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**CHARACTERISATION AND FOOD APPLICATIONS OF FRAFRA POTATO
(*Solenostemon rotundifolius*) FLOUR**

This thesis is submitted to the University of Ghana, Legon, in partial fulfillment of the requirement for the award of **PHD in FOOD SCIENCE Degree**

by

**CROSSBY OSEI TUTU
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DECLARATION

I affirm that apart from the references to studies, which I have cited, this study, “**CHARACTERISATION AND FOOD APPLICATIONS OF FRAFRA POTATO (*Solenostemon rotundifolius*) FLOUR**” was carried out by me in the Department of Nutrition and Food Science, School of Biological Sciences, University of Ghana, Legon. No part of the study has been submitted for the award of a degree elsewhere.



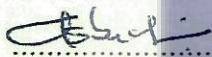
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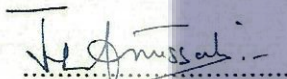
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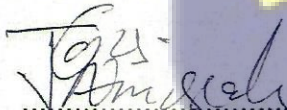
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ABSTRACT

Frafra potato (*Solenostemon rotundifolius*) is an underutilised climate-resilient tuber crop commonly cultivated in the tropics, including Ghana. Several accessions of Frafra potato have been identified and bred to broaden its application in different food products. This study characterised the starch and flour made from ten (10) Frafra potato (FP) accessions, from Ghana (released) and Burkina Faso (unreleased), in terms of their starch (FPS) and flour (FPF) yield, and their physicochemical and functional characteristics. FPS yields were similar ($p < 0.05$) and ranged from 35 to 39% dry matter. FPS also had similar ($p < 0.05$) colour but differed in paste clarity, ranging from 51 to 63% of the FPS gels. The starches from all accessions displayed similar ($p < 0.05$) amylose/amylopectin ratio, syneresis %, granule types and shapes. Differences were observed in the thermal properties of the starches, even though XRF and FTIR spectra revealed them to be A-type starches, which is typical of root crops. The variations in granule size and thermal properties between FPS likely affect FP's cooking and eating quality.

The released accessions had significantly higher ($p < 0.05$) protein and ash, ranging from 5.1 to 8.7% and 5.1 to 6.5%, respectively, relative to the unreleased accessions. Four proteins commonly found in tuber crops (lipoxygenase, patatin, sporamin and tarin) and protease inhibitors (Bowman-Birk PIs) were identified in FPF based on their molecular weight (SDS-PAGE). Amino acids analyses (LC/MS) of the FPF identified seven (7) essential amino acids (Valine, Tryptophan, Threonine, Histidine, Methionine, Lysine, and Isoleucine). FPF showed similar functional properties, and their slurries exhibited typical shear-thinning pseudoplastic flow. Particle size analyses of FPF showed them to be generally fine particles, mostly passing through sieve size 100 μ m, and their sorption behaviour was characterised by a maximum allowable 10% EMC at about 0.5 a_w . Considering their fine particle size distribution, relatively high protein content and other functional properties, FPF was used as a replacement for wheat flour in the processing of gluten-free bread. The results showed that the application of dough conditioners (egg-gelatin powder combination) followed by Transglutaminase treatment could technologically be used to develop gluten-free bread from FPF with comparable attributes as that obtained from wheat flour (WTB). The dough and bread structure showed that the dough conditioners technologically mimicked the gluten-like network, as demonstrated by textural and dough properties. SEM revealed the improved network matrix and well-embedded starch granules in FPB comparable to WTB.

A panel of nine (9) trained assessors were used to assess six (6) bread samples, five of which were gluten-free, and one was typical wheat bread, using the Quantitative Descriptive Analysis (QDA®) method. The products were differentiated in appearance by brown crust (top and bottom), smooth bottom crust and homogeneous bottom crust and by a minor bitter flavour note perceived in the gluten-free products. The top and bottom crusts of WTB and B14 had the lowest intensity (lightest) of brown colour in the sample set, but they differed statistically ($p \leq 0.05$) from each other. All the bread samples smelled and tasted like typical wheat bread. Sensory profile of the products correlated strongly with the colour and dough properties of FPF. Thus, in developing bread from FPF, attributes of colour and dough properties should be considered.

The study showed that FPF has characteristics that make it suitable for gluten-free bakery applications and can help address nutrition and food security in Ghana and Sub-Saharan Africa by promoting its utilisation.



DEDICATION

This thesis is primarily dedicated to my mother, Nancy Osei Tutu and my lovely nieces and nephews for their support and prayers. I also dedicate it to my Supervisory Committee, the BASSAW family, the Department of Family and Consumer Sciences and friends who cared and supported my education. The Almighty God bless and keep you. A special dedication to my late father, Ing. Crosby Osei Tutu, whose diligence inspired me to have a positive attitude to work and life.



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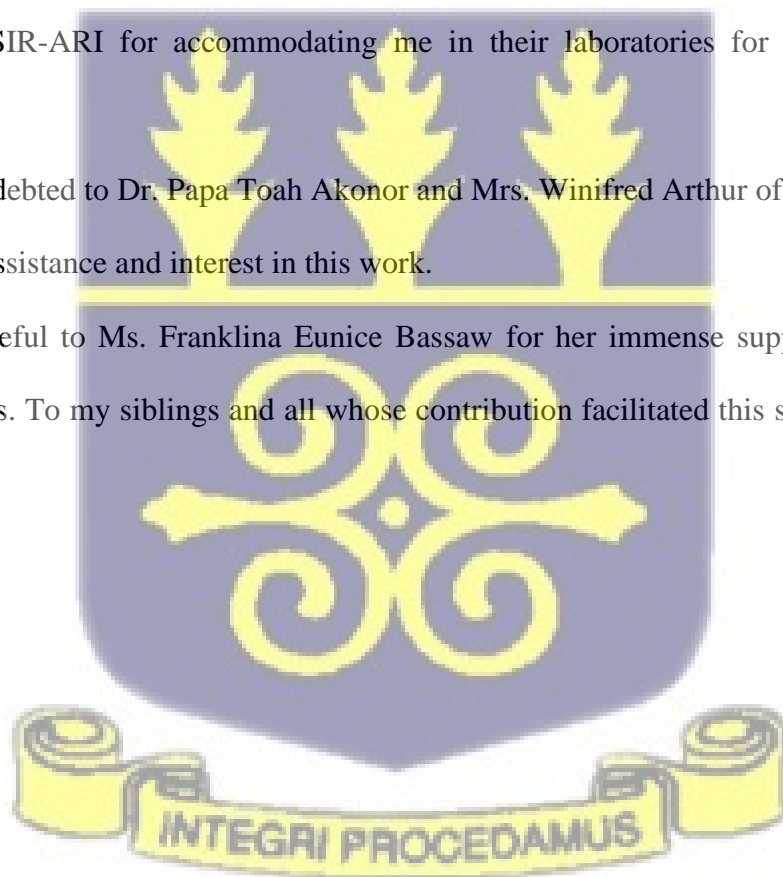
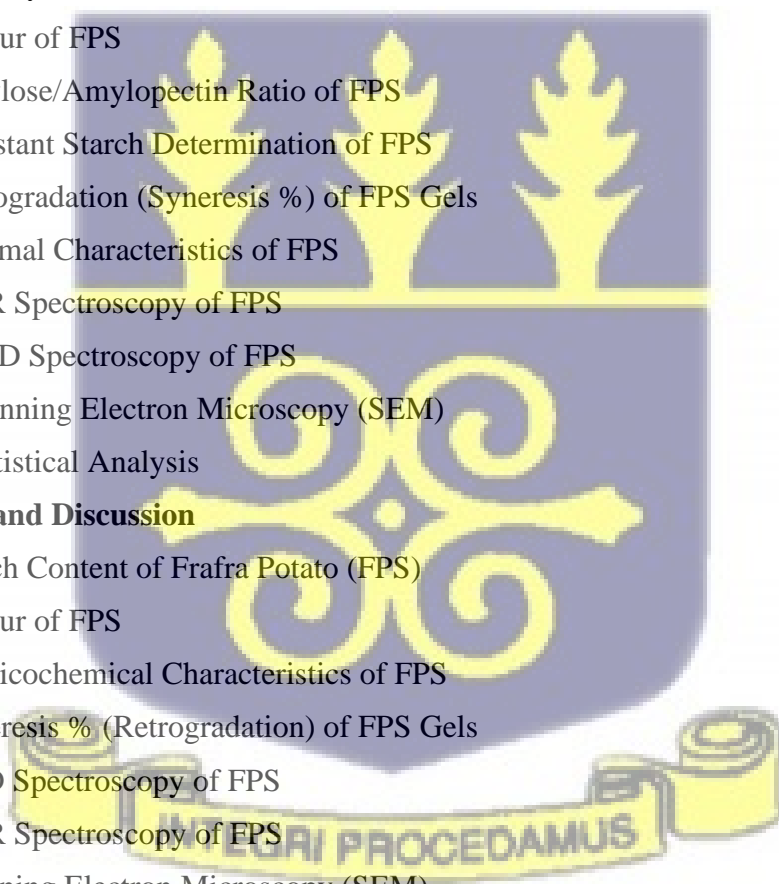


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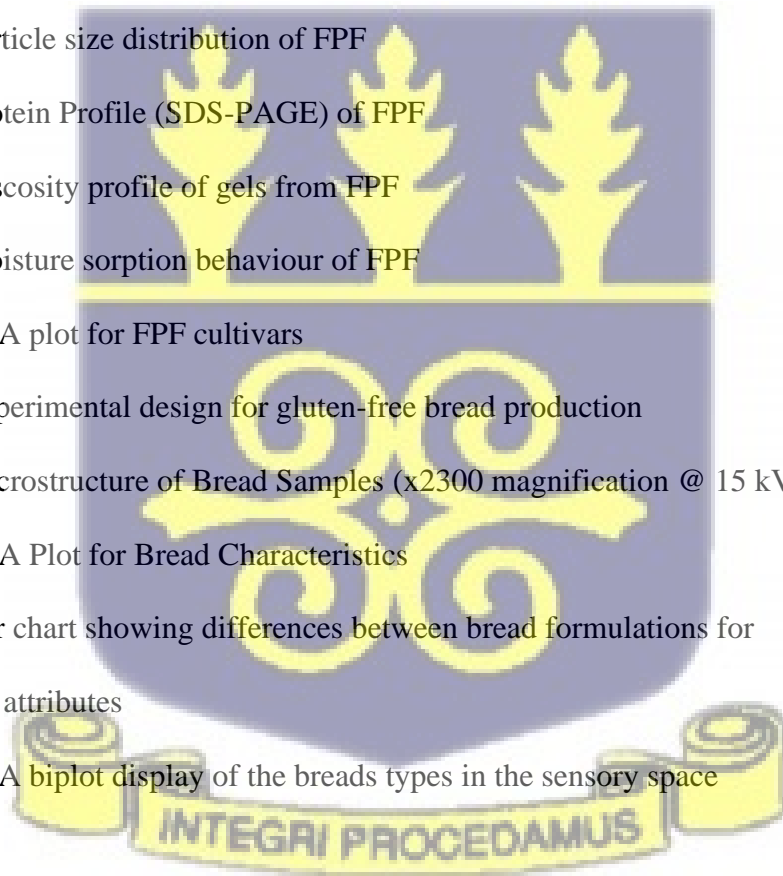
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LIST OF ACRONYMS

AACC	American Association for Clinical Chemistry
AOAC	Association of Official Analytical Chemists International
CSIR-ARI	Centre for Scientific and Industrial Research-Animal Research Institute
CSIR-FRI	Centre for Scientific and Industrial Research-Food Research Institute
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drugs Administration
FPB	Frafra potato bread
FPF	Frafra potato flour
FPS	Frafra potato starch
GSA	Ghana Standards Authority
SARI	Savana Agricultural Research Institute
SEM	Scanning Electron Microscope
TGase	Transglutaminase
USA	United States of America
USDA	United States Department of Agriculture
UK	United Kingdom
WF	Wheat flour
WB	Weight of bread
VB	Volume of bread
DB	Density of bread



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Climate change is disrupting all aspects of food and nutrition security globally, especially in developing countries. These countries must set up innovative ways to better manage their natural resources in order to alleviate hunger and poverty and make sure that people have access to nutritious food. Developing countries must adapt food production systems and processes to mitigate the demands of climate change for everyone to have safe and adequate food (FAO, 2008; Akanlu *et al.*, 2005). The effects of climate change, such as extreme temperatures, drought and unpredictable rainfall patterns are already causing significant losses to staple crops.

Consequently, it is imperative to adapt crop production systems by promoting climate-resilient crops. While these crops may not entirely be new to many communities, their production needs to be promoted and their utilisation extended to foods that are traditionally produced from non-climate resilient crops. Studies have explored the prospects of legumes such as yam bean and even fruits to formulate composites with wheat flour (Altan *et al.*, 2009; Alozie *et al.*, 2009) in pastry products such as cookies and composite bread.

Frafra potato (*Solenostemon rotundifolius*) is an underutilised climate-smart locally cultivated crop in Ghana and on the verge of extinction due to its low utilisation. Studies have shown the potential of such underutilised tubers (including sweet potato, yam and cassava) in the production of flour to partially or fully substitute wheat flour in pastry products (Osei Tutu *et al.*, 2019; Tortoe *et al.*, 2018; Komlaga *et al.*, 2012; Singh *et al.*, 2008). Wheat flour is widely patronized in Ghana, but it is expensive due to high importation costs. The successful substitution of wheat flour (partially or wholly) with locally processed (underutilised and climate-resilient) tuber flour in food applications will

have tremendous implications for food and nutrition security as well as for the local economy.

This study will investigate the suitability of Frafra potato flour (FPF) as a primary ingredient for gluten-free pastry products, with similar characteristics as wheat flour, with (or without) modifiers such as protein cross-linking agents.

1.2 Rationale

Changes in the climate have necessitated exploring the potential of neglected indigenous climate-smart crops on the verge of extinction to ensure food security in developing countries. Frafra potato is a nutritious locally cultivated climate-smart crop. The crop is a good source of carbohydrates, vitamin A, and micronutrients (zinc, iron, and calcium). Frafra potato has relatively higher protein, minerals and vitamin A concentrations compared to varieties of its close substitutes (sweet potato and yam); however, the utilisation of the crop is limited. Traditionally, it is consumed in the form of fried or boiled chunks. Current studies have explored the potential of its tuber flour as a partial substitute for wheat in pastry production. However, it has not been characterised extensively to understand its properties for culinary applications. Broad utilisation will be challenging without understanding the tubers' physicochemical, functional, and microstructural characteristics. Understanding the characteristics of Frafra potatoes and using them to promote the utilisation of the tuber crop can help address food and nutrition security, especially in the communities where the crop is cultivated. It could also provide food producers with a valuable alternative to wheat flour. Considering the nutritional benefits of Frafra potato, this study will characterise and investigate the potential food applications of Frafra potato flour.

1.3 Main Objective of Study

The main objective of this study was to process and characterise Frafra potato flour as an ingredient for food applications.

1.4 Specific Objectives of Study

The specific objectives of this study were to determine the:

1. Yield, physicochemical and microstructural characteristics of Frafra potato starches
2. Physicochemical, functional, and rheological characteristics of Frafra potato flours
3. Characteristics of gluten-free bread made from Frafra potato flours
4. Sensory profile of gluten-free bread made from Frafra potato flours



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Agronomy of Frafra Potato

Frafra potato (*Solenostemon rotundifolius*) is an annual herbaceous plant belonging to the family of Lamiaceae. This family includes more than 200 species comprising ornamental, medicinal plants, and edible tubers crops (Nanéma *et al.*, 2009; Nkansah, 2004). *Solenostemon* is a tropical genus of Lamiaceae. *S. rotundifolius* is believed to have originated in Central or East Africa. It spread early throughout tropical Africa and into South-East Asia (India, Sri Lanka, Malaysia, and Indonesia) (Nanéma *et al.*, 2009; Nkansah, 2004). It is cultivated on a small scale in numerous West African nations (Burkina Faso, Ghana, Mali, Niger, Nigeria, and Togo) (Tetteh and Guo, 1997). *S. rotundifolius* is variously referred to as Chinese potato, Sudan potato, country potato, Frafra potato, Hausa potato, Zulu round potato, innala, fabirama, or pessa (Nanéma *et al.*, 2009). It develops well in districts getting yearly precipitation between 700- and 1000-mm. Yields usually range from 7 to 15 t/ha; however, they may reach 18 to 20 t/ha, 30 t/ha, or 45t/ha under favourable conditions (Tortoe *et al.*, 2018; Nanéma *et al.*, 2009; Nkansah, 2004; Tetteh and Guo, 1997). Frafra potato (*Solenostemon rotundifolius*) belongs to the *Solenostemon* genus. The plant's scientific name (*rotundifolius*) means 'with round leaves'. It is occasionally described as *Coleus dysentericus* (Tindall, 1983), *Coleus rotundifolius* (Bejoy *et al.*, 1990; Mohankumar and Nair, 1990; Vasudevan and Jos, 1992), *Coleus parviflorus* (Abbiw, 1990; Yayock *et al.*, 1988). It is white, red or dark-brown tubers cultivated in some West African countries such as Nigeria as a staple and in India as a vegetable. It is considered a staple in Ghana because it is eaten as a main meal (Akanlu *et al.*, 2005; Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997). It is a neglected crop of minor economic importance due to post-harvest

constraints and the threat of genetic erosion by high yielding close substitutes (sweet potato (*Ipomoea batatas*), yam (*Dioscorea* spp.) varieties) in Ghana.

2.1.1 Frafra Potato Varieties

There are two main types of varieties of Frafra potato. They include the traditional and improved varieties. (Tortoe *et al.*, 2018). Table 2.1 shows ten common varieties of Frafra potato cultivated in Ghana.



Table 2.1: Physical characteristics of some common varieties of Frafra potatoes cultivated in Ghana

Variety	Local name	Status (improved/traditional)	Shape / Colour
AC0102	“Nutsugah Piesa (Nutsugah’s Frafra potato)”	Improved	Oblong/Light brown
UE009	“Garu Piesa (Frafra potato from Garu)”	Traditional	Elliptical/Brown
UW010	“Jirapa Piesa (Frafra potato from Jirapa)”	Traditional	Oblong/Light brown
UW001	“Wa Piesa (Frafra potato from Wa)”	Traditional	Elliptical/Brown
UW022	“Naachem-Tiir (hope for the youth)”	Improved	Obovate/Brown
UE023	“Manga-Moya (well-done manga station)”	Improved	Fairly round/Brown
UE019	“Nangode Piesa (Frafra potato from Nangode)”	Traditional	Obovate/Brown
UW020	“Maa-Lana (I cannot believe how far I have made it.)”	Improved	Elliptical/Brown
UE021	“WAAPP Piesa 1 (WAAPP Frafra potato)”	Improved	Elliptical/Dark Brown
AC0201	“PGRRI Piesa (Frafra potato from PGRRI)”	Traditional	Fairly round/Brown

Source: Tortoe et al., 2018.

2.1.2 Frafra Potato Production and Varietal Distribution in Ghana

In Ghana, Frafra potato is mainly cultivated on a subsistence level in northern Ghana, specifically in districts such as Builsa, Kassena-Nankana, Frafra, Lawra-Nandom, Jirapa-Lambussie, Nandawli, and Wa. Frafra potato ranks highest among the tuber crops in Ghana

in protein and some micronutrients (Tetteh and Guo, 1997; Sugri *et al.*, 2013; Tortoe *et al.*, 2018).

Frafra potato is usually cultivated as a mono-crop but may sometimes be intercropped with yam, okra, maize, rice, sorghum, cowpea and bambara groundnut. It is usually grown on mounds and sometimes on ridges but not on flat surfaces. Tubers are primarily used for propagation, but softwood stem cuttings can also be used. Farm sizes range between 0.05 and 1.2 ha. When it is very much developed, 50 kg of potatoes may be harvested from just one of the small hills (Akanlu *et al.*, 2005; Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997). A wild type referred to as "Tug-piece", meaning "shrub Potato", has been identified by farmers. In descending order of importance, constraints to production include rapid tuber deterioration in storage, lack of suitable planting materials, pests and diseases, and insufficient soil moisture for maturing the crop (Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997).

Frafra potato does not require fertile soil to flourish or develop well. They can do well on marginal lands, even though they do better on soils rich in organic matter. They do not require chemical fertilizers and can be planted like other root tuber crops and harvested when required. They are developed from seed-tubers that have started sprouting (Sugri *et al.*, 2013; Quainoo and Bayorbor, 2002). After harvesting, farmers store the undersized tubers to utilise as seed-tubers.

In some cases, the seed-tubers are dried and put into a pot sealed with manure until the next season or stored in an ambient environment to keep them as a seed (Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997). In the Upper East region for example, farmers utilize millet straw to cover the seed-tubers and store them in a cool, dry place. After sprouting the tubers are planted on raised beds. The crop is planted between October to December. Frafra potato takes approximately 100 days from planting until maturity and the yield ranges

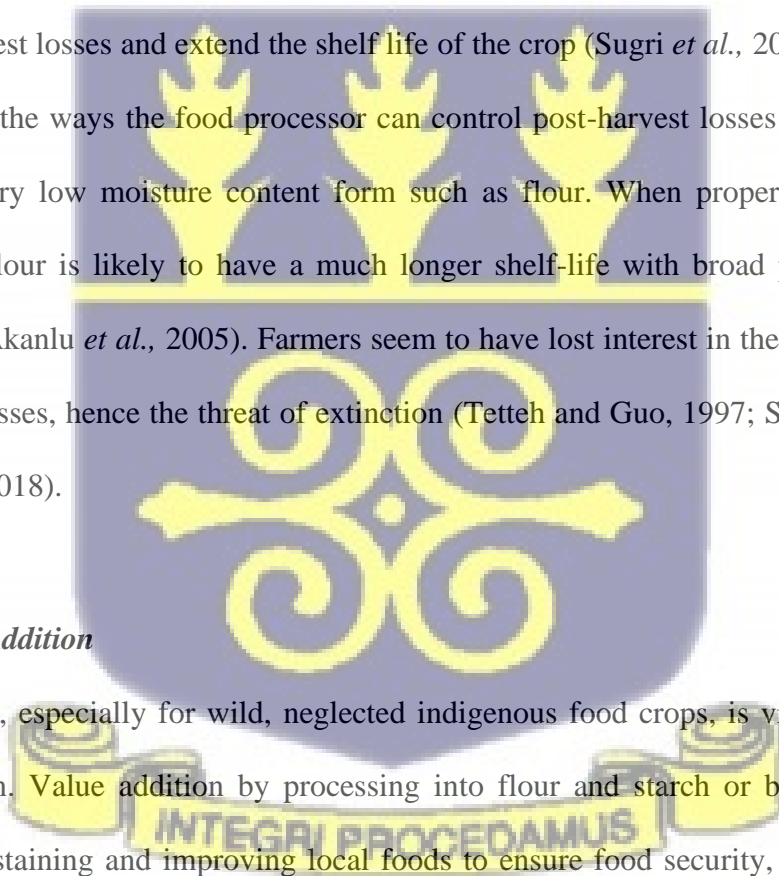
between 5 and 15 t/ha with natural and excellent planting conditions. Planning the soil to develop Frafra potatoes is exceptionally monotonous and work seriously. Making raised edges or hills sometimes requires more cultivated hands, depending on the measure of the cultivation. The sprouted tubers are planted at a 15 cm spacing (Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997).

