need of further instrumental characterization including rheology, colour studies against a commercial benchmark. Studies of shelf life including other quality parameters (taste, colour). Possibility to mix different proteins fortifying ingredients to improve nutritional profile. However, these were not available to the researcher at the time of the bench work.

Furthermore, in vivo trials are needed to test the physiological effects of the porridges in individuals. The test is to assess the postprandial glucose response of eating RTE banana porridge on healthy participants. One limitation of green banana (and banana in general) is the low protein content, relative to carbohydrate. For this reason, it was decided to investigate fortification using plant-based ingredients. These were chosen for their availability in the UK market. Initial assessment suggests these fortificants could be easily available in Nigeria. The results suggested that soya would be the most promising forticant to increase not only protein content, but also quality. This was followed by hemp, which is a promising candidate as, unlike soya, it is not classed as an allergen. Micronutrient analysis could only be conducted on moringa leaf and tomato fibre

powders. These were interesting as fortificants, as they could provide different minerals. Further analysis of all fortificants is needed to do a deeper evaluation on micronutrients.

4.3 Potential for commercialization

The processing methods used in this thesis to make RTE porridge were simple and low-scale. No additives were used. This simple processing is referred to as 'minimally processed', highly desirable by consumers. This means that they could be adopted at domestic scale by banana growers and consumers to extend the shelf-life of green banana. As seen in figure 1.4., bananas are often badly handled, with rapid ripening happening during transport and in the markets. This could be a challenge for the quality and consistency of the product. Indeed, bruised and/or damaged bananas tend to brown, soften and rot.

This call for collaboration with a company that could buy green bananas directly from farms and that could invest in larger scale processing equipment and distribution channels to reach consumers. This kind of collaborative venture is in high need in Nigeria, and could result in employment for rural workers. Looking into the future, there could be big market opportunities for this kind of product in Africa and globally.

4.4 The role of food processing in food security

Fortified RTE banana porridge has the potential to improve food security particularly in low and middle-income countries, and could present good opportunities for export market. As banana is well grown in Africa and subtropical countries and is perennial fruit crop, there would be enough raw material for industry to use and with this; there will be reduced banana wasting. Processing banana alone will not make Nigeria food secure. This is because banana can

only account for one-tenth of food production needed, then 90% is left unsolved. It will be proper to say that all the food produced in Nigeria should be properly processed and preserved, so that the shelf life of these products will be extended. Take for instance most of the fruits like water lemons, oranges, pineapples; etc could be processed into concentrates and powders. All cereals and legumes should be harvested and processed to prevent weevils attacking them. Indigenous Nigeria foods that could be processed and preserved include Yam Dioscorea rotundata, Yellow yam Dioscorea cayennensis, water yam Dioscorea alata, maize Zea mays, mung beans Vigna unguiculata, cocoyam Colocasia esculenta' ,cassava Manihot esculenta , tomato Solanum lycopersicum, onions allium cepa, carrot Daucus carota, ugu leaf Fluted pumpkin, Ora leaf Pterocarpus mildbraedi, green leaf Spinach and sorghum Sorghum bicolor.

4.5 Future for banana

There is need to keep the consumption of banana fruit going on throughout the entire world due to its nutritional benefits. For this to be done, it is important to note that the most common variety of banana (*Musa* Cavendish) has been affected by a fungal disease called Fusarium wilt. This is a recently emerged strain of *Fusarium oxysporum*, known as Tropical Race 4 (TR4) by infecting the banana plants' root and vascular system resulting in no nutrient absorption leading to death of the banana plant. The second disease is called Black Sigatoka (Jeger et al., 1995). The fungus *Pseudocercospora fijiensis* that attacks the plants' leaves, causing cell death that affects photosynthesis and leads to a reduction in fruit production and quality. Putting together all the effects of these two diseases on Musa Cavendish, it is possible we will not have banana crops in the near future. This has resulted to an urgent need to look for solution on how to

overcome these two diseases with little or no effect on the consumers of this banana.

Again, there should be rethinking of moving towards diversity of bananas in the areas where bananas are grown. In these areas, genetic diversity could be used to fight the attach of banana fungi infestation. This could result in the use of wild or coloured banana varieties. Diversity of bananas could be achieved now through these methods or by simply using their suckers. After all, the coloured bananas are more nutritious than the Cavendish bananas. One of the most important thing is that any method that they are to use in solving this banana problem should be a method that will ensure sustainability of banana in the entire world. Genetic modification or gene editing could be tried also.

4.6 Covid-19 Impact Statement

The research was heavily impacted by the global covid-19 pandemic and associated national lock downs. During the second year of PhD, the laboratory was shot down for up to eleven months. I was not able to do any lab work during the lock down. Some experiments were delayed (e.g. chapter 3) and the sensory work (planned chapter 4) had to be abandoned altogether. I have two school-age children who stayed at home with me during the lock down and I looked after them. I was still unable to do some reasonable desk-based work at home because they were talking, playing and disturbing me at home. In addition, I have family in Nigeria, and I was not able to see them for a long time. At a time, I lost hope of completing the programme because I do not know when the school will reopen again. Even when the school reopened, it was not possible to do the sensory evaluation due to limitations of lab occupancy and social distancing. Due

to Covid-19, I could not meet the deadline of my thesis submission. I had to apply for an extension, a new CAS letter and visa, all of which took time.