2.1.3 Post-harvest Losses and Strategies for Controlling Post-Harvest Losses

Frafra potato has a relatively low yield and high post-harvest loss due to its perishable nature (Akanlu *et al.*, 2005). Its perishability is due to the high moisture content and size of the tubers. Therefore, breeders and food processors need to develop a protocol to control the high post-harvest losses and extend the shelf life of the crop (Sugri *et al.*, 2013; Tortoe *et al.*, 2018). One of the ways the food processor can control post-harvest losses is to process the crop into a very low moisture content form such as flour. When properly packaged and stored, tuber flour is likely to have a much longer shelf-life with broad potential in food applications (Akanlu *et al.*, 2005). Farmers seem to have lost interest in the crop due to high post-harvest losses, hence the threat of extinction (Tetteh and Guo, 1997; Sugri *et al.*, 2013; Tortoe *et al.*, 2018).

2.1.4 Value Addition

Value addition, especially for wild, neglected indigenous food crops, is vital to promoting their utilisation. Value addition by processing into flour and starch or by fortification is essential to sustaining and improving local foods to ensure food security, especially in the rural areas where 80% of poor, hungry people live (Akanlu *et al.*, 2005; FAO, 2008).



2.2 Importance of Frafra Potato

The significance of Frafra potatoes as a staple food has been supplanted by other tuber crops such as cassava (*Manihot esculenta*), yam (*Dioscorea spp.*), and sweet potato (*Ipomoea batatas*). The crop serves as an essential food crop, especially during the dry season in the northern parts of Ghana (Nkansah, 2004). The crop's potential in meeting the nutritional needs of consumers and replenishing soil fertility cannot be overemphasized (Sugri *et al.*, 2013; Nkansah, 2004; Tetteh and Guo, 1997).

2.2.1 Nutrient Composition of Frafra potato

Frafra potato is an essential source of dietary nutrients. The nutritional profile does not vary significantly among the varieties (Nkansah, 2004). However, ecological factors (geographic) and the stage of maturity during harvest may influence the nutritional composition of the crop (Nkansah, 2004). According to Tetteh and Guo (1997), nutritionally, the crop is mainly a carbohydrate food. The tubers contain considerable sums of calcium, iron and beta-carotene (vitamin A precursor), as Nkansah (2004) reported. It has also been reported that Frafra potato, with its relatively high nutritional advantage, could serve as an excellent alternative to flour in the bakery industry (Akanlu *et al.*, 2005; Sugri *et al.*, 2013; Tortoe *et al.*, 2018). Table 2.2 shows the composition of Frafra potato.

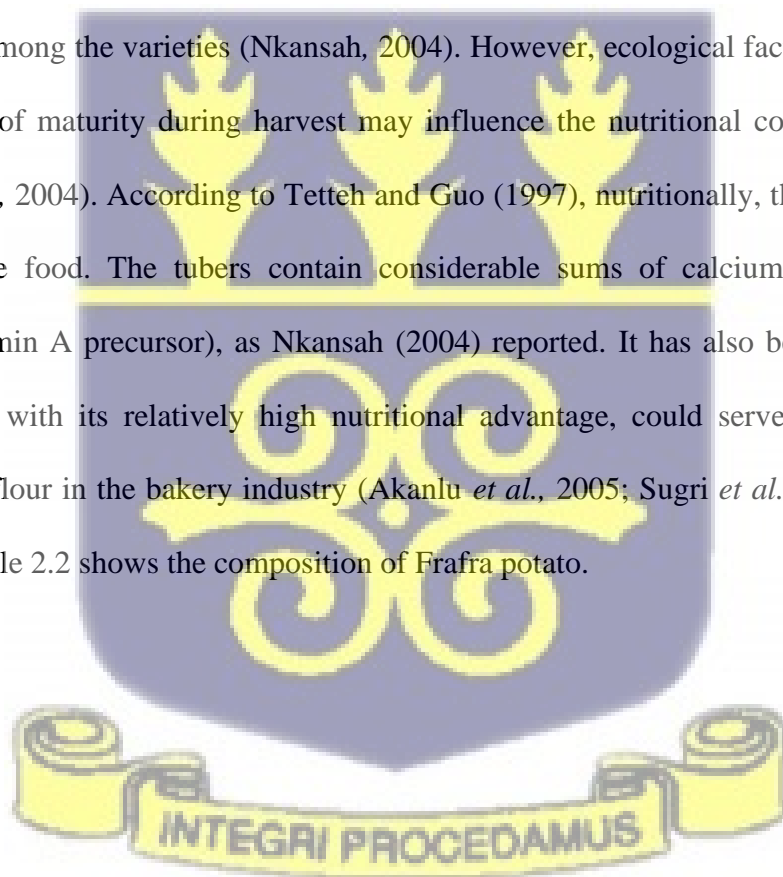


Table 2.2: Composition of the Frafra potato per 100 g edible portion

Composition	Per 100g	Source
Energy	94 kcal	(Tetteh and Guo, 1997)
Carbohydrate	21.9 g	
Protein	7.9 g	
Fat	0.2 g	
Fibre	1.1 g	
Calcium	17 mg	(Nkansah, 2004)
Iron	0.6 mg	
Thiamin	0.05 mg	
Riboflavin	0.02 mg	
Niacin	1.0 mg	
Ascorbic acid	1.0 mg	
Water	75.6 g	(Tetteh and Guo, 1997)

While the tuber is mainly a starchy food, it is also rich in protein (7.9 g/100 g). Compared with sweet potato (0.8 g/ 100 g), yam (1.8 g/100 g), cassava (0.7 g/100 g), Frafra potato ranks highest in protein and other nutrients among the tuber crops in Ghana (Tetteh and Guo, 1997; Sugri *et al.*, 2013; Tortoe *et al.*, 2018).

2.2.2 Agricultural Significance of Frafra Potato

Frafra potato is a potential source of income for farmers, especially in the northern parts of Ghana (Akanlu *et al.*, 2005). It is grown on a subsistence level on tiny portions of millet farming lands (Sugri *et al.*, 2013). The farmers use it in crop rotation programs to replenish soil nutrients since it makes less demand for soil nutrients (Tetteh and Guo, 1997). The crop develops well in unfavourable conditions and is not labour intensive as the crop does well with very little attention. It is a potential industrial raw material and may be prepared into different nourishment items (Akanlu *et al.*, 2005).

2.3 Utilisation of Frafra Potato

It is consumed in various forms depending on the food preferences and eating habits of consumers. The tubers are a staple food in West Africa and an alternative food (vegetable) in countries such as India, where part of their production is used for animal feeding (Sugri *et al.*, 2013; Tetteh and Guo, 1997).

According to Tetteh and Guo (1997), the potatoes are more often than not broiled, browned or steamed, and served as an early morning meal. This is popularly called ‘children food’ by the local people of Northern Ghana (Tetteh and Guo, 1997).

Newborn babies are fed with porridge made from Frafra potatoes (Sugri *et al.*, 2013; Akanlu *et al.*, 2005; Tetteh and Guo, 1997). The potatoes are added to vegetable soup and cakes, which is believed to protect infants from diseases. They are also boiled and mashed and used like yam balls. Most of these foods are no longer prepared, and the knowledge of how to prepare them is diminishing because it has not been extensively documented (Tetteh and Guo, 1997).

2.4 Flour and Composite Flour Making

2.4.1 Cereal Flour

Cereals such as maize, rice, and millet are used for flour; however, wheat is a widely used cereal for flour production globally. Wheat (*Triticum spp.*) is one of the earliest food crops cultivated since the beginning of human civilization. There are various varieties of wheat, namely hard or soft, spring or winter wheat and red or white wheat (Mongi *et al.*, 2011; Pasha *et al.*, 2011; FAO, 2009).



The great demand for wheat comes from increasing population, urbanization and changing food habits and preferences (Euromonitor, 2011). Wheat's characteristic protein and gluten content make it the best cereal for flour production and the most suitable primary constituent in bread and other baked foods (Mongi *et al.*, 2011; Pasha *et al.*, 2011).

2.4.1.1 Importance of Wheat Flour

Wheat is considered a great source of protein, minerals, B-group vitamins, and dietary fibre, an excellent nutritional component of food (Komlaga *et al.*, 2012; Sanful *et al.*, 2010). Apart from human and animal consumption, wheat can be used for brewing wheat beer. In addition, wheat is incorporated into food production as a partial or complete substitute for meat and makes wheat straw composites (Seibel, 2006). In the face of the increased price of petroleum and allied biofuel, wheat is being used for bioethanol production (FAO, 2009).

2.4.1.2 Nutritional Significance of Wheat flour

The grain is usually oval and brown approximately 2 - 3% germ, 13 - 17% bran and 80 - 85% mealy endosperm (Kumar *et al.*, 2011). The bran consists of dietary fibre, which makes up 53% of its structure; it also contains vitamin B, protein, and trace minerals. The outer layer of the endosperm (the aleurone layer) is abundant in proteins. In contrast, the inner endosperm, which is the source of the wheat flour, is made up of energy-yielding starch, fats, major B vitamins such as riboflavin, niacin and thiamin and protein such as albumins, globulins, gliadins and glutenins that will form gluten (makes up 47% of the total protein content in wheat). This gives wheat flour the desired pasting properties that make it excellent in foods such as bread, pasta, and noodle production (Sramkova *et al.*, 2009; Javed *et al.*, 2012).

2.4.1.3 Economic Impact of Wheat Flour Patronage in Ghana

In Ghana, all the wheat consumed is obtained from imports, making Ghana one of the largest wheat importers in Sub Saharan Africa (SSA) (Washington *et al.*, 2012). According to Washington *et al.* (2012), Ghanaians consumed an estimated 827,000 metric tons (MT) of wheat mainly as flour, with 80% of wheat flour utilised for bread making. In contrast, the remaining 20% was utilised for cakes and other baked goods. Three wheat-milling companies exist in Ghana with an add up capacity of 1,500 tons per day, but this caters for less than 10% of the total demand required to sustain the baking industry in Ghana. The demand for wheat in Ghana is soaring, resulting in more significant expenditure on wheat importation despite increases in world prices (USDA, 2021).

Ghana spent about \$167.1 million on wheat importation from 2019 to 2020. Wheat flour consumption is likely to increase to support increasing population growth, meaning the amount spent on wheat importation is predicted to increase (USDA, 2021).

With the steady increase in the consumption of wheat flour in Ghana, adoption of underutilised local crops such as Frafra potato will go a long way in reducing the import bill of the country and add value to the use of native crops, on the verge of extinction, to sustain them in the food chain. This will eventually alleviate poverty by generating income for the local farmers and contributing to food security.

2.4.2 Tuber Flour Production in Ghana

There have been extensive studies on the potential of tuber flour in Africa and Ghana. Among these is the Gratitude Project (G.P.) through the Food Research Institute (CSIR-FRI) developed technologies to produce high-quality cassava, yam, and Frafra potato flour (Osei Tutu *et al.*, 2019; Tortoe *et al.*, 2018; Gratitude Project, 2015). Osei Tutu *et al.* (2019) and

Tortoe *et al.* (2018) investigated the potential of Frafra potato flour in making composite bread and ‘koose’ (a local snack in Ghana). Ofori *et al.* (2009) also investigated the potential of sweet potato flour in food applications.

Cassava and yam are important food security crops in most of sub-Saharan Africa and Asia because their farming framework expands the capability of manageable cultivation despite drastic environmental changes (Gratitude Project, 2015). After harvesting, losses in cassava and yam are huge and come in three structures; physical waste, monetary waste, and wastes from the biowaste. Out of the products that can be derived from the tubers, High-Quality Flour (HQF) stands out because of the potential of utilizing the tubers on a large scale, which will accelerate the development of the country's agricultural sector. Possible uses of HQF include instant *fufu* preparation, composite flour (with wheat flour) for producing pastry products and other culinary applications (Tortoe *et al.*, 2018; Gratitude Project, 2015). Figure 2.1 shows a flow chat of tuber flour processing.



2.4.2.1 *Tuber Flour Processing*

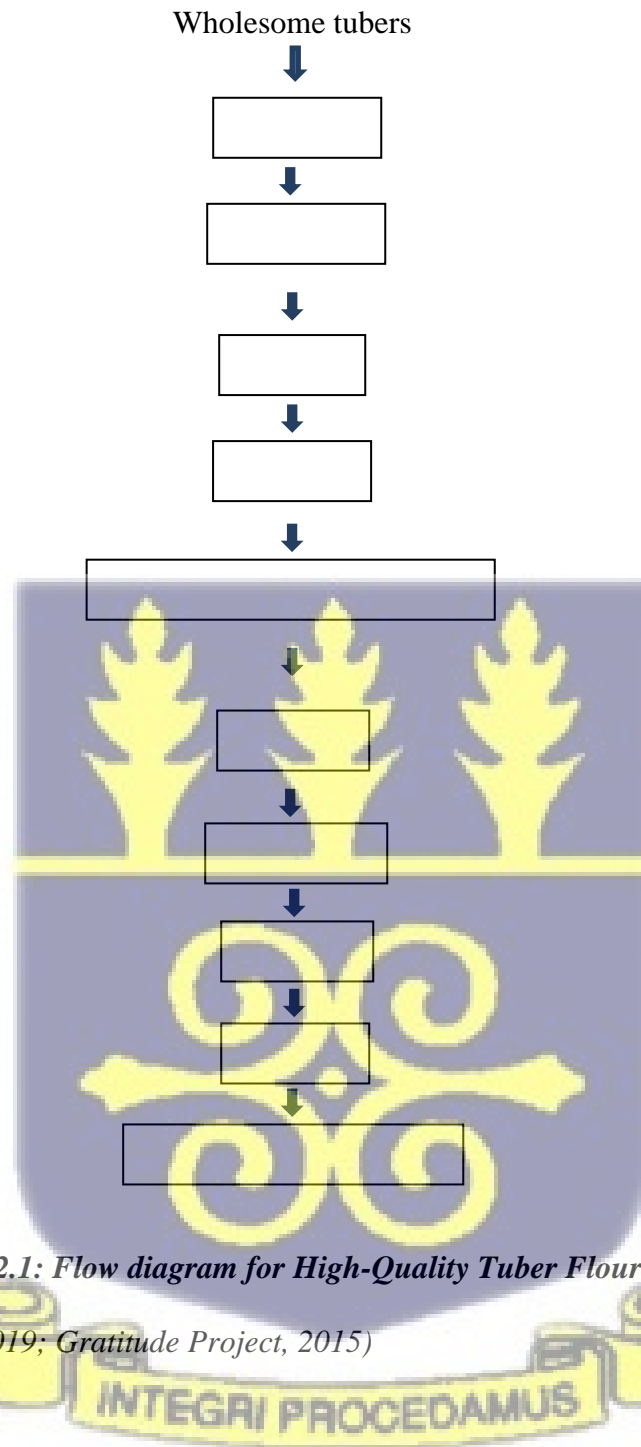


Figure 2.1: Flow diagram for High-Quality Tuber Flour production

(Osei Tutu et al., 2019; Gratitude Project, 2015)

2.4.2.1.1 *Washing, Weighing, and Peeling*

The tubers are washed to clean the soil residue on the tubers. They are weighed to determine their weight before processing. They are peeled to remove the outermost skin, which is not

edible, and washed again afterwards to ensure any exposure to dirt during the peeling process is gotten rid of (Tortoe *et al.*, 2018; Gratitude Project, 2015).

2.4.2.1.2 Cutting and Blanching

The peeled tubers are then blanched to prevent enzymatic browning by denaturing enzymes that may catalyze such a reaction and destroy microorganisms on the tubers (Tortoe *et al.*, 2018; Gratitude Project, 2015).

2.4.2.1.3 Drying and Milling

Drying is done in a controlled environment in an oven at a desirable temperature such as 50 - 60 °C for 10 – 12 hours, depending on the thickness of the tuber slices. The dried tuber slices are then milled in a laboratory mill and sieved to obtain the desired fineness of flour (Tortoe *et al.*, 2018; Gratitude Project, 2015).

2.4.2.1.4 Packaging and Storage

The flour is packaged in an airtight container and stored in a cool, dry place to prevent moisture absorption, which will cause the flour to grow mouldy, hence causing the flour to deteriorate (Tortoe *et al.*, 2018; Gratitude Project, 2015).

2.4.2.2 Importance of Tuber Flour Production

Tuber flour production contributes greatly to controlling post-harvest losses in tuber cultivation. Tuber flour production improves tubers' post-harvest management, reducing physical losses through value-added processing (Gratitude Project, 2015; Ofori *et al.*, 2009; Tortoe *et al.*, 2018).



2.4.2.3 Economic Impact of Tuber Flour Production

Approximately 30% of cassava and 60% of yam are lost annually, which occurs throughout Ghana's food chain. The waste associated with cassava and yam comes in various forms, as peeling losses can be 15 - 20% or higher. Tuber waste often has no economic value, making processing this waste into good form a negligible business plan. Tuber flour production improves the post-harvest management of tubers, leading to reduced economic losses through value-added processing and valorization of waste products (Gratitude Project, 2015; Ofori *et al.*, 2009; Tortoe *et al.*, 2018).

2.4.3 Composite Flour

Several institutions and organizations such as CSIR-FRI and the Food and Agriculture Organization of the United Nations (FAO) have examined the potential of composite flour in the face of population increases and wheat flour consumption over the years. This has mainly been because of rapid urbanization, changing food habits, the need for variety, coupled with a tremendous increase in population (Gratitude Project, 2015; Ofori *et al.*, 2009). The FAO has supported research intending to develop composite flour and alternative flour to reduce the over-dependence on wheat, especially by developing countries that are most affected by the trade-in wheat and countries who, because of climate conditions, cannot cultivate wheat to sustain the needs of their populations (Seibel, 2006; Fellers and Bean, 1988; Dendy, 1992).

Composite flour is made up of partial or total substitution of wheat flour with flour from local food crops such as tubers (cassava, cocoyam, sweet potato, yam) and protein-rich flour (soy, peanut) and cereals (maize, rice, millet) that serve as a means of diversifying and improving utilisation of local agricultural food products (Akubor *et al.*, 2013; Seibel, 2006;

Akubor, 2004). The secondary flour obtained can be incorporated into various staple foods. Yam, sweet potato, cocoyam, tapioca, cassava, Frafra potato, cowpea, African yam bean, and many other indigenous crops have been used to form a composite with wheat flour to improve the nutritional and fibre content of flour and flour products, combat dietary-related challenges such as protein-energy malnutrition (PEM) in Sub-Saharan Africa as well as reduce the over-dependence on wheat (Yadav *et al.*, 2012; Sanful *et al.*, 2010; Olaoye *et al.*, 2006). The extensive studies conducted on tuber flours provide a solid background for investigating the feasibility of processing Frafra potato into flour and assessing its potential in baking applications.

2.4.3.1 Importance of Composite Flour

There is adequate literature on composite flour globally; all the studies conducted investigated the feasibility of using composite flour in the confectionary and bakery industries (Alam *et al.*, 2014; Ali and Halim, 2013; Amadou *et al.*, 2013). The Gratitude Project (2015), experimented with yam-wheat, and cassava-wheat composite flours in bread production. Results revealed that 10% - 20% substitution of yam or cassava flour produced which was rated alike with 100% wheat flour bread. Also, research involving the use of cowpea-wheat composite flour in the cake, “chin-chin” and “puff-puff” production was investigated, 10% - 50% substitution of cowpea flour resulted in food products with similar sensory and physical properties as the control 100% wheat flour. It showed that the replacement of wheat flour in baked foods was achievable and resulted in products that satisfied consumers (Alozie *et al.*, 2009). Tuber-cereal composite flour has also been employed to produce various products (Alam *et al.*, 2014; Ali and Halim, 2013). In addition, 10% substitution of either cocoyam flour, cassava flour, plantain flour or breadfruit flour resulted in bread which scored 81% - 100% during sensory analysis, with cocoyam being the

closest (91%) to the control 100% wheat flour bread (Olaoye *et al.*, 2006; Mongi *et al.*, 2011). Several studies have reported on composite flour technology with varying levels of success (Gratitude Project, 2015; Chinma *et al.*, 2012; Ofori *et al.*, 2009; Ikegwu *et al.*, 2010; Yadav *et al.*, 2012; D'Appolonia, 1997).

The incorporation of local food crops was feasible and increased the resultant flour's nutritional properties and the final product's health benefits. These studies will serve as an excellent bedrock for investigating the potential of Frafra potato-wheat composite flour in baking applications.

Yam-wheat composite flour showed a corresponding increase in B-complex vitamins, calcium, potassium, iron, phosphorous and manganese content of the flour and bread produced with increased yam flour substitution, and this can be attributed to the high levels of these nutrients in yam; the resultant bread is of nutritive significance in many emerging countries where individuals can barely have enough money for nutritionally adequate meals (Olaoye *et al.*, 2006; Ndife *et al.*, 2011). In another study, it was found that lysine and tryptophan are the limiting amino acids in wheat protein; thus, incorporation of African yam bean flour in wheat flour complemented the limiting amino acids in wheat flour, thereby producing cakes of better nutritional quality with health benefits (Altan *et al.*, 2009). Frafra potato flour which is rich in energy, dietary fibre, vitamin A, E was mixed with Moringa flour rich in provitamin A, vitamin B, iron, manganese, and zinc was incorporated into wheat flour. This increased the protein, fat, crude fibre, and mineral content of the resultant composite flour while sodium content decreased from 189.20/100mg to 13.69mg/100mg. This resulted in composite bread samples that contained appreciable minerals, were low in starch and had high protein digestibility (Chinma *et al.*, 2012). Composite flour prepared from various roots and tubers, legumes, and cereals can supply the required amount of amino acids, fibre and minerals resulting in numerous health benefits.

Ohimain (2014) posited that implementing the composite flour program in five selected countries would promote the cultivation of 700,000 new hectares of indigenous tuber crops for local grain production, generate 51,000 new jobs, US \$110 million in value-added products and \$100 million in foreign exchange. These findings make it imperative for Ghana to adopt a composite flour program. It will utilize indigenous crops and reduce expenditure on wheat importation while contributing to overall health and wellbeing. However, it will also improve the livelihood of farmers and society in general.

2.5 Chemical Characteristics of Cereals and Root Tubers

Cereals and root tubers have carbohydrates (starch), protein, and minerals as their primary constituents. However, some root tubers (Frafra potato) have large amounts of beta carotene, a precursor of vitamin A (Tortoe *et al.*, 2018).

2.5.1 Starch

Starch is a carbohydrate that is abundant in nature and is one of the essential sources of energy for humans and non-food applications. It is an odourless, bland, delicate whitish substance made by green plants (Abo-El-Fetoh *et al.*, 2010; Wurzburg, 1995). The starch molecule's arrangement comprises numerous glucose units joined by glycosidic bonds. All vegetables deliver it as an energy store.

In 2012, plant-based starch generation worldwide was evaluated to be at around 75 million tons (Aprianita *et al.*, 2013). Starches can differ broadly in how rapidly they thicken, how much they thicken, the quality of the thickening, and their flavour after thickening. Choosing one starch over the other implies understanding its properties and how it behaves when

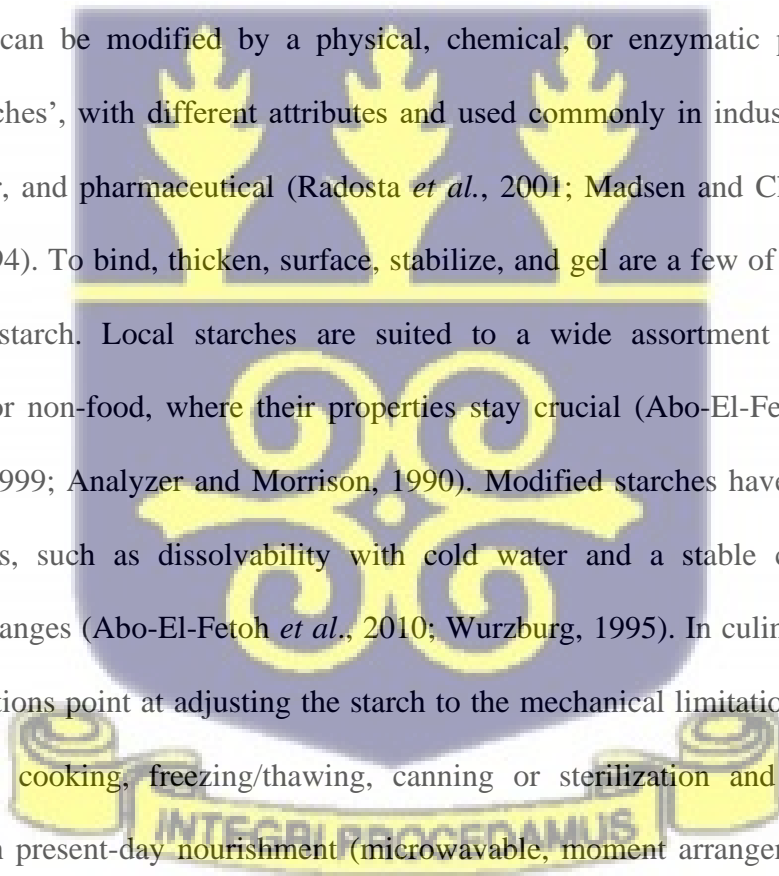
utilised for a particular reason (Abo-El-Fetoh *et al.*, 2010; Ikegwu *et al.*, 2010; Radosta *et al.*, 2001). Starches may be categorized into the following:

2.5.1.1 Native Starch

Once extracted, native starch could be a white bland and unscented powder that is insoluble in alcohol or cold water, which is utilised broadly within the culinary and paper industry essentially for thickening and binding purposes (Radosta *et al.*, 2001; Madsen and Christensen, 1996; Jane *et al.*, 1994).

2.5.1.2 Modified Starch

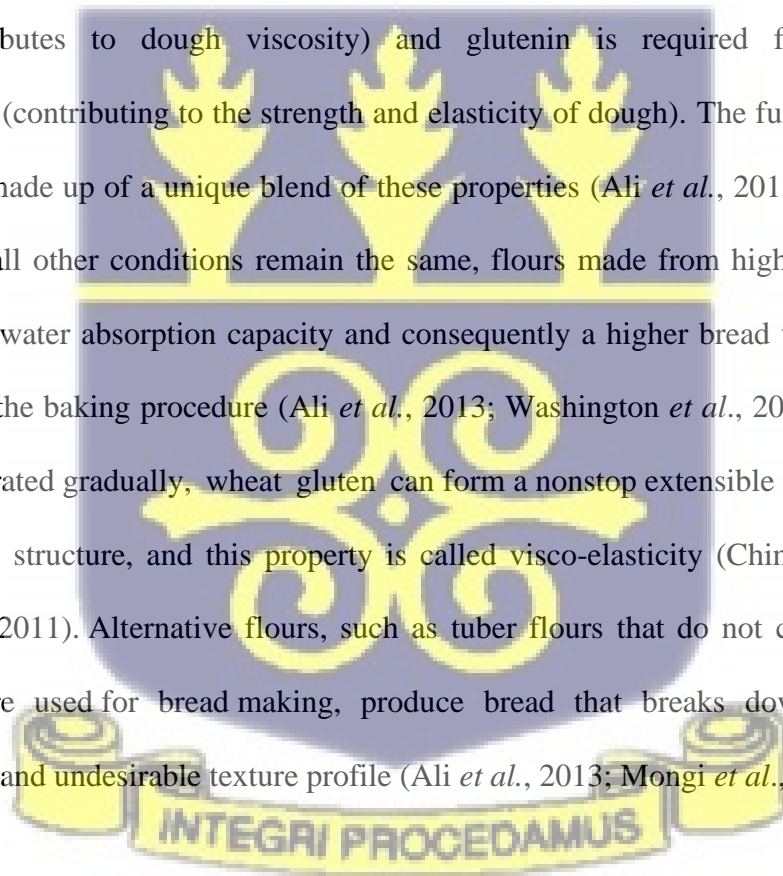
Native starch can be modified by a physical, chemical, or enzymatic process to make 'modified starches', with different attributes and used commonly in industries such as the culinary, paper, and pharmaceutical (Radosta *et al.*, 2001; Madsen and Christensen, 1996; Jane *et al.*, 1994). To bind, thicken, surface, stabilize, and gel are a few of the conventional capacities of starch. Local starches are suited to a wide assortment of applications, nourishment, or non-food, where their properties stay crucial (Abo-El-Fetoh *et al.*, 2010; Roger *et al.*, 1999; Analyzer and Morrison, 1990). Modified starches have been useful for their properties, such as dissolvability with cold water and a stable consistency with temperature changes (Abo-El-Fetoh *et al.*, 2010; Wurzburg, 1995). In culinary applications, the vital alterations point at adjusting the starch to the mechanical limitations coming about for case from cooking, freezing/thawing, canning or sterilization and to create them consistent with present-day nourishment (microwavable, moment arrangements, and ultra-high temperatures) (Abo-El-Fetoh *et al.*, 2010; Radosta *et al.*, 2001). One of the destinations common to most of these changes is to restrain the characteristic propensity of starch to retrogress. Amid the cooking of soup, for instance, the local starch is hydrated in contact



with water. The starch granules extend, and the thickness of the arrangement increments as well, giving it a surface (Abo-El-Fetoh *et al.*, 2010; Radosta *et al.*, 2001; Roger *et al.*, 1999).

2.5.1.3 Wheat Protein

From bread baking to cookies, wheat protein content is an important concern for all end products. Wheat proteins include gliadins, albumins, globulins, and glutenin (Kumar *et al.*, 2011; Musa Özcan *et al.*, 2006). Gliadin and glutenin are gluten proteins that account for around eighty percent of total protein in wheat flour and are found in roughly similar levels. The most important factors of wheat flour's functional characteristics are gliadin and glutenin (Muyonga *et al.*, 2014; Washington *et al.*, 2012). A correct balance of gliadin (which contributes to dough viscosity) and glutenin is required for ideal dough characteristics (contributing to the strength and elasticity of dough). The functional qualities of dough are made up of a unique blend of these properties (Ali *et al.*, 2013; Washington *et al.*, 2012). If all other conditions remain the same, flours made from higher protein wheat have a higher water absorption capacity and consequently a higher bread volume capacity, depending on the baking procedure (Ali *et al.*, 2013; Washington *et al.*, 2012; Mongi *et al.*, 2011). Rehydrated gradually, wheat gluten can form a nonstop extensible and hermetically sealed flexible structure, and this property is called visco-elasticity (Chinma *et al.*, 2012; Mongi *et al.*, 2011). Alternative flours, such as tuber flours that do not contain sufficient gluten and are used for bread making, produce bread that breaks down easily, has a small volume, and undesirable texture profile (Ali *et al.*, 2013; Mongi *et al.*, 2011).



2.5.1.4 Root Tuber Proteins

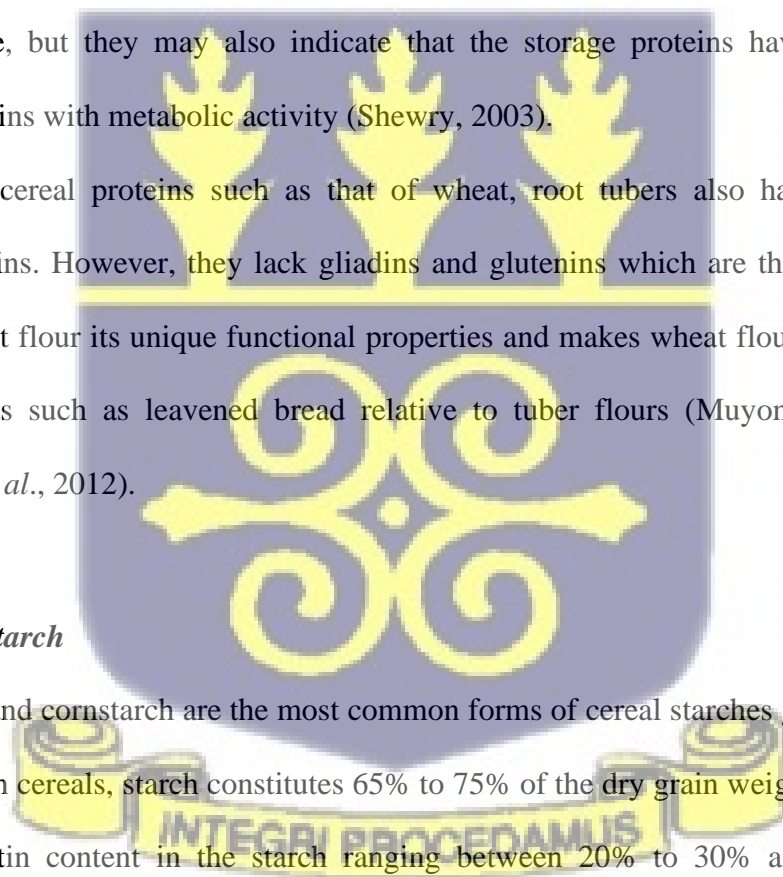
Root tuber usually contains storage proteins. Storage proteins can be defined as proteins whose primary role is to act as nitrogen, sulfur, and carbon stores. They may enable the plant

to survive periods of adverse conditions or between growing seasons and may provide nutrients to support the growth of new plants as seedlings (from seeds) or shoots (from tubers) (Alozie *et al.*, 2009; Shewry, 2003). They serve as a reservoir for nitrogen and sulfur, stored in more significant quantities under conditions of excess nutrient supply. They are also located in the cell in protein bodies which facilitates high-level accumulation without adverse effects on other cellular functions (Shewry, 2003). Most proteins in these groups have no known biological activity and are thought to function solely as storage proteins. Though they are related in their structures and evolutionary origins to groups of biologically active proteins, some 2S albumins have been shown to exhibit antifungal properties and inhibit serine proteinases when tested invitro. These activities may represent a substantial secondary role, but they may also indicate that the storage proteins have evolved from ancestral proteins with metabolic activity (Shewry, 2003).

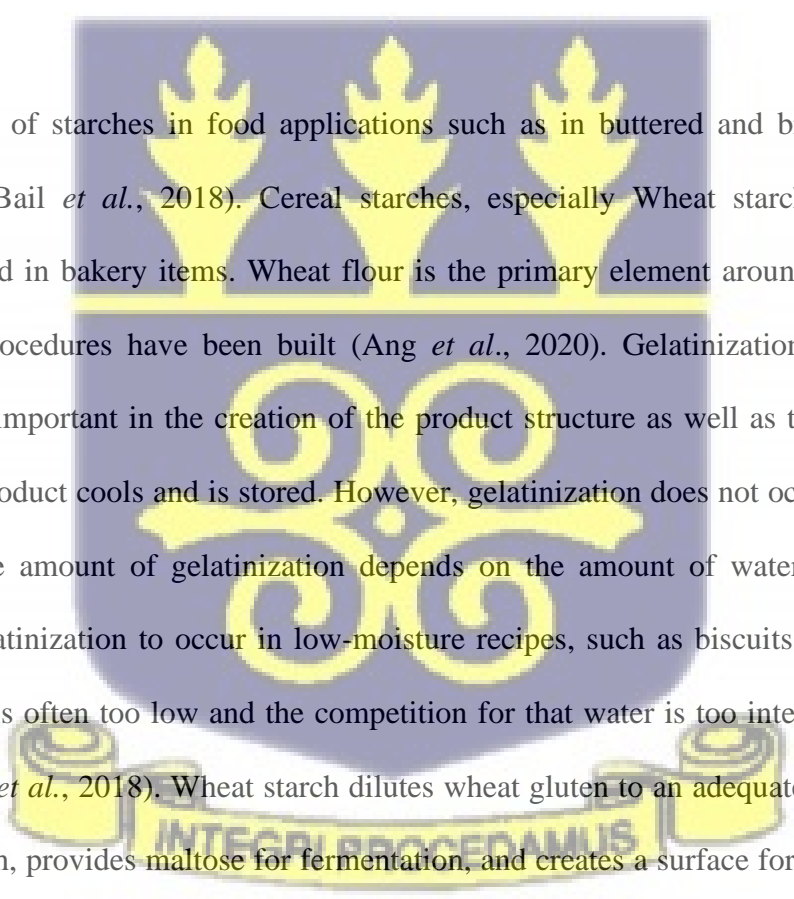
Compared to cereal proteins such as that of wheat, root tubers also have albumin and globulin proteins. However, they lack gliadins and glutenins which are the gluten proteins that give wheat flour its unique functional properties and makes wheat flour most suited for baked products such as leavened bread relative to tuber flours (Muyonga *et al.*, 2014; Washington *et al.*, 2012).

2.5.2 Cereal Starch

Wheat starch and cornstarch are the most common forms of cereal starches globally (Le-Bail *et al.*, 2018). In cereals, starch constitutes 65% to 75% of the dry grain weight, with amylose and amylopectin content in the starch ranging between 20% to 30% and 70% to 80% respectively. Previously, wheat with more than 28% amylose was classified as high amylose, but other studies have reported utilising wheat with 38% amylose. In recent years, scientists have developed a wheat variety with an 85% amylose concentration (Ang *et al.*, 2020).



Structurally, cereal starches' semicrystalline nature has been shown to be mostly an A type crystallinity, with diffraction pattern of X-ray showing characteristic peaks at 2θ angles ($\lambda = 0.15405$ nm): 10.1, 11.3, 14.9, 17, 18.1, 23, 23.9, and 26 degrees (Ang *et al.*, 2020; Le-Bail *et al.*, 2018). Cereal starches have medium-sized starch granules, with an average diameter of 10 μm , that gelatinize at higher temperatures than root starches (Abo-El-Fetoh *et al.*, 2010). However, once that temperature is reached, thickening happens very rapidly. These starches are nearly unadulterated starch, with cornstarch being a more efficient thickener than wheat starch. Cereal starches contain higher fats and proteins, making sauces thickened with these starches opaque and matte-like. These starches tend to have a particular cereal taste once cooked (Abo-El-Fetoh *et al.*, 2010; Radosta *et al.*, 2001; Roger *et al.*, 1999).

The image features a large, semi-transparent watermark of the University of Ghana crest in the background. The crest is a shield-shaped emblem with a blue field. At the top, there are three golden flames. Below the flames is a golden scroll with the Latin motto 'INTEGRUM PROCEDEMUS'. The shield is surrounded by a golden border.

The main role of starches in food applications such as in buttered and breaded foods is adhesion (Le-Bail *et al.*, 2018). Cereal starches, especially Wheat starch, is still most commonly used in bakery items. Wheat flour is the primary element around which baking recipes and procedures have been built (Ang *et al.*, 2020). Gelatinization occurs during baking and is important in the creation of the product structure as well as the changes that occur as the product cools and is stored. However, gelatinization does not occur in all baked goods, and the amount of gelatinization depends on the amount of water available. For significant gelatinization to occur in low-moisture recipes, such as biscuits and pastes, the water content is often too low and the competition for that water is too intense (Ang *et al.*, 2020; Le-Bail *et al.*, 2018). Wheat starch dilutes wheat gluten to an adequate consistency in leavened dough, provides maltose for fermentation, and creates a surface for strong bonding with wheat gluten. It allows for bread expansion during partial gelatinization and sets the loaf structure during baking. When it comes to the cooling stage, it creates a hard network

that prevents the loaf from collapsing and gives the baked product structural and textural features (Le-Bail *et al.*, 2018; Radosta *et al.*, 2001).

Retrogradation, which symbolizes the gel form by the reassociation of amylose chains, is another cooling event. During storage, this process involves the development of organized structures. Retrogradation is essential since it might be a desired end point in some applications, but it also causes bread to become stale and starch pastes to become unstable. The granules size distribution, and the amylose/amylopectin ratio of wheat starches makes them more suited to baking than other starches such as those from root tubers (Ang *et al.*, 2020; Le-Bail *et al.*, 2018).

2.5.3 Root and Tuber Starch

In root tubers, starch constitutes about 12% to 69%, with amylose and amylopectin content in the starch ranging between 13% to 49% and 51% to 87% respectively (Abo-El-Fetoh *et al.*, 2010; Radosta *et al.*, 2001). Previously, root tuber starches' semicrystalline nature were thought to be mainly B type crystallinity, with diffraction pattern of X-ray showing characteristic diffraction peaks at 2θ angles ($\lambda = 0.15405$ nm): 5.6, 10.1, 11.3, 14, 14.9 17, 19.5, 22, 26, and 23.9 degrees (Ang *et al.*, 2020; Le-Bail *et al.*, 2018). However, recent studies have shown that to be inaccurate as there are some root tuber starches that are of the A type crystallinity, which gives characteristic peaks at 2θ angles ($\lambda = 0.15405$ nm): 10.1, 11.3, 14.9, 17, 18.1, 23, 23.9, and 26 degrees, just like most cereal starches (Ang *et al.*, 2020; Le-Bail *et al.*, 2018). Root and tuber starches are larger-grained starches, with granule size ranging from about 14 to 42 μ m, that gelatinize at generally lower temperatures, with higher paste clarity (50 to 80%) compared to starches from cereals. Sauces thickened with root tuber starches are more translucent and glossier, and they have a silkier mouthfeel. These root starches do not perform as well as cereal starches to longer cooking time and so

they are best utilised to thicken sauces toward the end of cooking (Aprianita *et al.*, 2013; Abo-El-Fetoh *et al.*, 2010; Radosta *et al.*, 2001). Due to the absence of gluten in root tubers, when their flours are used in breaded foods, they do not give the baked product structural and textural features in cereals such as wheat flour (Ang *et al.*, 2020; Aprianita *et al.*, 2013).

2.6 Utilisation of Cereal and Alternative Flours

2.6.1 Cereal Flours

Cereal flours are made from cereals such as wheat, rye, and rice. They are widely used globally for confectionary, bakery, and pastry purposes (Ali *et al.*, 2013). Flours made from cereals such as rye and rice contain significantly less gluten when compared to wheat flour. Gluten gives wheat flour its unique and superior bakery and pastry quality. For example, in bread production, gluten enables the dough to rise during proofing by trapping carbon dioxide (CO₂) produced by the action of yeast from escaping (Ali *et al.*, 2013; Washington *et al.*, 2012). Gluten also gives baked foods structure, but it can make them hard if overworked. Hence, flours from cereals such as rye work well in cookies, cakes, biscuits, pancakes, and anything else that must be tender rather than chewy (Aprianita *et al.*, 2013). Wheat flour works well in chewy breaded foods; however, wheat flour products are controversial due to the gliadins and glutenins in wheat, a type of wheat protein. Gluten intolerance and Celiac disease are rising steadily; hence there is the need to explore gluten-free flour and its products (Ali *et al.*, 2013).

Even though cereal flours have excellent performance in baking applications, there is the need to sometimes resort to alternative or composite flours, especially in developing countries where the importation of all-purpose flour, which is made from wheat, is costly. Alternative or composite flours made from indigenous root tuber crops have the potential in

bakery applications to lessen the over-dependence on wheat flour, which is expensive (FAO, 2014; Ali *et al.*, 2013; Aprianita *et al.*, 2013). Table 2.3 shows the nutrient profile of wheat flour.