Chapter 5

Conclusion and recommendations

5.1 Conclusion

This study is the first to process green banana fruit into green banana flour (GBF), and used this produced GBF to develop ready to eat (RTE) banana porridge. After development, the RTE green banana porridge was fortified with five plant-based ingredients (soya, hemp and pea protein isolates, moringa leaf powder and tomato fibre powder). The fortification was done in other to increase the low protein content of RTE banana porridge from 5% to 14% by all the fortificants, but tomato fibre increased protein content of RTE banana porridge to 11%. This is in other to meet the 25% target contribution to protein requirement of the targeted groups. These groups include the child and adult (male and female).This method of fortification used was able to show that irrespective of the number of fortificants chosen, the same quantity of protein will be required during fortification from each of the fortificants but the quantities of the fortificants to give this quantity are different. This can be seen in Appendix 4.

Protein digestibility and quality were also investigated and soy protein isolate ingredient has been found to be the most promising fortificant for green banana porridge, based on colour and nutritional properties. Hemp is also promising, as it is not classed as an allergen. Moringa leaf could provide a source of iron, while tomato fibre could provide beta-carotene. Minimally processed products with fortification could play a role in reducing food and nutrition insecurity in some countries, alongside other indigenous foods in counties like Nigeria.

Based on this research, it has been demonstrated that a safe, nutritious and fortified green banana RTE porridge has been successfully developed using

simple, minimal processing methods. The porridge is rich in carbohydrates, and carbohydrate digestibility is affected by processing.

5.2 Recommendations

More work should be done on this research to enable this project yield into a physical edible food that can be consumed by people. This will require more funding, so that those areas where more works are still needed will be worked upon.

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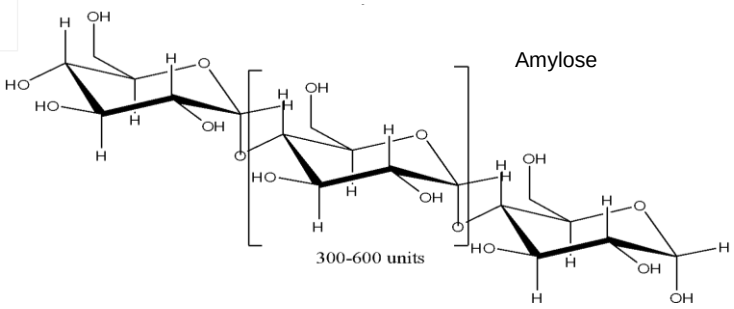
Appendix 1

Starch composition and structure

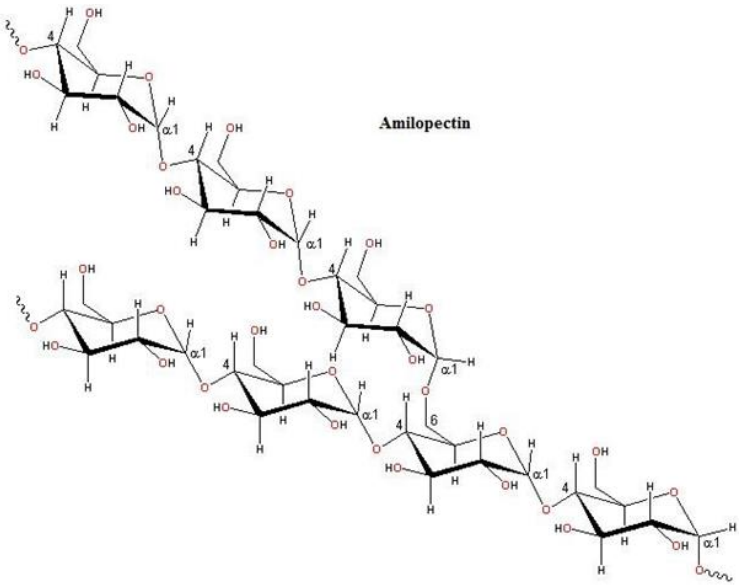
A)



-Glc a1-4 Glc a1-4

20 – 30% linear chain, C1 – C4 300- 600-glucose unit and tightly pack.

B)



70 – 80% linear and branch chain, 1,000,000 – glucose unit, branch at every 20 unitlooselypack.thtp://glyco3d.cermav.cnrs.fr/mol.php?type=polysaccharide&molecule=2406

Appendix 2

Photos of package ingredients materials used

A 

B 

C 

D 

E 

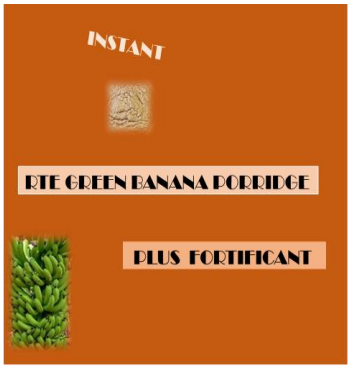
F 

Packaged materials bought.

A = Green banana flour. B = Hemp protein powder

C = Pea protein powder. D = Moringa *oleifera* powder

E = Tomato fibre. F = Soya protein powder

G 

G = Packaged RTE green banana porridge plus fortificant produced.

Appendix 3

Green banana and plantain

Green banana mixed with plantain

Green banana with long different sizes of the heads.

Appendix 4

Fortification results of green banana flour -based ready-to-eat porridge with **fortificants for meeting target consumers adult (male and female)**

Nutrient	Quantity
GBF-based RTE(g)	100
GBF- based Protein contribution(g)	4.88
% protein contribution to protein req.	8.87
25% Target protein contribution (g)	14
Moringa protein contribution (g)	9.12
Quantity of moringa needed(g)	23.56
Hemp protein contribution (g)	9.12
Quantity of hemp needed (g)	18.24
Soy protein contribution (g)	9.12
Quantity of soy needed (g)	10.1
Tomato fibre (TF) protein contribution (g)	9.12
Quantity of TF) Needed(g)	61.45
Pea protein contribution (g	9.12
Quantity of pea Needed(g)	11.69