Table 2.3: Nutrient Profile of Wheat Flour

Nutrient	Amount	Unit	Derived By
Water	11.92	g	
Energy	364	kcal	Calculated
Energy	1523	kJ	
Protein	10.33	g	
Total lipid (fat)	0.98	g	
Ash	0.47	g	
Carbohydrate, by difference	76.31	g	Calculated
Fiber, total dietary	2.7	g	
Sugars	0.27	g	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Calcium, Ca	15	mg	
Iron, Fe	1.17	mg	
Magnesium, Mg	22	mg	
Phosphorus, P	108	mg	
Potassium, K	107	mg	
Sodium, Na	2	mg	
Zinc, Zn	0.7	mg	
Copper, Cu	0.144	mg	
Manganese, Mn	0.682	mg	
Selenium, Se	33.9	µg	Analytical
Vitamin C, total ascorbic acid	0	mg	
Thiamin	0.12	mg	
Riboflavin	0.04	mg	

Niacin	1.25	mg	
Pantothenic acid	0.438	mg	
Vitamin B-6	0.044	mg	
Folate, total	26	µg	
Folic acid	0	µg	“Assumed zero (Insignificant amount or not naturally occurring in a food, such as fibre in meat).”
Folate, food	26	µg	
Folate, DFE	26	µg	Calculated
Choline, total	10.4	mg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Vitamin B-12	0	µg	“Assumed zero (Insignificant amount or not naturally occurring in a food, such as fibre in meat).”
Vitamin B-12, added	0	µg	“Assumed zero (Insignificant amount or not naturally occurring in a food, such as fibre in meat).”
Vitamin A, RAE	0	µg	“Calculated
Retinol	0	µg	Assumed zero (Insignificant amount or not naturally occurring in a food, such as fibre in meat).”
Carotene, beta	0	µg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Carotene, alpha	0	µg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Cryptoxanthin, beta	0	µg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Vitamin A, IU	0	IU	Calculated
Lycopene	0	µg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Lutein + zeaxanthin	18	µg	“Based on another form of the food or similar food; Concentration adjustment; No

			adjustment; Retention factors not used. “
Vitamin E (alpha tocopherol)	0.06	mg	“Based on another form of the food or similar food; Concentration adjustment; No adjustment; Retention factors not used.”
Vitamin E, added	0	mg	“Assumed zero (Insignificant amount)”

Source: Washington et al., 2012.

2.6.2 Alternative Flours

Alternative flours refer to food ingredients other than traditional milled cereal flours, such as root and tuber flours (Aprianita *et al.*, 2013). They aim to improve the nutritional value of foods and satisfy the interest of producers and consumers to explore new textures, flavours, and aromas. These flours do not have the unique attributes of cereal flours, such as gluten, to make, for example, a loaf of bread without mixing with other gluten-rich flours (wheat flour) or dough conditioners. However, they are nutritious and rich in vitamins and micronutrients (Ali *et al.*, 2013; Aprianita *et al.*, 2013).

Studies have shown the potential of alternative flours in bread and noodles production using protein cross-linking enzyme (Transglutaminase) and dough conditioners (egg and gelatin powders). The enzyme and dough conditioners were used to mimic the visco-elasticity property of gluten and the gluten-like matrix in wheat flour products to improve the gluten-free dough properties as well as bread and noodle texture and structure. The gluten-free products made had some physicochemical and sensory characteristics similar to wheat flour noodles and bread. However, the wheat flour products had a superior texture profile relative to the gluten-free ones (Han *et al.*, 2021; Alencar *et al.*, 2017; Ali *et al.*, 2013).

The use of alternative flours is gaining interest in the food industry to offset the negatives of traditional wheat flour (Ali *et al.*, 2013). The alternative flour category is still in the development phase. Innovations are now possible due to advances in food science and technology to produce textures like wheat flour and much greater consistency than in the

past. Alternative flour products have been improved by using transglutaminases and gelatins to achieve results like those of traditional wheat flour products (Aprianita et al., 2013). As the alternative flour industry grows, the flours become more accessible, consistent, and widespread (Ali et al., 2013; Aprianita et al., 2013). These flours will also be more sustainably produced and processed with cleaner technologies. Rising interest in alternative flours will ensure a new set of sustainable and nutritionally dense ingredients to product developers.).

2.6.2.1 *Transglutaminases*

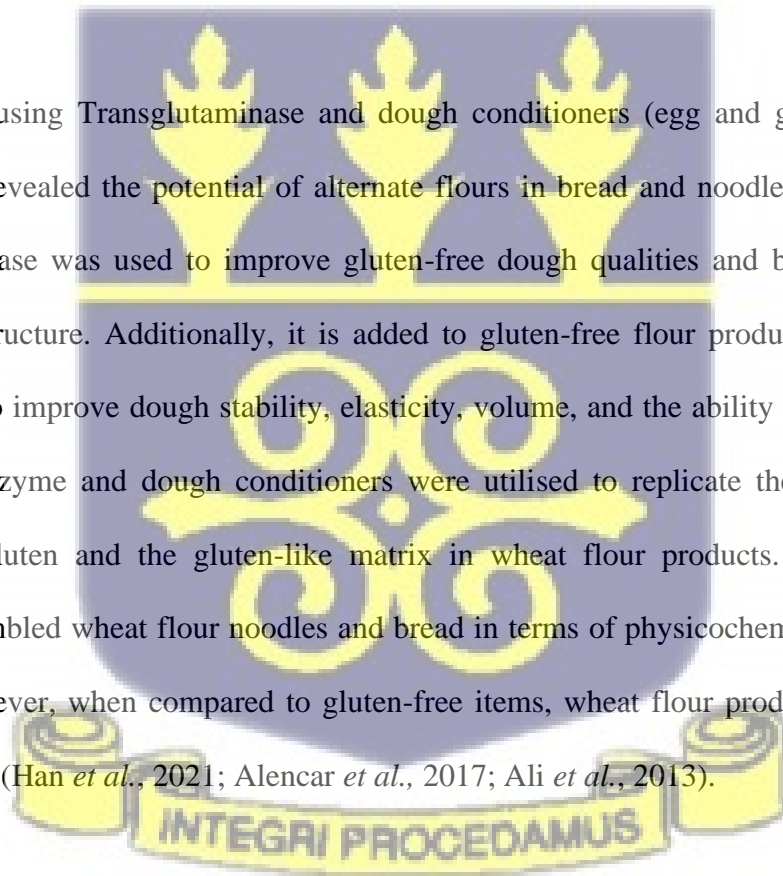
Transglutaminases, previously commonly referred to as “meat glue”, are enzymes found naturally in animals and plants (Griffin *et al.*, 2002). They are an extensively distributed group of enzymes that catalyze the post-translational modification of proteins by forming covalent bonds. This occurs either through protein cross-linking through lysine bonds or by incorporating primary amines at selected peptide-bound glutamine residues (Kieliszek *et al.*, 2014).

The transglutaminases used in food applications are produced either from the blood clotting factors of animals like cows and pigs or bacteria derived from plant extracts. It is usually sold in powder form. The covalent bonding property of transglutaminase makes it a valuable ingredient for food product developers (Kieliszek *et al.*, 2014; Griffin *et al.*, 2002). As its nickname suggests, it works like glue, holding together proteins found in everyday foods like meat, baked and pastry products (USDA, 2017). This enables food producers to improve the sensory characteristics of foods or develop new products, such as gluten-free baked products, by binding proteins in gluten-free flour to improve dough stability, elasticity, volume, and the ability to absorb water (Kieliszek *et al.*, 2014).

2.6. 2.1.1 Uses of Transglutaminases in Food Applications

It is used in various foods, including sausages, chicken nuggets, yoghurt, and cheese. Studies have shown that adding transglutaminase to chicken sausages made from various chicken parts improves texture, water retention and appearance (Kieliszek *et al.*, 2014; Griffin *et al.*, 2002). Chefs in high-end restaurants use it to produce innovative dishes like spaghetti made from shrimp (Kieliszek *et al.*, 2014). Since transglutaminase effectively fuses proteins, it is commonly used to create one piece of meat from multiple pieces. For example, a high-volume restaurant serving buffet-style meals may serve steak made by binding cheaper meat cuts with transglutaminase (Kieliszek *et al.*, 2014; Griffin *et al.*, 2002). It is also used in manufacturing cheese, yoghurt, and ice cream (USDA, 2017; Kieliszek *et al.*, 2014).

Additionally, using Transglutaminase and dough conditioners (egg and gelatin powders), studies have revealed the potential of alternate flours in bread and noodles manufacturing. Transglutaminase was used to improve gluten-free dough qualities and bread and noodle texture and structure. Additionally, it is added to gluten-free flour products, such as root tuber flours, to improve dough stability, elasticity, volume, and the ability to absorb oil and water. The enzyme and dough conditioners were utilised to replicate the visco-elasticity property of gluten and the gluten-like matrix in wheat flour products. The gluten-free products resembled wheat flour noodles and bread in terms of physicochemical and sensory features. However, when compared to gluten-free items, wheat flour products had a better texture profile (Han *et al.*, 2021; Alencar *et al.*, 2017; Ali *et al.*, 2013).



2.6.2.1.2 Safety Concerns over the use of Transglutaminases

With a nickname like “meat glue”, it is not surprising that there are safety concerns over the use of transglutaminase in food. There is no scientific research directly linking transglutaminase to an increased risk of disease, although research in this area is ongoing. The FDA classifies transglutaminase as GRAS (generally recognized as safe), and the USDA deems the ingredient safe to use in meat and poultry products (FDA, 2017; USDA, 2017). The European Union banned transglutaminase in foods, such as meat and poultry, in 2010 over safety concerns. However, it is considered safe for use in bakery or pastry products and must be listed in the ingredients (Kieliszek *et al.*, 2014).

2.6.2.2 Gelatin

Gelatin is a product derived from collagen. It can be made from animal parts by boiling them in water. The extracted gelatin is a lustrous, colourless, flavourless food ingredient that is soluble in warm water and takes on a gel-like rheological property when it cools. This makes it a suitable gelling agent in food processing, in products such as cereal bars, toffees, gluten-free bakery and pastry goods (Hegazy and El-Sayed, 2014). Unlike Transglutaminases, Gelatin is classed as a food and not subject to the food additives legislation globally. The worldwide gelatin production amounts to about 300,000 tons per year (roughly 300 thousand kilograms). It is available as supplements in powder or granule form and can also be purchased in sheet form (FDA, 2017; EFSA, 2006). Although gelatin contains 98-99% protein, it has less nutritional value than many other complete protein sources (Hegazy and El-Sayed, 2014). Common amino acids found in mammalian gelatin is shown in Table 2.4.

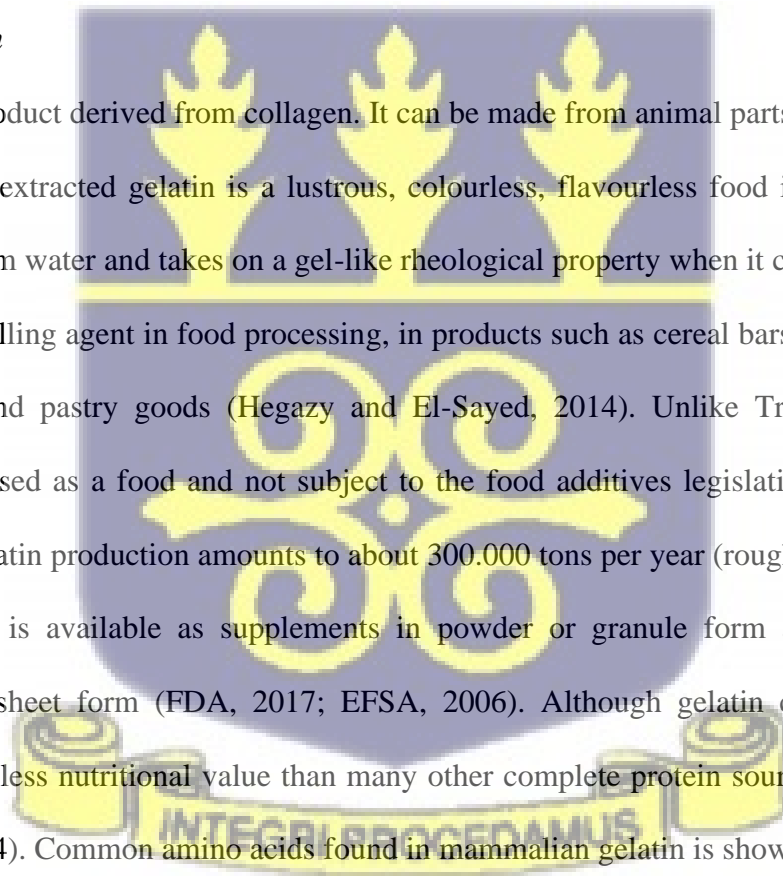


Table 2.4: Amino Acids Profile of Gelatin

Amino acids	Percentage
Proline, or hydroxyproline	25%
Glycine	27%
Glutamic acid	11%
Arginine	8%
Alanine	8%
Other essential amino acids	16%
Other non-essential amino acids	12%

Source: Eastoe, 1955

The exact amino acid concentration differs depending on the type of tissues utilised and the preparation method (Eastoe, 1955). Although the human body can make it, studies have shown that the body will not usually make enough to cover its needs (Hegazy and El-Sayed, 2014). This means it is vital to eat enough food. The nutrient content of the remaining 1 per cent to 2 per cent varies but comprises water and small amounts of vitamins and minerals like sodium, calcium, phosphorus, and folate. Nevertheless, gelatin is not a rich source of vitamins and minerals. Instead, its health benefits result from its unique amino acid profile (Eastoe, 1955).

2.6.2.2.1 Uses of Gelatin in Food Applications

Due to gelatin's unique amino acid profile is helpful in combination with transglutaminases in meat or gluten-free food products' development. It is widely used in home cooking to improve the rheological properties of food. It is usually stirred into smoothies, gravies, stews, and soups (Hegazy and El-Sayed, 2014; Eastoe, 1955). It is mainly used as a gelling

agent in cooking; different gelatin is used in a wide range of food products (FDA, 2017; EFSA, 2006). Good examples of gelatin foods include marshmallows, gelatin desserts, candy corn, and confections such as fruit snacks. Gelatin can be utilised as a stabilizer, thickener, or texturizer in nourishments such as yoghurt, cream cheese, and margarine. It is additionally utilised in fat-reduced foods to recreate the mouthfeel of fat and make volume. It is utilised to generate a few sorts of soup dumplings and sautéed and steamed dumplings. Gelatin is utilised for the clarification of juices (such as apple juice) and vinegar. Isinglass, a sort of gelatin, is gotten from the swim bladders of fish and utilised as a fining agent for wine and lager (Hegazy and El-Sayed, 2014; Eastoe, 1955).

Studies have highlighted the possibility of alternative flours in bread and noodles manufacturing using gelatin powder and Transglutaminase to improve the texture and structure of gluten-free bread and noodles. It was also used to increase dough stability, elasticity, and volume in gluten-free bread and noodles. The visco-elasticity property of gluten and the gluten-like matrix in wheat flour products were replicated using the enzyme and dough conditioners (gelatin and egg powders). The gluten-free products were similar to wheat flour noodles and bread in terms of physicochemical and sensory properties. On the other hand, Wheat flour products exhibited a better textural profile when compared to gluten-free items (Han *et al.*, 2021; Alencar *et al.*, 2017; Ali *et al.*, 2013).

2.6.2.2.2 Safety Concerns over the use of Gelatin

Gelatin is made from animal parts from the meat industry, including skin, bones, and connective tissue. The U.S. Food and Drug Administration (FDA) in 1997 began monitoring the potential risk of transmitting animal diseases, especially bovine spongiform encephalopathy (BSE), commonly known as mad cow disease (FDA, 2016 and 2017). An FDA study from that year posited that steps such as heat, alkaline treatment, and filtration

could effectively reduce the level of contaminating agents. However, there is no scientific evidence to suggest that these treatments would effectively remove the BSE infectious agent is present in the source material (FDA, 2017). On March 18, 2016, the FDA finalized three previously issued interim final rules intended to reduce further the potential risk of BSE in human food (FDA, 2016). The final rule clarified that gelatin is not considered a prohibited cattle material is manufactured using the expected industry processes specified (FDA, 2016). Gelatin Manufacturers of Europe (GME) posited that the risk associated with bovine bone gelatin is shallow or zero. In 2006, the European Food Safety Authority (EFSA) stated that the BSE risk of bone-derived gelatin was small and that it recommended removal of a request, in 2003, to exclude the skull, brain, and vertebrae of bovine origin older than 12 months from the material used in gelatin manufacturing (EFSA, 2006).

2.7 Physico-Chemical Characterisation of Flour and Composite Flour

2.7.1 Physical Characteristics of Flour

2.7.1.1 Particle Size Distribution

According to Aprianatia *et al.* (2013), the particle size of flours affect their properties and is a valuable indicator of quality and performance. The size and state of flour particles impact movement, viscosity, and compaction properties. Bigger and more circular particles will commonly flow more effortlessly than tiny and less circular particles (Aprianatia *et al.*, 2013; AACC, 2000). Tiny particles break up more rapidly and promptly to higher suspension viscosities than bigger ones. Smaller particle sizes and higher surface charges will usually enhance flour samples' suspension and emulsion steadiness (AACC, 2000). For numerous reasons, it is vital to measure and control the particle size distribution of some foodstuffs since they affect the outcome of end products (Abo-El-Fetoh *et al.*, 2010). The

most evident case is milling (or size reduction by another innovation), where the operation's objective is to reduce the particle size of food to a desirable requirement (Aguilar *et al.*, 2014). Numerous other size reduction operations and techniques likewise require laboratory estimations to track changes in particle size, including pulverizing, homogenization, emulsification, and micro fluidization (AACC, 2000). Detachment steps, such as screening and sifting, might be checked by measuring particle size before the procedure. Determining the particle size of flour requiring mixing is common since flour with similar and narrower distributions have similar effects on flavour, texture, and mouthfeel, and vice versa (AACC, 2000).

The particle size distribution considers the complete range of sizes in the sample. Sieve analysis, where powders or flours are separated on sieves of various diameters, is the most common method of assessing particle size distribution (Aguilar *et al.*, 2014). The particle size may be expressed as a range analysis, in which the amount in each size range is listed in order. It may also be presented in a cumulative form, in which the total of all sizes retained or passed by a single sieve is taken as the size for a given range of sizes (Abo-El-Fetoh *et al.*, 2010). Range analysis is suitable when a particular ideal mid-range particle size is being sought. In contrast, cumulative analysis is used to control the amount of undersized or oversized particles (Aguilar *et al.*, 2014).

Particle size and distribution have a significant impact on food ingredient functionality. The size of flour particles influences the viscosity and compaction qualities of flours. Larger, more circular particles will often flow more freely than smaller, less circular particles. Smaller particles break up more rapidly and result in higher suspension viscosities than larger particles (Aprianatia *et al.*, 2013; Abo-El-Fetoh *et al.*, 2010). The suspension and

emulsion stability of flour samples is usually improved by smaller particle sizes and higher surface charge. It impacts the reactivity of particles involved in chemical reactions and their behaviour when they come into contact with water (Aguilar et al., 2014; Aprianatia et al., 2013). The particle size distribution of particulate food ingredients must be measured and controlled for these and other reasons, as it affects finished products outcome. Understanding the physical and chemical properties of food ingredients requires knowledge of particle size distribution. Depending on the functional features presented, finely processed flours may be suitable for use in baking and flour mix production. The similarities in the least gelation concentrations seen in various flours could be related to the processed flours' particle size distribution (Aprianatia *et al.*, 2013; Abo-El-Fetoh *et al.*, 2010).

2.7.1.2 Colour and Total Colour Difference

The tristimulus system is often used to determine food colour. It is a system for visually matching a colour against the three primary colours, red, green, and blue, under standardized conditions; the three results are denoted as X, Y, and Z, respectively, and are known as tristimulus values. The tristimulus values can also be used to determine a sample's visually perceived colour scheme. Even though they are calculated so that the values show a sample's reflectivity when visually compared with a standard white surface by an average viewer under average daylight, these values specify not only colour scheme but also visually perceived reflectance (Ohimain, 2014; Minolta, 1991).

Colour represents flour's chemical makeup and manufacturing history while also influencing function and the sense of nutritional value and safety. Flour's colour is affected by many factors, including variety, ageing, milling processes, and bleaching and maturing agents, resulting in a spectrum of colours ranging from brownish to creamy yellow to whitish. As a

result, from the beginning of modern flour manufacturing, the colour of flour has been an important research topic (Kareem *et al.*, 2015; Andrés-Bello *et al.*, 2013; Aprianita *et al.*, 2013).

Colourimeters are well-suited for analysing fine powders' colour, providing the precise data needed to perform quality inspections. Colourimeters use sophisticated spectral technology to measure the colour of flour, allowing for the formulation of colour standards and ensuring that all products fall within set tolerances. The Colour parameters of interest in flour are the L^* (lightness from darkness), a^* (redness), b^* (yellowness). These are scores that can be used to estimate total colour difference (ΔE^*) (Kareem *et al.*, 2015; Ohimain, 2014).

The colour of flour samples ultimately affects the colour and appearance of products made with the flour (Kareem *et al.*, 2015; Aprianita *et al.*, 2013). Food processors need to assess the colour of flour samples to make adequate provision to ensure the colour of the finished product is not affected negatively, as this would discourage patronage of the flour products (Aprianita *et al.*, 2013).

2.7.2 Chemical and Functional Characteristics of Flour

2.7.2.1 Proximate Composition

The proximate composition of flour may be determined according to AOAC methods (AOAC, 2005). Parameters such as moisture (method 925.40), crude fat (method 948.22), protein content (method 955.52), and ash (method 935.52) can be established, and carbohydrate can be estimated as the difference between the sum of the other components and 100%.

2.7.2.2 pH

pH is an essential parameter in foods because it impacts pigments (such as chlorophyll, carotenoids, anthocyanins) responsible for fruit colour, vegetables and meat colour (Mridula, 2011). Colour and texture are vital qualities and central points influencing tactile observation and buyer approval of baked goods, and these may be affected by pH (Andrés-Bello *et al.*, 2013; Mridula, 2011). pH has a pronounced impact on water absorption capacity and the tenderness of food products. The pH value affects many characteristics and processes such as protein characteristics as denaturing, gelification, enzymatic activities, development and death of microbes, incubation or the deactivation of microbial spores and biochemical effects such as Maillard reaction (Nasir *et al.*, 2010; Musa Özcan *et al.*, 2006). Therefore, information on pH impacts and its control amid production is vital to create safe and high-quality value-added foods.

2.7.2.3 Water and Oil Absorption Capacity

Proteins have both hydrophilic and hydrophobic properties, allowing them to interact with both water and oil in foods. Variations in oil and water absorption capacities could be attributed to differences in protein concentration, degree of interaction with oil and water, and possibly conformational characteristics (Olliver *et al.*, 2003; Pomeranz, 1998). Water absorption capacity is an essential functional attribute for flours in the development of buttered and breaded foods, as high water absorption capacity may ensure product quality (Ohimain, 2014; Popov-Raljić *et al.*, 2013). The ability of flour to absorb water is a valuable indicator of whether it can be incorporated into aqueous food formulations, particularly those involving dough handling. The amount of water available for gelatinization is indicated by water absorption capacity (Ohimain, 2014; Jacob and Leelavathi, 2007). Water is a significant element of all foods. The interaction of water with other food constituents,

particularly macromolecules such as proteins and polysaccharides, determines foods' rheological and textural properties (Olliver *et al.*, 2003). The physicochemical properties of proteins are altered by water. Many functional properties of proteins rely on water-protein interactions, including swelling, solubility, thickening, water-holding capacity, gelation, and foaming. Flour has been shown to reduce hydration and air incorporation, resulting in denser and less spongy products. The particle size distribution and milling quality of flour influence its water absorption (Popov-Raljić *et al.*, 2013; Pasha *et al.*, 2011).

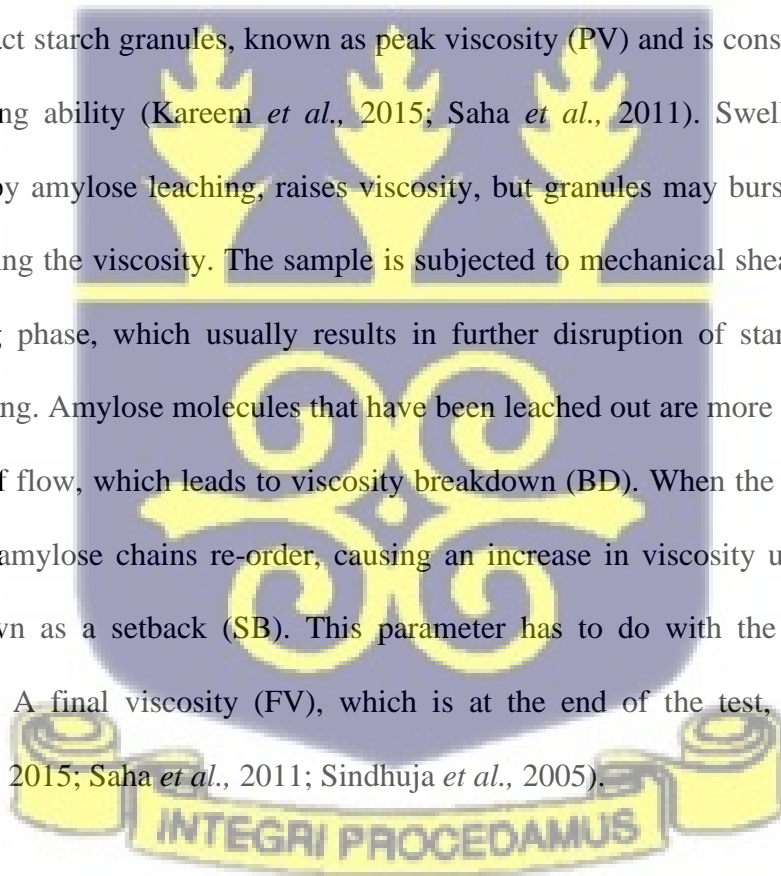
Oil absorption indicates the behaviour of the food during processes such as baking, roasting, and frying. If a food ingredient has a high oil absorption capacity, it will likely become soggy and require a short contact time in oil, such as deep-frying (Ohimain, 2014; Pasha *et al.*, 2011). The ability of flours to absorb fat is known to be important in food formulation and sensory characteristics such as mouth-feel and flavour. The high value of some flours' oil absorption capacity demonstrated that it improves mouthfeel when used in food preparations such as bread. Low fat absorption capacity of flour means lower flavor retention, which could be due to a low hydrophobic protein content when compared to flours with superior binding lipids (Popov-Raljić *et al.*, 2013; Pasha *et al.*, 2011).

Flavour, aroma, and texture are critical sensory characteristics of flour products and significantly affect sensory perception and consumer acceptance of flour products (Ohimain, 2014; Jacob and Leelavathi, 2007). These sensory characteristics of the food product are influenced by the water and oil absorption capacity of the flour used to produce the products. Products made with good oil absorption capacity are likely to have good flavour and aroma, imparted by the relatively high fat absorbed into the product (Olliver *et al.*, 2003). On the other hand, water absorption tends to influence the volume of the product (Popov-Raljić *et*

al., 2013; Pasha *et al.*, 2011). Therefore, water and oil absorption capacity and control during processing are necessary to produce safe, high-quality, value-added products.

2.7.2.4 Pasting Properties

The pasting properties of flour reveal its starch structure. When starch granules are heated above their gelatinization temperature in the presence of water, they absorb the water and swell, increasing viscosity (Kareem *et al.*, 2015; Saha *et al.*, 2011; Spies, 1990). Swelling is the arrangement of a three-dimensional framework (Tester and Morrison, 1990). In the Brabender Viscoamylograph, the temperature at the commencement of this rise in viscosity is known as the pasting temperature (PT). The paste's viscosity rises to the greatest number of swollen-intact starch granules, known as peak viscosity (PV) and is considered a measure of water-binding ability (Kareem *et al.*, 2015; Saha *et al.*, 2011). Swelling of granules, accompanied by amylose leaching, raises viscosity, but granules may burst after additional heating, lowering the viscosity. The sample is subjected to mechanical shear stress during a 95 °C holding phase, which usually results in further disruption of starch granules and amylose leaching. Amylose molecules that have been leached out are more or less aligned in the direction of flow, which leads to viscosity breakdown (BD). When the sample is cooled to 50 °C, the amylose chains re-order, causing an increase in viscosity until a gel forms, which is known as a setback (SB). This parameter has to do with the amylose chains' retrogradation. A final viscosity (FV), which is at the end of the test, is also recorded (Kareem *et al.*, 2015; Saha *et al.*, 2011; Sindhuja *et al.*, 2005).



Pasting properties are influenced by factors such as granule size distribution, granule volume fraction, granule-granule interaction, and amylose content (Sindhuja *et al.*, 2005; Singh *et al.*, 1993). Some cereal flours were classified into three groups (low, medium, and high

amylose content) to investigate the relationship between their amylose content and pasting properties. The temperatures at the onset, peak, and end of gelatinization were found to be highly positively correlated with amylose levels. Compared to high amylose flours, low amylose flours had a lower degree of retrogradation. Among the flours tested, low amylose flours had the highest peak viscosity and breakdown, as well as the lowest setback and pasting temperature (Kareem *et al.*, 2015; Saha *et al.*, 2011). Pasting properties are vital parameters needed to decide the usefulness of starch in flour for certain products and determine the types of starch sources for various purposes (Singh *et al.*, 2008; Shahzadi, 2004).

2.7.2.5 Starch Characterisation

Some functional food additives are commonly used in the food industry, including native starches and altered starches with high moisture-retention capacity and to give an end product with a desired appearance and texture (Bemiller, 1997). Each food item has its focal points and drawbacks; knowing them allows food product developers to attain the most excellent in utilising such added substances in particular handling conditions. Potato, corn, wheat, rice, custard, and other starches are utilised to get modified starches. The starches are handled utilizing physical, chemical, or enzymatic processes (Radosta *et al.*, 2001; Roger *et al.*, 1999). The starch properties depend on their physical and chemical characteristics, the granule size, the rate of dispersion, the amylose/amylopectin ratio, and mineral substance (Bemiller, 1997). Starch granules' shapes and sizes are the characteristics of their botanical root. The granules' frame may change from circular and adjusted to oval (Wurzburg, 1995; Madsen and Christensen, 1996).

Five main modifications can be highlighted from the changes in native starches: gelatinization, depolymerization, oxidation, stabilization (without cross-linking polymeric

chains), and formation of cross-linked starches (Roger *et al.*, 1999). Potato starch is one of the common polysaccharides utilised as thickeners and stabilizers. Potato starch has been broadly utilised in sausage due to its lower gelatinization temperature. Considering that pasteurizing sausage takes place at lower temperatures (70–72°C), there is a chance that any other type of starch granules would be incapable of gelatinating in such conditions (Buleon *et al.*, 1998).

Food product developers need to assess the type of starch to adopt appropriate technologies to fully take advantage of those properties or make modifications, where necessary, to achieve the desired product quality (Sarteshnizi *et al.*, 2015).

2.7.2.6 Gel Properties

A gel is an intermediate phase of a substance between a solid and a liquid. They are characterised by a lack of fluidity and elastic deformability. Gel formation is achieved by setting a particular pH, adding specific ions, or heating or cooling. Starch and protein gels are commonly prepared by heating with water. The type of gel formed is dependent on the molecular properties and solution conditions (Adebowale and Lawal, 2004). Least gelation concentration (LGC) is one of the techniques used to assess gel properties of flours. LGC is a measure of the minimum amount of flour required to form a gel in each volume of water. It is qualitatively measured as the minimum sample concentration at which the gel does not slide along test tube walls in an inverted position (Moure *et al.*, 2006).



Protein gels are highly hydrated systems containing up to 98% water. The stability of a gel network against thermal and mechanical forces is dependent on the number of types of cross-links formed (Adebowale and Lawal, 2004). A minimum protein concentration, known

as the least concentration endpoint (LCE), is required to form a self-standing gel network. Several intrinsic and extrinsic factors, such as temperature, pH, size, affect gel stability (Saha *et al.*, 2011; Sindhuja *et al.*, 2005). Another critical factor is protein concentration. The lower the least gelation concentration, the better the gelling ability of the proteins because proteins gels have aggregates of denatured molecules (Moure *et al.*, 2006; Adebowale and Lawal, 2004).

Also, starch gels are formed by heating with water, causing the starch granules to swell. As a result, the water is absorbed gradually and irreversibly. The reaction produces a gel, which is utilised to provide a pleasant texture in sauces, puddings, creams, and other food products (Kareem *et al.*, 2015; Saha *et al.*, 2011; Sindhuja *et al.*, 2005). The starch gel system has a fluid and translucent texture, with a high paste clarity (PC) and makes them more suitably for food systems in which high transparency is desired. In contrast, protein gels are primarily opaque with low paste clarity; this makes them more suited to food systems where transparency is not required, such as baked foods (Kareem *et al.*, 2015).

2.7.2.7 Bulk Density

Bulk density indicates the weight of powdered samples, such as flour, in unit volume, including intergranular air space. A high bulk density is vital in packaging and transportation, and it is desirable because it can significantly reduce costs (Saha *et al.*, 2011). Placing a known quantity of flour into a graduated cylinder and applying sufficient pressure to pack the content in the cylinder while recording the final volume is a common method of estimating the bulk density of flours (Sarteshnizi *et al.*, 2015). A variety of factors influence the bulk density of flours. The amount of air entrapped in the flour particles, the overall density of the particle, the air between the individual flour particles, and the particle size

distribution are all examples of these factors (Aprianita *et al.*, 2013). Flour's high bulk density suggests that it is suitable for buttered and breaded foods and for use as a thickener in food products. On the other hand, low bulk density would be advantageous in the formulation of complementary foods because it helps to reduce paste thickness in food systems, which is an important factor in convalescent and child feeding (Sarteshnizi *et al.*, 2015; Aprianita *et al.*, 2013).

2.7.2.8 Swelling Power

Swelling power is the maximum increase in volume and weight that starch undergoes when allowed to swell freely in the water when heated. The swelling capacity is a function of the process conditions, nature of the material and the type of treatment (Kareem *et al.*, 2015; Saha *et al.*, 2011; Spies, 1990). Biopolymers such as starch and proteins contribute to the development of this property. The swelling of insoluble proteins corresponds to the hydration of soluble proteins by inserting a water molecule between the peptide chains. This results in an increase in volume and other changes in the physical properties of the protein. The amount of water taken up by the swelling action can amount to multiple protein content (dry weight). The swelling power of starch and flour increases with temperature (Kareem *et al.*, 2015; Saha *et al.*, 2011; Spies, 1990). The degree of swelling and the amount soluble depends on the starch species. It has been proposed that bonding forces within the granules of starch affect swelling power. Consequently, highly associated starch granules, with an extensive and strongly bonded micellar structure, should display relative more excellent resistance to swelling (Andrés-Bello *et al.*, 2013)

2.7.2.9 Foaming Properties

Foaming properties are used as indices of whipping characteristics, which is an essential quality in flours. The formation of foam involves the incorporation of air into an aqueous phase. In the case of foam, water molecules surround air droplets (Andrés-Bello *et al.*, 2013). A protein that is utilised to form stable foam must rapidly diffuse to the interface and unfold in such a manner as to lower the interfacial tension between the air and water phase. Once the foam is formed, it has been observed that specific influences such as capillary pressure (capillary drainage), gravitational force and mechanical disturbances (for example, vibrations) decrease its stability. However, high surface viscosity and properties of constituent proteins significantly promote foams' stability (Andrés-Bello *et al.*, 2013).

Foam capacity and foam stability of food ingredients such as flours are often determined in food formulations. This provides information on the suitability of the ingredient for food products intended and its behaviour during processing and storage. For example, good foam volume and stability are desirable properties in the preparation of 'akara', as they significantly influence the final product's texture and, therefore, its acceptability (Ikegwu *et al.*, 2010). The sponginess of 'akara' is attributed to high air incorporation during mixing. The high foam capacity of some flours makes them suitable aerating agents in food systems such as 'akara' and 'moi moi', which require the reproduction of stable high foam volumes when whipped. Low foaming capacity could be due to inadequate electrostatic repulsions, lesser solubility, and excessive protein-protein interactions. A higher value for foam stability indicates highly hydrated foams, and decreases in foaming stability might be due to denaturation (Ikegwu *et al.*, 2010).

2.7.3 Water in Food (Water Activity a_w)

An essential property of water in food processing is the water activity (a_w) of food. Water activity is the proportion of the vapour pressure of a food product to the vapour pressure of pure water at the same temperature. The water activity of foods depicts the degree to which the water is accessible to support the biochemical activities of microorganisms (Andrade *et al.*, 2011). Controlling water in food by drying, freezing, or adding sugar or salt is able to preserve and control food quality and safety. Water activity levels of foods can predict the stability and safety of food concerning microbial activity, biochemical reaction rates, and physical properties. Hence, water activity is an essential property for food safety. Free water in food molecules supports the growth of bacteria, yeasts, and moulds (fungi) (Andrade *et al.*, 2011). Figure 2.2 shows a global stability map of foods, indicating stability as a function of water activity (a_w).



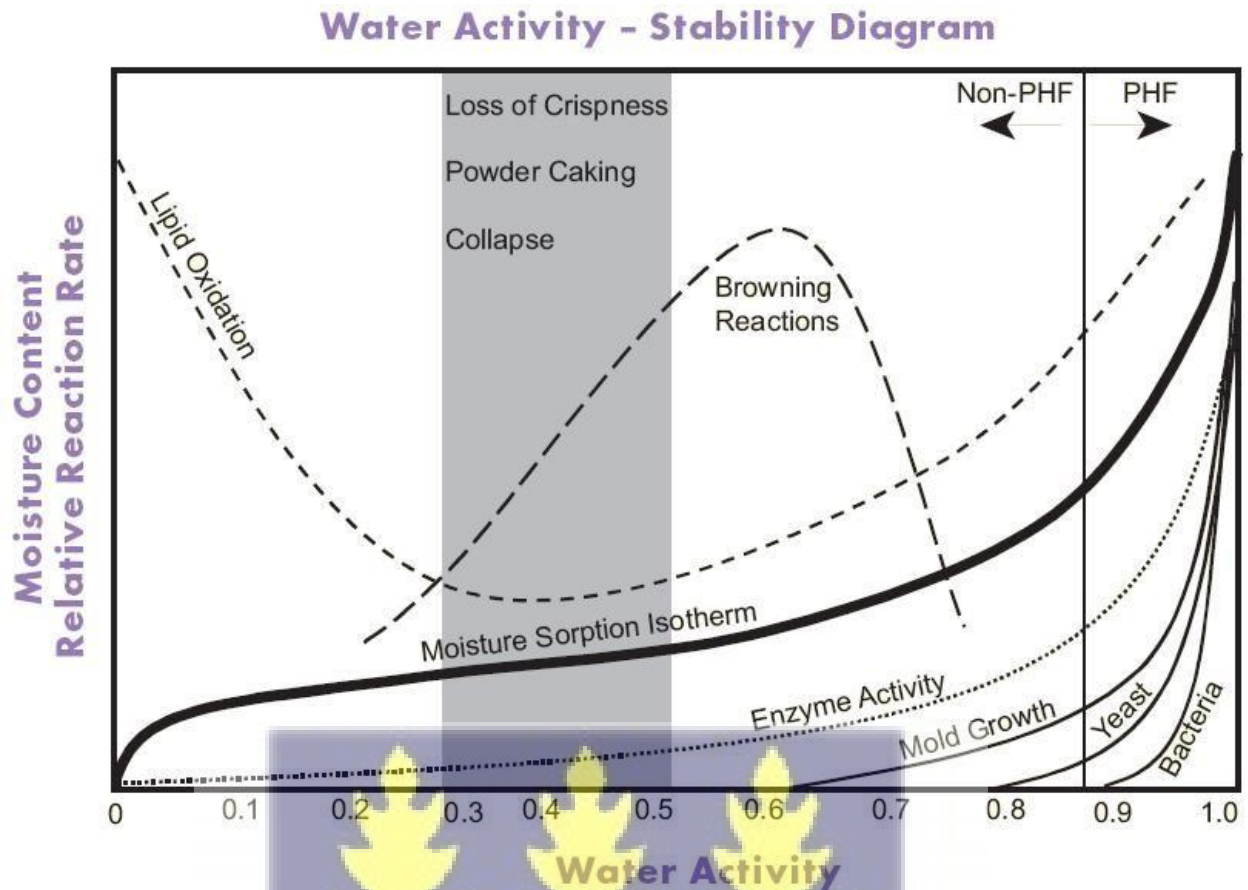


Figure 2.2: Water Activity – Stability Diagram

Source: Andrade *et al.*, 2011

Water's ability to act as a solvent, medium and reactant increases with increasing water activity. Measuring the water activity of foods enables predicting the types of microorganisms that are likely to be the source of spoilage and infection in the food. Water activity plays a significant role in determining physical properties such as texture, enzymes, and shelf-life of foods (Andrade *et al.*, 2011). Hence, controlling water activity is a meaningful way to maintain physical, chemical, and microbiological stability. The values of equilibrium moisture content of biological products depend mainly on the temperature and relative humidity of the species or variety of the product. The point at which a sample stops absorbing moisture from or releasing moisture into the surrounding air is its equilibrium moisture content. Physiological maturity and history of the product and how the equilibrium

was obtained (absorption or desorption) influences the equilibrium moisture content (Andrade *et al.*, 2011).

2.7.3.1 Moisture Sorption Isotherm

A moisture sorption isotherm describes the relationship between water activity and the equilibrium moisture content for a food product at constant pressure and temperature. The moisture sorption isotherm of products provides vital data on storage stability and extrapolation of shelf-life, indicating the humidity-water activity related to a given temperature (Andrade *et al.*, 2011).

Several models have been suggested in the literature for expressing the relationship between the equilibrium moisture content and the water activity. Among them, the Brunauer-Emmett-Teller (BET, 1938) and SMITH (1947) models have been applied successfully to starchy foods, including flours (Andrade *et al.*, 2011).

The principal methods for obtaining sorption isotherms for foods are gravimetric, manometric and hygrometric (Basu *et al.*, 2006). The gravimetric method exposes the samples to atmospheres of saturated salt solutions or sulfuric acid solutions of known relative humidity. This method is a lengthy and laborious process involving a series of repetitive weighing (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.2 Types of Sorption Isotherm

All food materials have a unique shape of sorption isotherm at different temperatures. The precise shape is based on the food item's physical structure and chemical composition and the extent of water-binding within the food. The physical and chemical properties and

sorption isotherm provide indices for food material characterisation and storage stability in varying humidity conditions. The absorption of water can occupy one or several layers. The best-known model for interpreting sorption isotherms is that of Brunauer *et al.* (1938) based on multilayer absorption (Andrade *et al.*, 2011). Five types of isotherms are described by BET in figure 2.3 as follows:

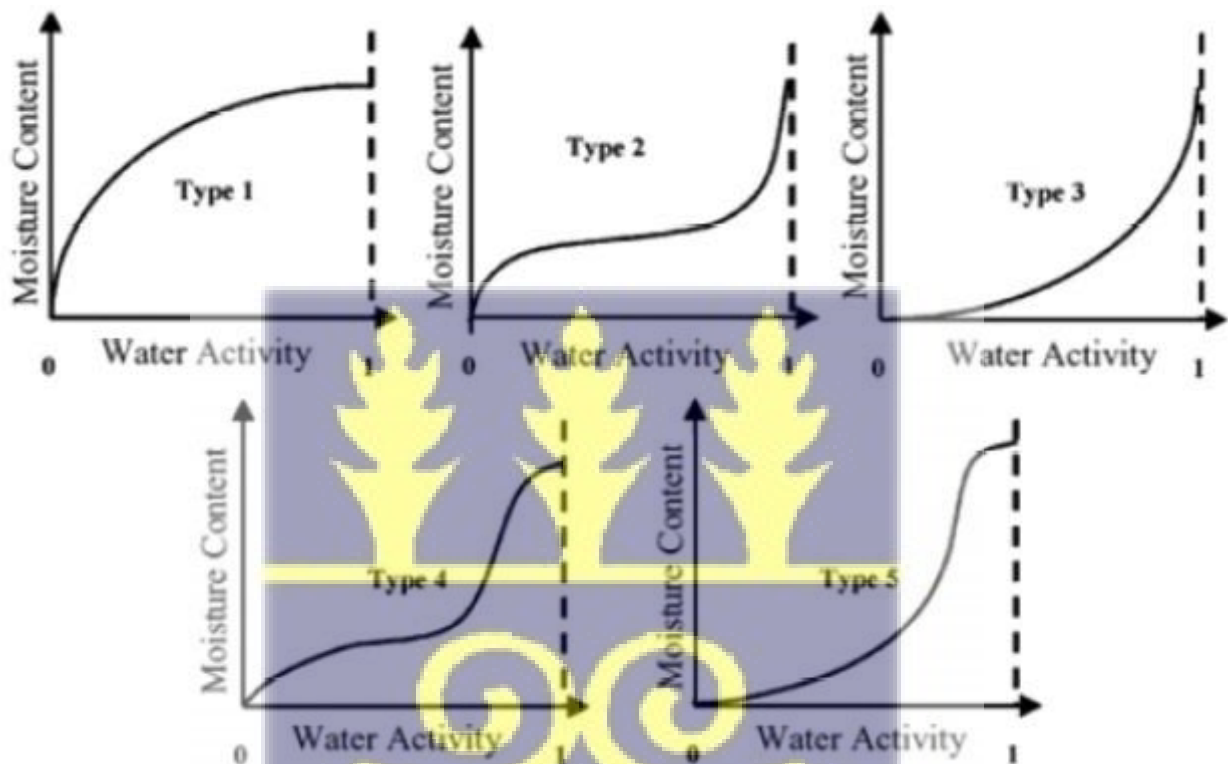


Figure 2.3: Five types of isotherms

Source: Andrade *et al.*, 2011

Type 1 is the well-known *Langmuir isotherm*, obtained by the monomolecular absorption of gas by porous solids in a finite volume of voids. Type 2 is the *Sigmoid isotherm*, obtained for soluble products and shows an asymptotic trend as water activity tends towards 1. Type 3, known as the *Flory-Huggins isotherm*, accounts for the adsorption of a solvent like glycerol. Type 4 isotherm describes the adsorption by a swellable hydrophilic solid until maximum hydration of sites is reached. Type 5 is the BET *multilayer adsorption isotherm*,

observed for water vapour adsorption on charcoal and related to types 2 and 3 isotherms. The two isotherms most frequently found for food products are Types 2 and 4 isotherms (Andrade *et al.*, 2011; Basu *et al.*, 2006).

It is reported that BET is successful only in a water activity range below 0.5. Although its applicable water activity range is small, it is still a helpful equation as it provides an essential parameter of M_0 . The goodness of fit of the data to the equation is evaluated by the coefficient of determination (R^2) criteria. It is well-known that the fit becomes better as the coefficient of determination approaches 1. The smaller the standard errors or residuals, the better the fit. (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3 Models of Isotherms

Several equations describe the sorption isotherm of various foods (Table 5). However, no one equation provides an accurate result throughout the range of water activities and all types of foods. Some of the equations are empirical, while others are based on theory. Table 2.5 shows some of the commonly used isotherm models and equations.

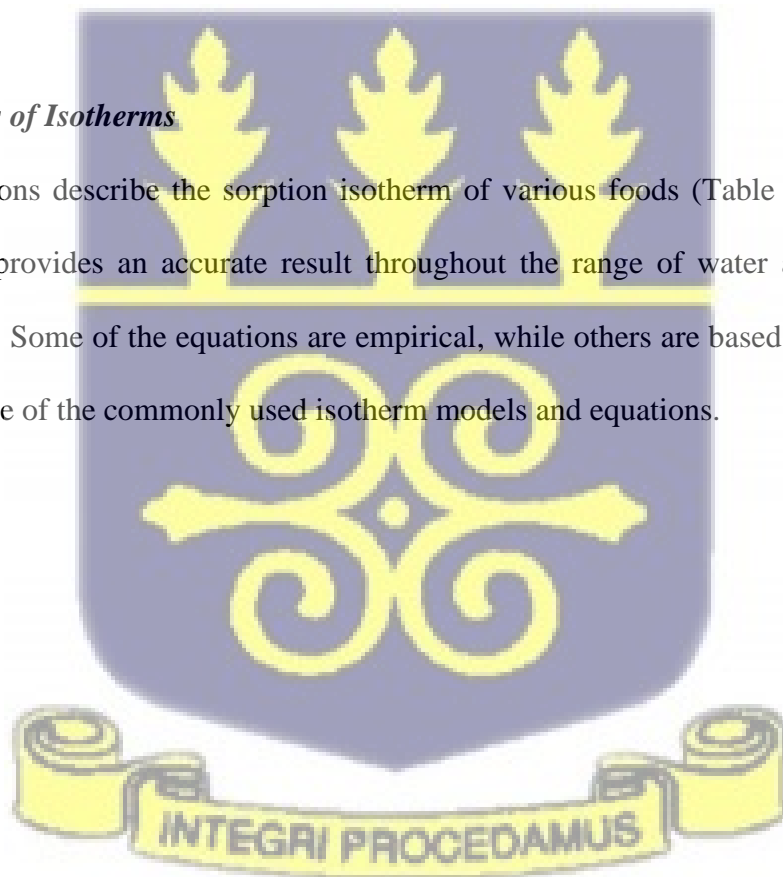


Table 2.5: Some Isotherm Equations for Some Foods

Model	Equation	Foods
BET (1938)	$A_w / [M(1-A_w)] = 1 / M_0C + [(C-1) / M_0C]A_w$	Cereals, fruits, vegetables, starchy foods
GAB (1983)	$M = [XmCK(A_w)] / [1-K(A_w)][1- K(A_w) + C(A_w)]$	Grains, dairy products, nuts, fruits
OSWIN (1946)	$M = A[a_w / (1 - a_w)]^B$	Meat, vegetables, starchy foods
SMITH (1947)	$M = A - B \ln(1 - A_w)$	Cereals, starchy foods, nut
HENDERSON (1952)	$1 - A_w = \exp - (KM)^n$	Spices, starchy foods

Source: Basu *et al.*, 2006

2.7.3.3.1 BET Isotherm Model

This model is widely used in all fields of interest. It represents a primary goal in the explanation of multilayer sorption isotherms, chiefly Types II and III. The BET isotherm provides an estimation of the monolayer value of moisture adsorbed on the surface. The model equation gives a sigmoidal-type isotherm as the type observed experimentally for biological materials (Basu *et al.*, 2006). BET monolayer concept is a correct guide concerning various aspects of interest in dried foods. The monolayer moisture content of many foods has been reported to correspond with the physical and chemical stability of dehydrated foods. Determination of the monolayer value from the BET plot is the most critical aspect of the equation since the optimum stability of the food product is at its

monolayer coverage value, that is, moisture content of a product when the monolayer is completed (Andrade *et al.*, 2011; Basu *et al.*, 2006). The BET equation is generally expressed in the form:

$$\frac{1}{V_a \left(\frac{P_0}{P} - 1 \right)} = \frac{C-1}{V_m C} \times \frac{P}{P_0} + \frac{1}{V_m C}$$

or

$$A_w/[M(1-A_w)] = 1/M_o C + [(C-1)/M_o C] A_w$$

The general linear form of the BET equation is $A_w/[M(1-A_w)]$ versus A_w plot named as the BET plot, where monolayer value M_o and constant C are evaluated from the plot and the intercept of the line (Andrade *et al.*, 2011; Basu *et al.*, 2006). Where M is the equilibrium moisture content, M_o is the monolayer moisture content, A_w is the water activity, and C is the constant related to the net heat of sorption. The constants are estimated based on the linearization of the equation.

2.7.3.3.2 GAB Isotherm Model

The Guggenheim-Anderson-de Boer (GAB) model is a semi-theoretical multimolecular localized homogeneous adsorption model and has been considered the most acceptable fit model for numerous food materials over a range of relative humidity and is expressed as:

$$M = \frac{M_o C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)}$$

or

$$M = [X_m C K (A_w)]/[1 - K(A_w)][1 - K(A_w) + C(A_w)]$$

Where X_m (M_o) is the GAB monolayer moisture, K and C are related to the temperature (C and K are related to interaction energies). The GAB isotherm equation is an extension of the two-parameter BET model, which considers the modified properties of the sorbate in the

multilayer region and bulk liquid properties through the introduction of a third parameter K. If K is less than unity, lower sorption than that demanded by BET model is predicted and allows the GAB isotherm to be successful up to relative humidity of 0.90. When K is equal to 1 and if K is greater than 1, the sorption becomes infinite at a relative humidity value less than unity which is physically unsound. It has been suggested to be the most versatile sorption model available in the literature (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3.3 Oswin Isotherm Model

Oswin (1946) developed an empirical sorption isotherm model equation using a mathematical expression. The Oswin model is a good fit model for the sorption of various foods. The Oswin equation is an empirical model which is a series expansion for sigmoid shaped curves, and it is expressed as:

$$M_w = C \left(\frac{a_w}{1 - a_w} \right)^n$$

or

$$M = A [a_w / (1 - a_w)]^B$$

Where M is the equilibrium moisture content, A and B are constants obtained from the intercept and the slope of the linear plots of log M versus log $[a_w / (1 - a_w)]$, respectively. Oswin's model equation is the best model for explaining the isotherms of starchy foods and a relatively good fit for vegetables and meat (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3.4 Smith Isotherm Model

This is an empirical model developed by Smith (1947) to explain the final curved section of water sorption isotherm of biopolymers with high molecular weight. It is reported that this equation could be used between 0.5 and 0.95 relative humidity. The Smith model is expressed as:

$$X_e = a - b (\ln(1 - a_w))$$

or

$$M = A - B \ln(1 - A_w)$$

Where A and B are constants for the material, the A parameter represents moisture bound to the surface, the quantity of water in the first sorbed fraction. The parameter B represents the moisture in a unimolecular layer of typically condensed moisture, the quantity of water in the multilayer moisture fraction (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3.5 Henderson Isotherm Model

This model is also famous for relating water activity to the amount of water sorbed. The Henderson model is expressed as:

$$X_e = \left[\frac{\ln((1 - a_w))^{1/b}}{-a(T + c)} \right]$$

or

$$1 - A_w = \exp - (KM)^n$$

Where X_e is the equilibrium moisture content, A and B are constants. A linearized plot of $\ln(1 - a_w)$ versus moisture content X_e is often used to represent this type of isotherm (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3.6 Monolayer Capacity (M_0)

The measure of the sorption ability of starchy food is known as Monolayer moisture. It is the minimum amount of water bound to active sites and guarantees the stability of powder and flour during storage (Andrade *et al.*, 2011; Basu *et al.*, 2006). The prediction of M_0 values is

vital since the deterioration of foods is minimal below M_0 . This is because water is firmly bound to the food below M_0 and is not involved in any deteriorative reaction either as a solvent or as one of the substrates. The lower the monolayer moisture content, the more stable the food ingredient were during storage under the right conditions (Andrade *et al.*, 2011; Basu *et al.*, 2006). The higher the monolayer moisture content value, the less stable the food ingredient is, hence highly susceptible to spoilage.

The moisture level considered safest regarding good storage stability of foods is between the monolayer value and corresponding to a water activity of 0.60 – 0.64. Food product deteriorative characteristics such as non-enzymatic browning, lipid oxidation, enzymatic activity, loss of colouring pigments, texture, and other qualities are affected by high water activity (Basu *et al.*, 2006).

Beyond 0.68 a_w , water is deemed accessible in food ingredients and not affected by surface forces. In the case of foods with moisture levels of 13-16%, corresponding to water-activity of 0.60-0.75, various species of drought-resistant fungi can develop. Among flours above 0.75 water-activity, moulds develop rapidly, and heating may occur with a subsequent loss in product quality (Andrade *et al.*, 2011). Enzymes, such as amylases, peroxidases, are inactivated when the water activity is less than 0.85, whereas, below 0.90, bacterial growth is practically negligible. Yeasts usually grow when the water activity exceeds 0.70. It can be therefore concluded that the safe upper limit of moisture for food ingredients is between 6-13% for starchy foods and 4-6% for fat-rich foods corresponding to water-activity of about 0.60, with the most stable region being 0.20 – 0.60 a_w (Andrade *et al.*, 2011; Basu *et al.*, 2006).

Hence, sorption information is very useful in selecting suitable packages by carefully selecting water-vapour barrier packaging material and are helpful guides for increasing the shelf life by suitably reducing the equilibrium relative humidity of foods near to that corresponding to the monomolecular moisture level.

2.7.3.3.7 Specific Surface Area for Adsorption

The monolayer moisture can approximate shelf stability and efficient energy use in the drying process (Basu *et al.*, 2006). The monolayer moisture contents can be used to define the seeming sorption area (S_o , m²/g solid) using the following equation:

$$S_o = (1/M_s)N_o A M_o$$

Where M_s = relative molecular mass of water, N_o = Avogadro's number (6.023×10^{19} molecules/mol), A = surface area of 1 water molecule (1.05×10^{-19} m²), and M_o = monolayer value (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.7.3.3.8 Heat of Sorption

Heat sorption, enthalpy and entropy of sorption are critical thermodynamic factors drawn from moisture sorption isotherms (Andrade *et al.*, 2011). The net heat of sorption gives valuable data on the heat and free energy changes during moisture sorption in food. Several studies have reported that the equilibrium moisture content and heat sorption for various food ingredients are related. Due to the theoretical basis of the Clausius Claypeyron equation, it is frequently used in estimating the total heat of sorption of farm produce, as well as the effects of the temperature of the environment and water activity at a constant amount of sorbed water (Andrade *et al.*, 2011; Basu *et al.*, 2006). The equation is expressed as follows:

$$q_{st} = -R \frac{\partial \ln a_w}{\partial (1/T)}$$

Where q_{st} is the isosteric heat of sorption (kJ/mol), R is the universal gas constant (8.314 kJ/mol K), a_w is the water activity, and T is the absolute temperature, Kelvin (Andrade *et al.*, 2011; Basu *et al.*, 2006).

2.8. Food Intolerance and Food Allergies

Physiological reactions toward certain foods are expected; however, most result from a food intolerance rather than a food allergy (Singh *et al.*, 2018). Even though a food intolerance can cause some of the same signs and symptoms as a food allergy, the two are frequently confused. A true food allergy triggers an immune response that affects multiple organs in the body. It can result in various symptoms (Alam *et al.*, 2014). An allergic food reaction can be severe or even fatal in some cases. On the other hand, food intolerance symptoms are usually mild and limited to digestive issues (Aguilar *et al.*, 2014). The following include examples of food intolerances:

- Lactose intolerance is a typical example of the absence of an enzyme required to digest a food (milk) fully.
- Irritable bowel syndrome (IBS). Cramps, constipation, and diarrhoea are all symptoms of this chronic condition.
- Food additive sensitivity Sulfites used to preserve dried fruit, canned goods, and wine can cause asthma attacks in sensitive people.
- Stress or psychological factors that reoccur. Sometimes just thinking about food can cause some people to feel sick. The reason for this is not fully understood.
- Furthermore, gluten intolerance is common in Celiac disease (Nindjin *et al.*, 2011).

2.8.1 Celiac disease

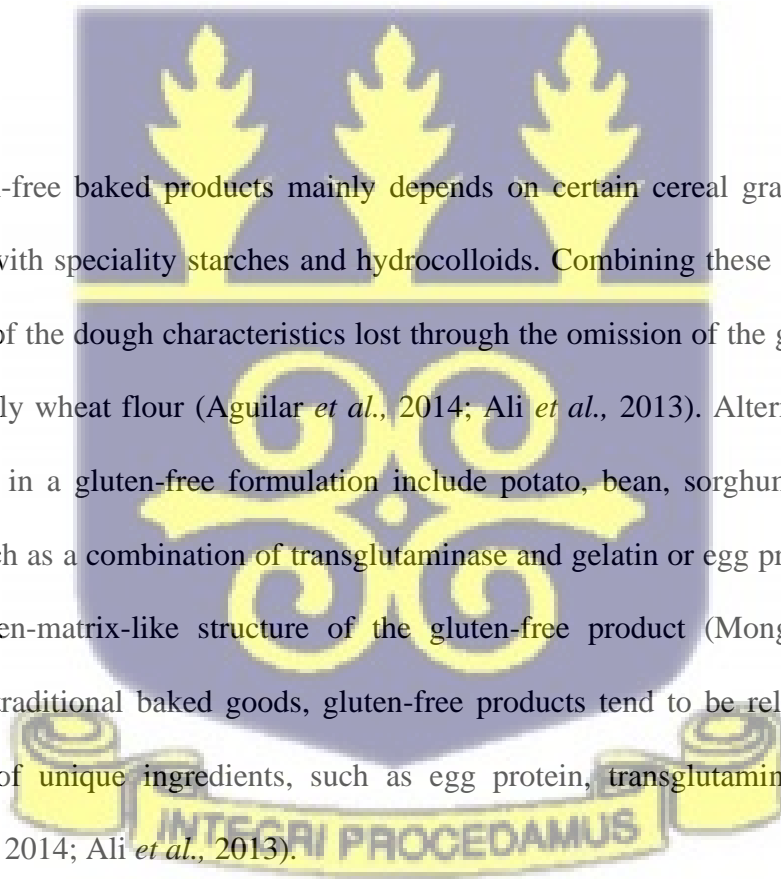
The current rapidly growing sector of the food industry is gluten-free baked products. These pastry products do not only accommodate those with medical needs, such as celiac disease and gluten intolerance, but they also provide for the millions of individuals who pursue variety and a gluten-free diet (Aguilar *et al.*, 2014; Ali *et al.*, 2013). The confectionery industry is increasingly paying attention to consumer needs for gluten free baked goods. The sales of these products are on a steady upsurge (Alam *et al.*, 2014). This may be due to the increased reported cases of Coeliac disease and gluten intolerance (Singh *et al.*, 2018; Nindjin *et al.*, 2011). Celiac disease is a digestive condition where a person has an adverse reaction to gluten; the immune system mistakes the components of gluten as harmful to the body and attacks them. This mutilates the superficial part of the small intestines, interrupting the body's nutrient absorption ability. The exact cause of the immune system's reaction in this manner is not entirely clear; however, a person's genetic makeup and the environment play a part in this disorder. On the other hand, gluten intolerance is an allergy triggered by gluten (Singh *et al.*, 2018; Aguilar *et al.*, 2014; Nindjin *et al.*, 2011).

2.8.2 Wheat proteins, development and composition of gluten

Gluten is made up of two proteins, namely gliadin and glutenin. The two proteins exist together with starch and endosperm of wheat and other cereal crops, such as barley and rye. Gluten is an insoluble protein known for giving kneaded dough its elasticity and adding chewiness to finished baked goods constituents (Mongi *et al.*, 2011; Spies,1990). It also helps trap gas bubbles resulting from the fermentation of sugars in the leavened dough, causing it to rise. During baking, gluten thickens, evening out the dough mixture and contributes to overall crumb structure and volume (Singh *et al.*, 2018; Nindjin *et al.*, 2011). Gluten is a vital functional component in baked foods. It enhances the dough's extensibility,

mixing tolerance, and gas-holding ability, which impacts product structure and volume. glutenin and gliadin (Ndife *et al.*, 2011). Glutenin is a rough, rubbery mass when it absorbs water, while gliadin forms a gelatinous, fluid mass when exposed to water. These make gluten exhibit cohesive, elastic, and viscous properties, referred to as the gluten matrix, by combining the characteristics of the two constituents (Mongi *et al.*, 2011; Ndife *et al.*, 2011). The gluten matrix, enclosing the starch granules and fibre fragments, is a major determining factor of the properties of dough (mixing tolerance, gas trapping ability, resistance to stretch, extensibility). The main challenge to removing gluten from a traditional dough system is changing from a plastic dough to a fluid dough. Resulting pastry foods are very different in appearance, texture, and eating quality (Aguilar *et al.*, 2014; Ali *et al.*, 2013; Singh *et al.*, 2008).

Making gluten-free baked products mainly depends on certain cereal grains like rice and corn blended with speciality starches and hydrocolloids. Combining these ingredients helps recover some of the dough characteristics lost through the omission of the gluten-containing material, usually wheat flour (Aguilar *et al.*, 2014; Ali *et al.*, 2013). Alternative flours that could be used in a gluten-free formulation include potato, bean, sorghum. Other binding ingredients such as a combination of transglutaminase and gelatin or egg protein can help to form the gluten-matrix-like structure of the gluten-free product (Mongi *et al.*, 2011). Compared to traditional baked goods, gluten-free products tend to be relatively higher in cost because of unique ingredients, such as egg protein, transglutaminase, and gelatin (Aguilar *et al.*, 2014; Ali *et al.*, 2013).



2.8.3 Development of Gluten-free Products

The quality of pastry products depends on the rheological properties of the dough, which is greatly influenced by the gluten matrix (Alam *et al.*, 2014; Moore *et al.*, 2006). The influence of gluten on dough rheological properties relate to the strength of the dough, the size and uniformity of the air cells within the dough, and the presence of a diverse matrix within the dough, hence, exhibit a less homogeneous colour (Olaoye *et al.*, 2006; Moore *et al.*, 2006). Dough from alternative flours produces more giant and less homogeneously sized air cells, hence, exhibit more significant heterogeneity and yields a longer, chewy texture (Ohimain *et al.*, 2014). Therefore, a robust gluten matrix replacement system is critical to developing the targeted pastry product similar to existing ones (Aguilar *et al.*, 2014; Ali *et al.*, 2013; Moore *et al.*, 2006).

The space that gluten occupies within the dough in, for example, a whole wheat bread also requires compensating adjustments. A helpful replacement technique must ensure that the volume and weight contributed by the gluten protein are replaced with a gluten-free alternative ingredient (Aguilar *et al.*, 2014; Nindjin *et al.*, 2011). An essential attribute of the gluten-replacing constituent is controlling water in the dough like water management in gluten dough. To postpone staling and prevent moulds in the bread, water activity must be controlled. The shelf life of gluten-free products tends to be equal to or less than that of a conventional wheat product, depending on the type of product and storage conditions (Ali *et al.*, 2013; Moore *et al.*, 2006). The deliberate addition of sugars helps to bind water and manage water-activity related changes while in storage. The sensory characteristics of common gluten-free ingredients usually do not result in a product that would be considered acceptable relative to wheat products. However, the probability of achieving acceptable sensory attributes of wheat products is far more remarkable due to advances in the

development of alternative techniques and ingredients (Ohimain *et al.*, 2014; Olaoye *et al.*, 2006; Moore *et al.*, 2006).

Existing gluten-free foods are relatively low in quality, exhibiting poor sensory appeal (flavour and texture) (Alam *et al.*, 2014; Moore *et al.*, 2006). To develop gluten-free bread, one can choose from several alternative flours. However, the challenge in developing an acceptable product depends on how the protein matrix can be mimicked by using alternative sources of proteins, and the softness or/ and resilience can be obtained by using different starches or gums (Han *et al.*, 2021).

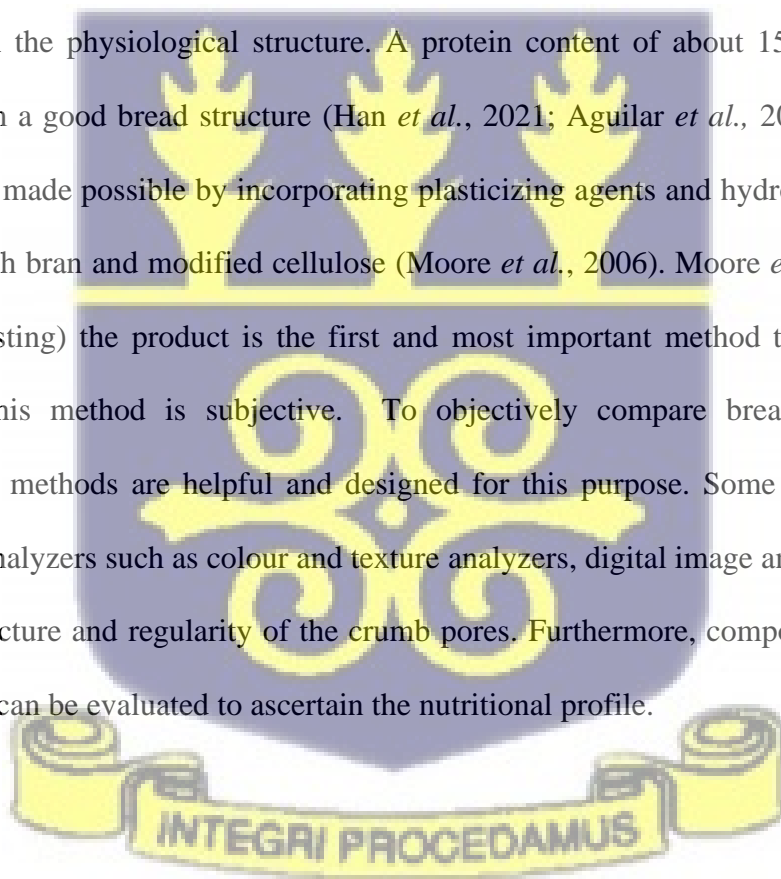
Replacing wheat flour is not easy, even though several alternative ingredients exist. Depending on the nature of the alternative flour, a method of “trial and error” can be used to establish an acceptable formulation (Moore *et al.*, 2006). The following include some ingredients that may be used for making gluten-free bread:

- Alternative cereal flours, Almond flour, tuber flours; to replace wheat flour
- Transglutaminase, gelatin, and egg albumin powders for protein cross-linking and film-forming
- Non-hydrogenated vegetable oils for tenderizing
- CMC (carboxymethylcellulose) or Xanthan gum, for film-forming and water binding
- Guar gum, for film-forming and water binding

It has also been reported that transglutaminase can help form a protein matrix that will improve the structure of gluten-free bread (Han *et al.*, 2021; Moore *et al.*, 2006). The efficiency of the enzyme is dependent both on the protein source and the level of enzyme concentration.

Gluten in wheat bread is responsible for the elastic characteristics of the dough and its baking capacity. Gluten forms a highly flexible viscosystem, stabilising and trapping air to give the desired porous structure typical of leavened bread. There are many alternative gluten-free flours, however, few of them have the required properties for baking because they lack the elastic rheological properties necessary for foam stabilization under ordinary baking conditions. There is the need to produce gluten-free bread due to the demand by the increasing number of people with celiac disease or gluten intolerance, as well as the high cost of wheat importation in Africa.

The microstructure of gluten-free dough revealed that the amount of protein was insufficient and trapped in the physiological structure. A protein content of about 15% to 20% was needed to form a good bread structure (Han *et al.*, 2021; Aguilar *et al.*, 2014). Good foam formation was made possible by incorporating plasticizing agents and hydrocolloids such as beta-glucan rich bran and modified cellulose (Moore *et al.*, 2006). Moore *et al.* (2006) posit that eating (tasting) the product is the first and most important method to evaluate bread quality, but this method is subjective. To objectively compare bread quality, some instruments or methods are helpful and designed for this purpose. Some of these include instrumental analyzers such as colour and texture analyzers, digital image analyzers to assess the crumb structure and regularity of the crumb pores. Furthermore, compositional analysis of the product can be evaluated to ascertain the nutritional profile.



2.9 Sensory Characteristics of Bread

Sensory assessment systematically analyses food using the human senses of sight, smell, taste, touch, and hearing. The qualities of food identified by the sense are called sensory

attributes or characteristics. These include the foods appearance, taste, aroma, sound and feel in the mouth (Stone *et al.*, 2012; Macfie, 1989). Sensory attributes serve as a quality guide to manufacturers for ensuring consistency in production. It is also a quality guide to the consumer in selecting foods. Food processors measure these attributes to determine product quality and consumer preference to manufacture an acceptable product at maximum production economy. Sensory parameters are measured to determine food conformity with established government or trade standards and food grades (Lawless and Heyman, 2010; Stone *et al.*, 1974).

Three standard methods have been employed to study the sensory characteristics of foods. These include descriptive, discriminative, and consumer testing (Stone *et al.*, 2012; Lawless and Heyman, 2010). Consumer acceptability tests have been used to collect data on likeness or dislike of products, whereas carefully screened and trained assessors have been used in descriptive tests. Understanding the factors that affect bread quality and developing a method to carefully study the descriptive sensory profiling through Quantitative descriptive analysis (QDA) have been explored by both industry and academia as part of research and development processes (Stone *et al.*, 2012; Lawless and Heyman, 2010).

2.10 Conclusion

Growing the world's food basket is key to ensuring global nutrition and food security in the changing climate, especially in developing countries. This has generated interest in the increased utilisation of neglected native climate-smart crops to ensure food and nutrition security in developing countries. Frafra potato is a locally cultivated climate-smart crop and may be used for flour and flour-based foods to lessen the over-dependence on wheat and reduce the cost of importation of other wheat-based products. The crop is high in nutrients

compared to other tubers (yam and sweet potato); however, it has not been characterised extensively to unearth its full potential in food applications. It deteriorates rapidly after harvest, and this discourages farmers from cultivating it on a large scale. Previous studies by Osei Tutu *et al.* (2019) have investigated the feasibility of developing Frafra potato flour to control post-harvest losses and formulate composite bread. Further studies must be conducted to establish its potential as an ingredient in food formulations.

2.11 References

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CHAPTER THREE

3.0 PHYSICOCHEMICAL AND MICROSTRUCTURAL CHARACTERISTICS OF FRAFRA POTATO STARCH

Abstract

Frafra potato (*Solenostemon rotundifolius*) is an underutilised climate-resilient tuber crop commonly cultivated in the tropics. Different accessions have been identified and bred to broaden its application in food. This study characterised the starches extracted from ten (10) accessions, from Ghana (released) and Burkina Faso (unreleased), in terms of yield, physicochemical, and microstructural characteristics. The starches did not differ significantly ($p < 0.05$) in colour (L^*) and amount (35 to 39% dry matter basis). Furthermore, there were no significant differences ($p < 0.05$) among them in amylose/amylopectin ratio, syneresis %, granule types and shapes, except for one of the unreleased accessions (E82), which had a significantly ($p < 0.05$) smaller granule size. There were, however, significant differences ($p < 0.05$) in paste clarity of the starch gels, ranging from 51 to 63% of the starch gels, as well as in the thermal properties of the starches. The XRD and FTIR spectra showed the starches to be A-type, typical of tuber starches, with relative crystallinities ranging between 30.5 to 33.5 %. The ten Frafra potato cultivars were clustered into two groups using PCA procedures; one group (Maa-Lana and Nutsugah) clustering on thermal properties of starch, while the other group (E82, E111, E132, E134, E145, Manga, Naachem-Tiir, and WAAPP) on paste clarity and change in temperature. The variations in granule size and thermal characteristics of the starches could impact the cooking and textural properties of Frafra potatoes.

KEYWORDS: *Frafra potato, starch, thermal properties, SEM, X-ray diffraction spectroscopy, FTIR spectroscopy*

3.1 Introduction

Frafra potato (*Solenostemon rotundifolius*) is a neglected climate-smart crop cultivated in parts of Africa and Asia. It is a valuable tuber crop mainly cultivated in Northern Ghana (Sugri *et al.*, 2013; Akanlu *et al.*, 2005; Quainoo and Bayorbor, 2002; Tetteh and Guo, 1997). The crop has excellent potential for ameliorating malnutrition and enhancing food and nutrition security in Ghana and other developing countries (Sugri *et al.*, 2013; Akanlu *et al.*, 2005; Quainoo and Bayorbor, 2002). Although it has not been extensively explored in industrial applications, it has the potential to act as a source of flour and starch (Osei Tutu *et al.*, 2019; Akanlu *et al.*, 2005; Tetteh and Guo, 1997). It can be processed into flour or starch and used as raw material in confectionery and pastry products (Osei Tutu *et al.*, 2019; Tortoe *et al.*, 2018; Peroni *et al.*, 2006).

Several new accessions of Frafra potato have been identified and developed through traditional and contemporary breeding methods (Sugri *et al.*, 2013; Nkansah, 2004; Quainoo and Bayorbor, 2002). The utilisation of these accessions of Frafra potato for food and industrial applications is primarily dependent on the properties of their starch, which are affected mainly by the amylose/amylopectin composition and by the molecular structure (Tortoe *et al.*, 2017; Aprianita *et al.*, 2013; Chinma *et al.*, 2012; Adebowale *et al.*, 2005; Ratnayake and Jackson, 2003). This study aimed to isolate starch from ten (10) Frafra potato accessions and determine their optical, functional, and microstructural characteristics for food applications.

3.2 Materials and Methods

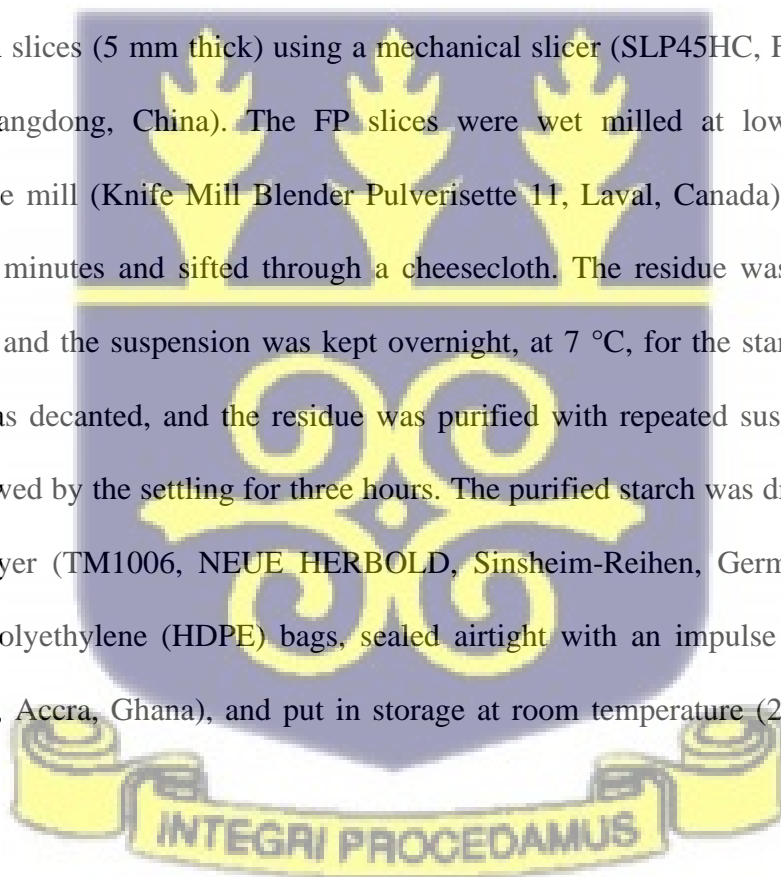
3.2.1 Source of Raw Materials

Approximately twenty-five (25) Kg each of ten (10) cultivars of Frafra potato (FP) were obtained from the Council for Scientific and Industrial Research (CSIR), Savanna

Agricultural Research Institute (SARI), Manga in the Upper East Region of Ghana. These cultivars were WAAPP Piesa, Maa-Lana Piesa, Naachem-Tiir Piesa, Nutsugah Piesa, Manga Moya Piesa (released cultivars from Ghana), E82, E111, E132, E134, E145 (unreleased cultivars from Burkina Faso). The tubers were transported (about 8 hours) to the food processing laboratory at CSIR-Food Research Institute, Accra, packed in perforated paper cartons (aeration) for processing.

3.2.2 Isolation of Frafra Potato Starch (FPS)

The procedure of Gayin *et al.* (2016) was used for starch isolation with modifications. Frafra potato tubers were washed in potable water, weighed, hand-peeled, washed, weighed, and sliced into thin slices (5 mm thick) using a mechanical slicer (SLP45HC, Foshan Sorumpor Electrical, Guangdong, China). The FP slices were wet milled at low speed using a laboratory-scale mill (Knife Mill Blender Pulverisette 11, Laval, Canada) with 1:2 w/v of water for two minutes and sifted through a cheesecloth. The residue was wet-milled and filtered thrice, and the suspension was kept overnight, at 7 °C, for the starch to settle. The supernatant was decanted, and the residue was purified with repeated suspension in water (1:2 v/v) followed by the settling for three hours. The purified starch was dried at 35 °C in a mechanical dryer (TM1006, NEUE HERBOLD, Sinsheim-Reihen, Germany), packed in high-density polyethylene (HDPE) bags, sealed airtight with an impulse sealer (“Oalink-QNS-3200HI”, Accra, Ghana), and put in storage at room temperature (25 °C ± 0.53) for further tests.



3.2.3 Starch yield of Frafra Potato

The yield of FPS and the proportion of peels, moisture and fibre removed during processing of the ten (10) varieties was estimated as a percentage of the unpeeled FP weight.

3.2.4 Colour of FPS

According to Stevenson *et al.* (2007), the colour of FPS was determined using a Chromameter (CR-400 Chroma Meter, Konica Minolta, Tokyo, Japan). The instrument was calibrated against the standard white tile ($L^*0 = 98.93$, $a^*0 = 0.31$, and $b^*0 = 4.63$) before use. FPS samples were contained in a transparent petri dish and covered with the same. FPS colour was also described using C^* (Chroma) and h^* (Hue angle) notations. Paste Clarity (PC) of FPS was determined as follows. Five millilitres (5 mL) of 1% starch suspension in 15 mL screw-capped centrifuge tubes were incubated in a boiling water bath for 30 minutes, with continual shaking. The starch solutions were cooled to room temperature ($25\text{ }^\circ\text{C} \pm 0.53$), and their transmittance (%) was measured against a water blank at 650 nm on a UV-VIS spectrophotometer (Shimadzu 1800, Tokyo, Japan).

3.2.5 Amylose/Amylopectin Ratio of FPS

The Amylose / Amylopectin ratio was determined using the method of Kowsik and Mazumder (2018), with modifications. 100 mg of FPS was dissolved in 9 mL of 90% dimethyl sulfoxide (DMSO) in screw-capped tubes and homogenized. The suspension was heated in a water bath at 85°C for 15 min, with continual shaking. The solution was allowed to cool to room temperature ($25\text{ }^\circ\text{C} \pm 0.53$) and diluted with water to twenty-five millilitres in a volumetric flask. Five millilitres of the dilute solution was pipetted into a separate 100 mL standard flask. 1 mL of 1M acetic acid was added, followed by 5 mL of iodine solution was added before making up to the mark with distilled water. The resulting solution was homogenized, allowing for 20min for colour development before absorbance measurement at 620 nm with a UV-VIS spectrophotometer (Shimadzu 1800, Tokyo, Japan). The total amylose content of each sample was inferred from the absorbance recorded (AR). The amylose content was calculated using the formula:

Total amylose content (%) = $20 \times 3.06 \times (AR)$. Amylopectin was calculated as the difference between the total amylose content and 100%.

3.2.6 Resistant Starch Determination of FPS

Total resistant starch was determined with a Megazyme-resistant starch assay kit following the procedure established by the manufacturer. 50 g of samples were dissolved and homogenized. Pancreatic α -amylase and amyloglucosidase were added and then incubated in a water bath, shaking for 16 hours at thirty-seven degrees Celcius (37°C). In that time, non-resistant starch was solubilized and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was completed by adding an equal volume of ethanol, and the RS was recovered as a pellet on centrifugation. The pellets were dispersed in 2M potassium hydroxide by stirring in an ice-water bath over a magnetic stirrer. This solution was neutralized with acetate buffer (pH of 4.5), and the starch was quantitatively hydrolysed to glucose with amyloglucosidase. D-Glucose was measured with glucose oxidase/peroxidase reagent, which was a measure of the resistant starch content of the samples.

3.2.7 Retrogradation (Syneresis %) of FPS Gels

Retrogradation of FPS gels was determined using the method of Ezekiel and Singh (2011). A 12% suspension of FPS was kept at 95 °C for fifteen minutes in a water-bath, cooled to 50 °C, and kept at this temperature for 15 min. Fifty millilitres was sampled into test tubes and kept at 4 °C. The retrogradation of pastes prepared from FPS was measured by determining the amount of water expelled during storage at 4 °C, known as syneresis. Retrogradation was expressed as Syneresis (%) and was computed as follows:

$$\text{Syneresis} = \frac{\text{weight of water expelled}}{\text{weight of sample}} \times 100.$$

3.2.8 Thermal Characteristics of FPS

Thermal characteristics (TC) of FPS were determined using a Thermogravimetric Analyzer (TGA-DSC SDT Q600 V20.9 Build 20, TA Instruments, New Castle, DE USA) following the procedure established by the manufacturer. 2.5 mg of FPS was weighed into an aluminium pan, and double deionized water (7.5 μL) was added. The pan was hermetically sealed and equilibrated at $25\text{ }^{\circ}\text{C} \pm 0.53$ for about one hour for moisture balance and then heated at the rate of five degrees Celcius per minute from twenty-five to one hundred degrees Celcius with a blank as reference. Thermal parameters such as onset (T_o), peak (T_p), conclusion (T_c) temperatures, range of gelatinization temperature ΔT ($\Delta T = T_c - T_o$) and enthalpy of gelatinization (ΔH) were recorded.

3.2.9 FTIR Spectroscopy of FPS

FTIR Spectroscopy of FPS was determined using a Fourier Transform Infrared (FTIR) spectrometer (UATR Two, PerkinElmer, United Kingdom 105024) with a deuterated triglycine sulfate detector, following the procedure established by the manufacturer. Original spectra were corrected by subtraction of the baseline in the region from 4000 to 500 cm^{-1} before deconvolution was applied using Resolutions Pro FTIR software version 5. The assumed line shape was Lorentzian with a half-width of 19 cm^{-1} and a resolution enhancement factor of 1.9. Intensity measurements were performed by recording the height of the absorbance spectra.

3.2.10 XRD Spectroscopy of FPS

XRD Spectroscopy of FPS was determined using an X-ray powder diffraction spectroscopy (Empyrean Series 2 XRD, Malvern Panalytical, United Kingdom). The starches were kept in a desiccator where a saturated solution of NaCl maintained a constant relative humidity of

75% for one week at $26\text{ }^{\circ}\text{C} \pm 0.41$. XRD recorded the X-ray diffraction spectra. The diffraction angle and intensity measurements were recorded.

3.2.11 Scanning Electron Microscopy (SEM)

Granular morphology of FPS was characterised by SEM (Phenom ProX World Desktop SEM + EDS, Thermo Fisher Scientific, USA) following the procedure established by the manufacturer. Samples were first coated with gold dust (15nm) using Emitech K550X Sputter Coater (Quorum Technologies Limited, Kent, UK). An accelerating voltage of 15 kilovolts was used for imaging at x2300 magnification. The average diameter of granules at $30\text{ }\mu\text{m}$ was reported as the granule size (GS) of FPS.

3.2.12 Statistical Analysis

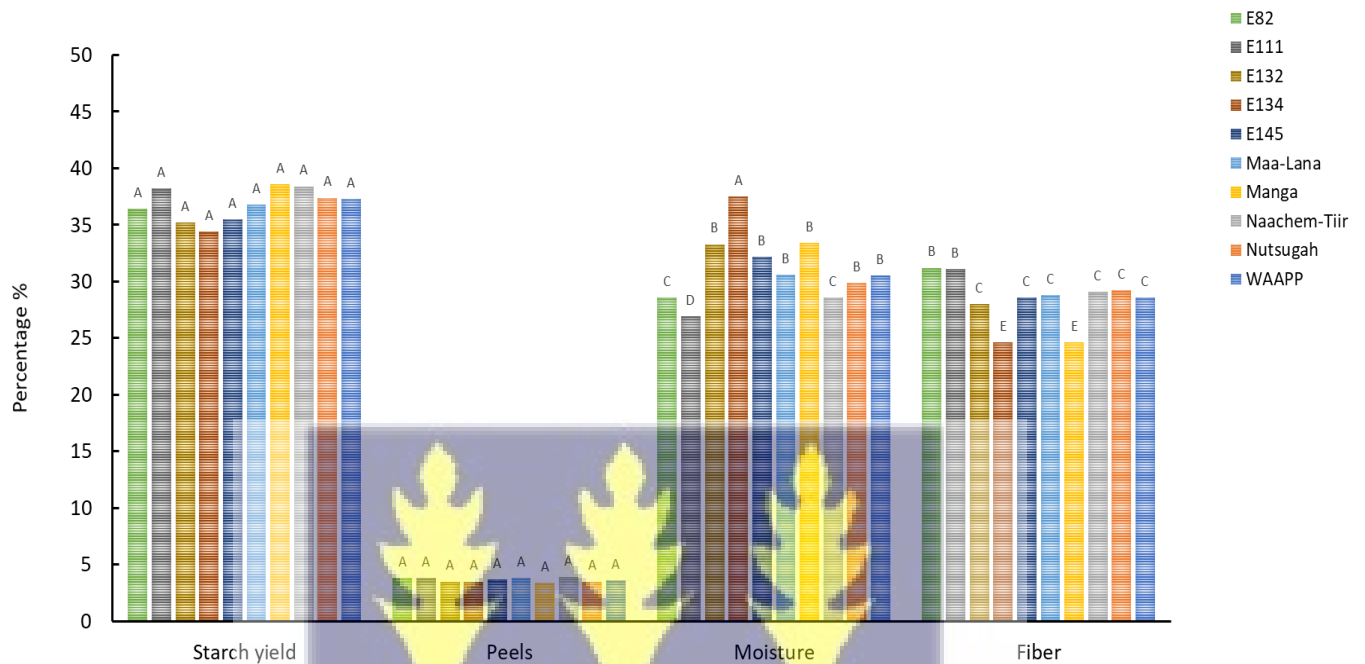
The data were subjected to Analysis of Variance (ANOVA) using “R” statistical software for Windows pc version 4.1.1 (R Project, Bell Laboratories, USA), and Duncan Multiple Range Test (DMRT) was performed to separate varieties with significantly different ($p < 0.05$) means. Principal components analysis (PCA), in XLSTAT 2018 for windows pc, was used to cluster samples with close associations based on their physicochemical properties.



3.3 Results and Discussion

3.3.1 Starch Content of Frafra Potato (FPS)

Starch extractability and proportion of peels, moisture and fibre removed from Frafra potato tubers are summarized in figure 3.1.



Bars, within attributes, with different letters are significantly different ($p \leq 0.05$)

Figure 3.1: Starch yield, moisture content and percentages of peels, and fibre from FP

The starch yield of the tubers ranged from 35 to 39 % (Figure 3.1). There was no significant difference ($p \leq 0.05$) between the starch yield and peels among the Frafra potato varieties. However, there were significant differences between moisture, ranging from 27% (E111) to 38% (E134) and fibre ranging from 25% (Manga) to 31% (E111) removed during processing among the Frafra potato varieties. The differences observed in the moisture content can be attributed to varietal differences reported in similar root and tuber starch isolation studies (Aprianita *et al.*, 2013; Peroni *et al.*, 2006). The starch content of the Frafra potatoes was lower than that of some varieties of other root and tuber crops, such as sweet potato, which

recorded starch yield ranging from 49 % to 77 % (Tortoe *et al.*, 2017). As a source of starch for food applications, the implications of a relatively lower starch yielding tuber are unfavourable as it will mean a relatively higher cost of production (Aprianita *et al.*, 2013; Peroni *et al.*, 2006).

Therefore, there is the need to enhance the starch content of Frafra potato accessions in Ghana by developing cultivars with improved starch yielding qualities. Higher starch yielding cultivars will help position Frafra potato as an affordable source of starch for food applications in Ghana and Sub-Saharan Africa.



3.3.2 Colour of FPS

The colour parameters of FPS is summarized in table 3.1

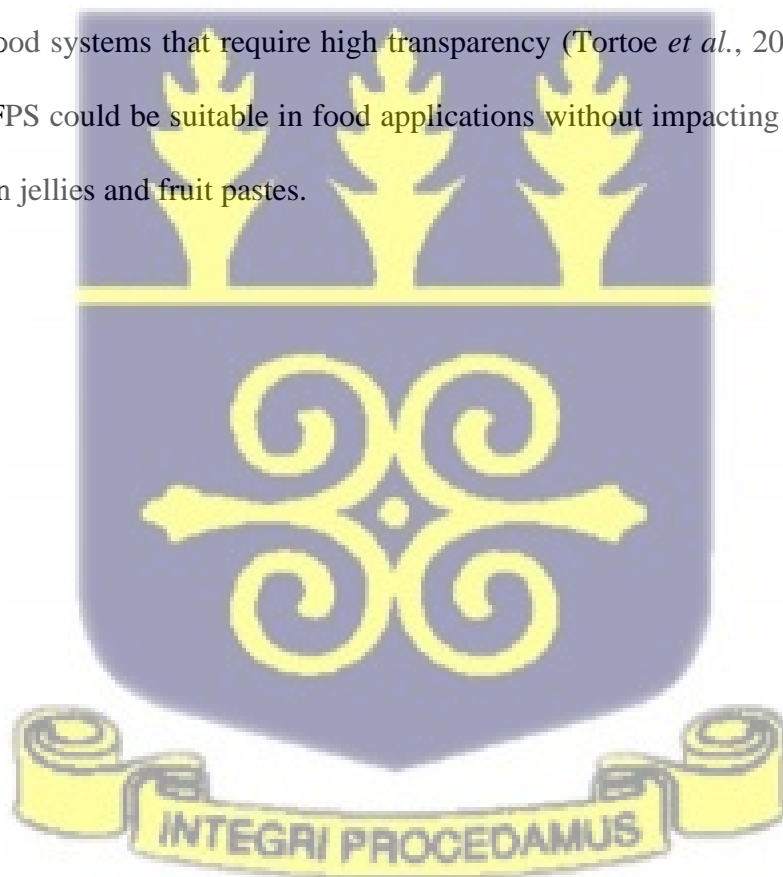
Table 3.1: Colour of FPS

Cultivar	L*	a*	b*	h*	C*	PC (%)
E 82	98.22 ± 0.11 ^a	0.20 ± 0.09 ^a	1.18 ± 0.12 ^a	80.38 ± 0.08 ^a	1.20 ± 0.16 ^a	51.43 ± 0.12 ^b
E111	98.32 ± 0.13 ^a	0.22 ± 0.02 ^a	1.22 ± 0.14 ^a	79.78 ± 0.12 ^a	1.24 ± 0.08 ^a	52.77 ± 1.94 ^b
E132	97.59 ± 0.04 ^a	0.20 ± 0.13 ^a	1.39 ± 0.10 ^a	81.81 ± 0.07 ^a	1.40 ± 0.09 ^a	57.73 ± 0.58 ^{ab}
E134	96.90 ± 0.09 ^a	0.24 ± 0.11 ^a	1.15 ± 0.09 ^a	78.21 ± 0.09 ^a	1.18 ± 0.12 ^a	58.57 ± 0.47 ^{ab}
E145	97.86 ± 0.12 ^a	0.22 ± 0.03 ^a	1.23 ± 0.14 ^a	79.86 ± 0.05 ^a	1.25 ± 0.18 ^a	58.60 ± 1.40 ^{ab}
Maa-Lana	98.11 ± 0.05 ^a	0.23 ± 0.05 ^a	1.15 ± 0.11 ^a	78.69 ± 0.07 ^a	1.17 ± 0.14 ^a	62.77 ± 1.94 ^a
Manga	97.49 ± 0.07 ^a	0.21 ± 0.15 ^a	1.28 ± 0.07 ^a	80.68 ± 0.16 ^a	1.30 ± 0.08 ^a	57.73 ± 0.06 ^{ab}
Naachem-Tiir	97.48 ± 0.10 ^a	0.22 ± 0.12 ^a	1.25 ± 0.12 ^a	80.02 ± 0.09 ^a	1.27 ± 0.15 ^a	58.77 ± 0.64 ^{ab}
Nutsugah	98.14 ± 0.09 ^a	0.21 ± 0.06 ^a	1.11 ± 0.13 ^a	79.29 ± 0.12 ^a	1.13 ± 0.12 ^a	57.13 ± 0.55 ^{ab}
WAAPP	98.13 ± 0.08 ^a	0.21 ± 0.10 ^a	1.17 ± 0.08 ^a	79.82 ± 0.05 ^a	1.19 ± 0.18 ^a	58.60 ± 1.40 ^{ab}

L* - lightness from dark, a* / -a* = redness / greenness, b* / -b* = yellowness / blueness, h* = Hue angle (dominant colour), C* = Chroma (colour intensity), PC = Paste clarity. "Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different (p ≤ 0.05)".



The L* values ranged between 97 to 98, indicating that the different cultivars' starches were white (Table 3.1). Except for the paste clarity (PC), the starches had similar ($p \leq 0.05$) L*, a*, b*, h*, and C*. They had a similar intensity of white colour when observed with naked eyes. The differences observed in the PC properties of the FPS cultivars may be attributed to variations in their carotenoid concentrations (Peroni *et al.*, 2006). PC is an essential property of starch gels, especially for food applications, and varies among different botanical sources or cultivars (Tortoe *et al.*, 2017). The results showed that the starch formed clear pastes characteristic of starches from most root and tuber crops (Tortoe *et al.*, 2017; Peroni *et al.*, 2006). Maa-Lana starch was the most transparent, with a transmittance of nearly 63 %, whereas E82 was less transparent, with about 51 % transmittance. PC is particularly beneficial in food systems that require high transparency (Tortoe *et al.*, 2017; Peroni *et al.*, 2006); hence FPS could be suitable in food applications without impacting the colour of the food, such as in jellies and fruit pastes.



3.3.3 Physicochemical Characteristics of FPS

The physicochemical properties of Frafra potato starches are summarized in table 3.2

Table 3.2: Physicochemical characteristics of FPS

Cultivar	Aml (%)	Amlp (%)	RS (%)	RC (%)	GS (μm)
E 82	16.3 \pm 1.13 ^a	83.7 \pm 0.03 ^a	17.4 \pm 1.45 ^c	30.5 \pm 0.01 ^a	11.5 \pm 1.74 ^b
E111	16.2 \pm 1.34 ^a	83.8 \pm 0.04 ^a	17.4 \pm 1.10 ^c	31.5 \pm 0.71 ^a	12.2 \pm 1.82 ^{ab}
E132	15.6 \pm 1.14 ^a	84.4 \pm 0.03 ^a	21.5 \pm 1.25 ^a	31.5 \pm 0.01 ^a	13.2 \pm 1.66 ^a
E134	17.0 \pm 0.03 ^a	83.0 \pm 0.05 ^a	17.1 \pm 1.56 ^c	32.5 \pm 0.71 ^a	12.6 \pm 1.53 ^{ab}
E145	17.0 \pm 0.03 ^a	83.0 \pm 0.07 ^a	18.4 \pm 1.67 ^b	31.5 \pm 0.71 ^a	12.4 \pm 1.98 ^{ab}
Maa-Lana	15.6 \pm 1.06 ^a	84.4 \pm 0.04 ^a	18.5 \pm 1.13 ^b	34.3 \pm 1.41 ^a	12.1 \pm 1.85 ^{ab}
Manga	15.9 \pm 2.04 ^a	84.1 \pm 0.03 ^a	19.2 \pm 0.56 ^{ab}	31.5 \pm 1.41 ^a	12.4 \pm 1.67 ^{ab}
Naachem-Tiir	16.9 \pm 0.04 ^a	83.1 \pm 0.06 ^a	20.2 \pm 0.87 ^a	33.4 \pm 0.01 ^a	12.6 \pm 1.92 ^{ab}
Nutsugah	15.9 \pm 1.04 ^a	84.1 \pm 0.04 ^a	20.5 \pm 1.01 ^a	33.3 \pm 0.01 ^a	13.4 \pm 1.31 ^a
WAAPP	15.6 \pm 2.15 ^a	84.4 \pm 0.13 ^a	20.8 \pm 0.55 ^a	33.5 \pm 1.41 ^a	13.8 \pm 1.01 ^a

Aml = Amylose, Amlp = Amylopectin, RS = Resistant starch, RC = Relative Crystallinity, GS = Starch Granule size. Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

There were no significant differences ($p \leq 0.05$) between the amylose/amylopectin content and the relative crystallinity among the starches of the FP cultivars (Table 3.2). However, there were significant differences ($p \leq 0.05$) in the resistant starch content among the cultivars, with the released cultivars of Frafra potato having higher resistant starch (19 % to 21 %) relative to the E-group cultivars (17 % to 18 %) except for E132, which had the highest (22 %) resistant starch. The granule sizes of FPS were significantly different, ranging from 11.5 μm to 13.8 μm ; however, most cultivars had similar average granule sizes. The similar granule sizes could be one of the reasons why FPS had similar paste

clarity (Table 3.1) since granules of the same size under the same conditions have similar transmittance, hence, similar paste clarity (Aprianita *et al.*, 2013; Tattiyakul *et al.*, 2007).

3.3.4 Syneresis % (Retrogradation) of FPS Gels

Retrogradation occurs when the amylose and amylopectin chains in cooked, gelatinized starch realign themselves to a crystalline structure as the cooked starch cools (Ezekiel and Singh, 2011). The syneresis % of FPS is summarized in table 3.3.

Table 3.3: Syneresis % of FPS Gels

Cultivar	Day 2	Day 4	Day 6	Day 8	Day 10
E 82	3.7 ± 1.3 ^a	9.1 ± 0.1 ^a	17.5 ± 0.4 ^a	21.4 ± 0.2 ^a	24.8 ± 0.2 ^b
E111	3.8 ± 1.1 ^a	9.3 ± 0.1 ^a	17.3 ± 0.1 ^a	21.1 ± 0.1 ^a	24.6 ± 0.1 ^b
E132	4.2 ± 0.3 ^a	9.4 ± 0.4 ^a	17.9 ± 0.2 ^a	22.4 ± 0.4 ^a	26.5 ± 0.2 ^a
E134	4.3 ± 0.2 ^a	9.1 ± 0.3 ^a	17.2 ± 0.2 ^a	22.1 ± 0.4 ^a	26.1 ± 0.4 ^a
E145	4.1 ± 0.1 ^a	9.2 ± 0.1 ^a	16.9 ± 1.2 ^a	22.3 ± 0.1 ^a	26.3 ± 0.1 ^a
Maa-Lana	4.1 ± 0.2 ^a	9.3 ± 0.3 ^a	17.6 ± 0.1 ^a	21.9 ± 0.3 ^a	25.6 ± 0.2 ^{ab}
Manga	4.2 ± 0.0 ^a	9.5 ± 0.2 ^a	17.3 ± 0.4 ^a	22.2 ± 0.1 ^a	26.0 ± 0.0 ^a
Naachem-Tiir	4.1 ± 0.4 ^a	9.6 ± 0.1 ^a	17.5 ± 0.2 ^a	22.1 ± 0.4 ^a	25.9 ± 0.4 ^{ab}
Nutsugah	4.3 ± 0.1 ^a	9.6 ± 0.4 ^a	17.8 ± 0.2 ^a	22.0 ± 0.0 ^a	26.2 ± 0.1 ^a
WAAPP	4.6 ± 0.1 ^a	9.8 ± 0.2 ^a	17.8 ± 0.1 ^a	22.5 ± 0.2 ^a	26.8 ± 0.4 ^a

“Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$)”.

The syneresis % value of the cooked pastes from the different FPS were comparable until day 10 when significant differences ($p \leq 0.05$) began to show (Table 3.3). The syneresis of cooked pastes from FPS increased progressively during storage, with cultivars recording syneresis ranging from about 4% on day 2 to 27% by Day 10. FPS cultivars had similar syneresis % till storage day 8; however, at storage day 10, cultivars E82, E111, Maa-Lana, and Naachem-Tiir recorded significantly lower syneresis % ($p \leq 0.05$). It was observed that

FPS cultivars containing large-sized starch granules, such as WAAPP, E132, and Nutsugah (Table 3.2), showed higher syneresis values at day 10. In contrast, those containing small-sized starch granules, such as E82 and E111 (Table 3.2), showed lower syneresis % at storage day 10. The observed syneresis % of FPS shows that the retrogradation properties are not different from starches from other tropical tubers such as cassava, sweet potato, and yam (Tortoe *et al.*, 2017).

3.3.5 Thermal Characteristics of FPS

The thermal characteristics of Frafra potato starch are summarized in table 3.4.

Table 3.4: Thermal characteristics of FPS

Cultivar	T ₀ (°C)	T _p (°C)	T _c (°C)	ΔT (°C)	ΔH _{gel} (J/g)
E 82	53.7 ± 0.08 ^b	59.6 ± 0.10 ^b	64.5 ± 0.08 ^b	10.8 ± 0.06 ^b	11.3 ± 0.04 ^b
E111	56.8 ± 0.14 ^b	62.4 ± 0.01 ^b	67.5 ± 0.07 ^b	10.7 ± 0.09 ^b	11.6 ± 0.02 ^{ab}
E132	55.7 ± 0.01 ^b	61.2 ± 0.09 ^b	66.4 ± 0.14 ^b	10.7 ± 0.04 ^b	11.8 ± 0.05 ^{ab}
E134	56.5 ± 0.05 ^b	62.1 ± 0.12 ^b	67.2 ± 0.01 ^b	10.7 ± 0.07 ^b	11.5 ± 0.03 ^{ab}
E145	55.8 ± 0.01 ^b	61.3 ± 0.01 ^b	66.9 ± 0.20 ^b	11.1 ± 0.02 ^b	11.2 ± 0.08 ^b
Maa-Lana	62.6 ± 0.32 ^a	67.2 ± 0.21 ^a	72.8 ± 0.91 ^a	10.2 ± 0.01 ^{bc}	12.7 ± 0.07 ^a
Manga	53.5 ± 0.16 ^b	60.9 ± 0.13 ^b	65.3 ± 0.01 ^b	11.8 ± 0.10 ^{ab}	12.4 ± 0.02 ^a
Naachem-Tiir	55.1 ± 0.22 ^b	61.2 ± 0.09 ^b	66.3 ± 0.06 ^b	11.2 ± 0.05 ^b	12.6 ± 0.06 ^a
Nutsugah	61.8 ± 0.41 ^a	66.4 ± 0.05 ^a	71.9 ± 0.11 ^a	10.1 ± 0.03 ^{bc}	11.9 ± 0.09 ^{ab}
WAAPP	53.4 ± 0.01 ^b	59.5 ± 0.18 ^b	65.4 ± 0.15 ^b	12.0 ± 0.02 ^a	12.8 ± 0.01 ^a

“T₀ = onset temperature, T_p = peak temperature, T_c = final temperature, ΔT = change in temperature, ΔH_{gel} = enthalpy of gelatinization (dry weight basis, based on starch weight). Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different (p ≤ 0.05)”.

FPS showed similar thermal properties (Table 3.4). However, two cultivars (Maa-Lana and Nutsugah) had significantly higher (p ≤ 0.05) onset, peak, and conclusion temperatures. These are the temperatures at which starch granules absorb water and swell, resulting in

increased viscosity, to the point where the number of swollen intact granules is maximum, and the holding period at which usually leads to further disruption of granules and amylose leaching, respectively. Also, WAAPP had a significantly higher ($p \leq 0.05$) gelatinization range, and Nutsugah had the least. The released Frafra potato cultivars had significantly higher ($p \leq 0.05$) enthalpies of gelatinization, except for Nutsugah, which was lower but similar to that of the unreleased E-group cultivars. The thermal attributes of starch are associated with several factors, such as the amylose/amylopectin content, granule size, ultrastructure of the starch granules and proportion and kind of crystalline organization (Gayin *et al.*, 2016; Ezekiel and Singh, 2011; Peroni *et al.*, 2006). In this study, FPS had similar amylose content (Table 3.2), crystal type (Figure 3.2), relative crystallinity (Table 3.2) and granule morphological structure (Figure 3.4), but their granule size distribution was different (Table 3.2), which might result in the different thermal characteristics in FPS. These parameters are useful in food processing; this is because gelatinization reduces the crystallinity of starch granules while increasing their non-crystalline amorphous content. This causes visible changes in the optical and rheological properties of food systems (Gayin *et al.*, 2016; Ezekiel and Singh, 2011).

3.3.6 XRD Spectroscopy of FPS

The importance of X-ray diffraction (XRD) in food analyses cannot be overstated. Polymorphism, crystallinity, and amorphism can all be determined using XRD. These parameters aid in controlling the texture and stability of foods under varying processing and storage conditions. XRD has aided in the study of common food ingredients such as starches and fats. XRD enhances FTIR and differential scanning calorimetry (DSC) studies on starch gelatinization in foods. XRD was used to characterise the X-ray diffraction pattern and crystal type of the Frafra potato starches in figure 3.3.

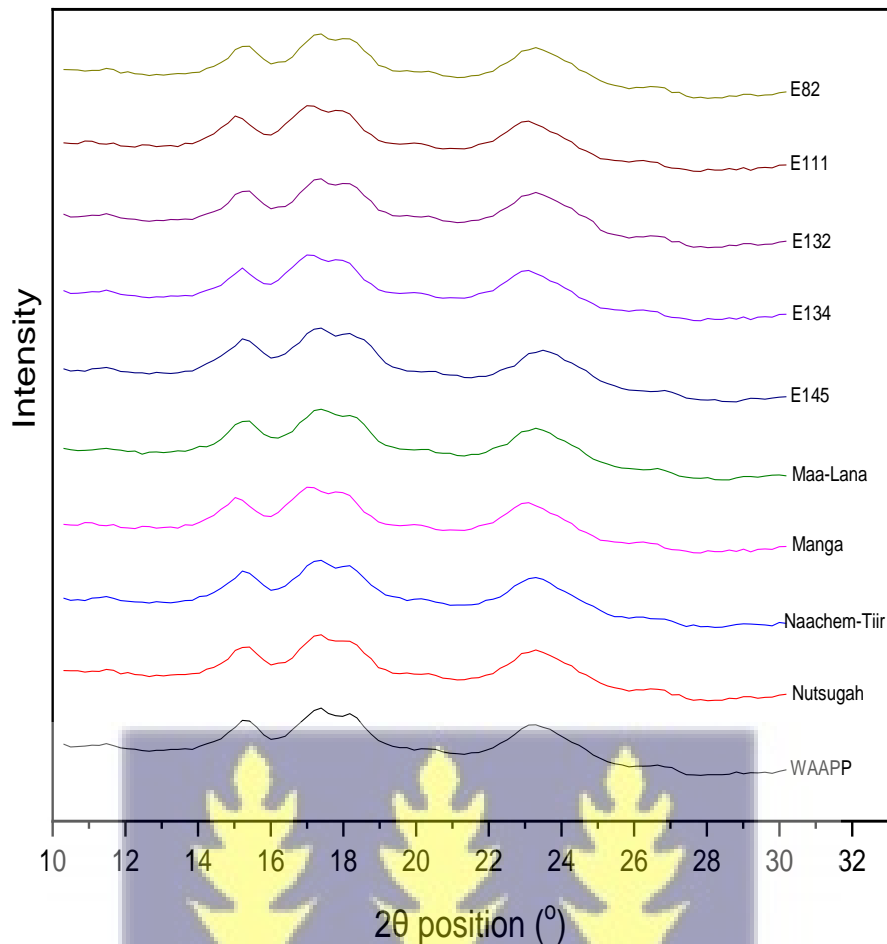


Figure 3.2: XRD spectra of FPS

According to the XRD patterns, three types of starch crystallinity are reported, known as A-, B- and C-type (Kowsik *et al.*, 2018; Gayin *et al.*, 2016; Huang *et al.*, 2015). C-type starch is a mixture of A- and B-type crystallinities and can be further classified to C_A-type (closer to A-type), C-type and C_B-type (closer to B-type) according to the proportion of A- and B-type allomorphs. A-type allomorphs show strong diffraction peaks at about 15° and 23° and an unresolved doublet at around 17° and 18° 2θ. However, B-type allomorphs show strong diffraction peaks at about 5° 2θ (Kowsik *et al.*, 2018; Huang *et al.*, 2015; Zhu *et al.*, 2011).

The XRD patterns for FPS (Figure 3.2) showed strong diffraction peaks at about 15° and 23° and an unresolved doublet at around 17° and 18° 2θ. The display of strong peaks at these sites is characteristic of A-type allomorphs, implying that the FPS cultivars are A-type

starches. The relative crystallinities of starches calculated from the ratio of diffraction peak area and total diffraction area were given in Table 3.2. FPS cultivars had similar ($p < 0.05$) relative crystallinity, typical of A-type starches (Huang *et al.*, 2015).

3.3.7 FTIR Spectroscopy of FPS

FTIR spectroscopy is helpful in starch modification for detecting changes in molecular and structural conformation (Kowsik *et al.*, 2018; Gayin *et al.*, 2016; Zhu *et al.*, 2011). It may also be used for ascertaining the quality of starch for use in culinary and pharmaceutical purposes (Kowsik *et al.*, 2018; Gayin *et al.*, 2016; Zhu *et al.*, 2011). The structural order of the external regions of FPS was characterised by FTIR spectra in figure 3.3.

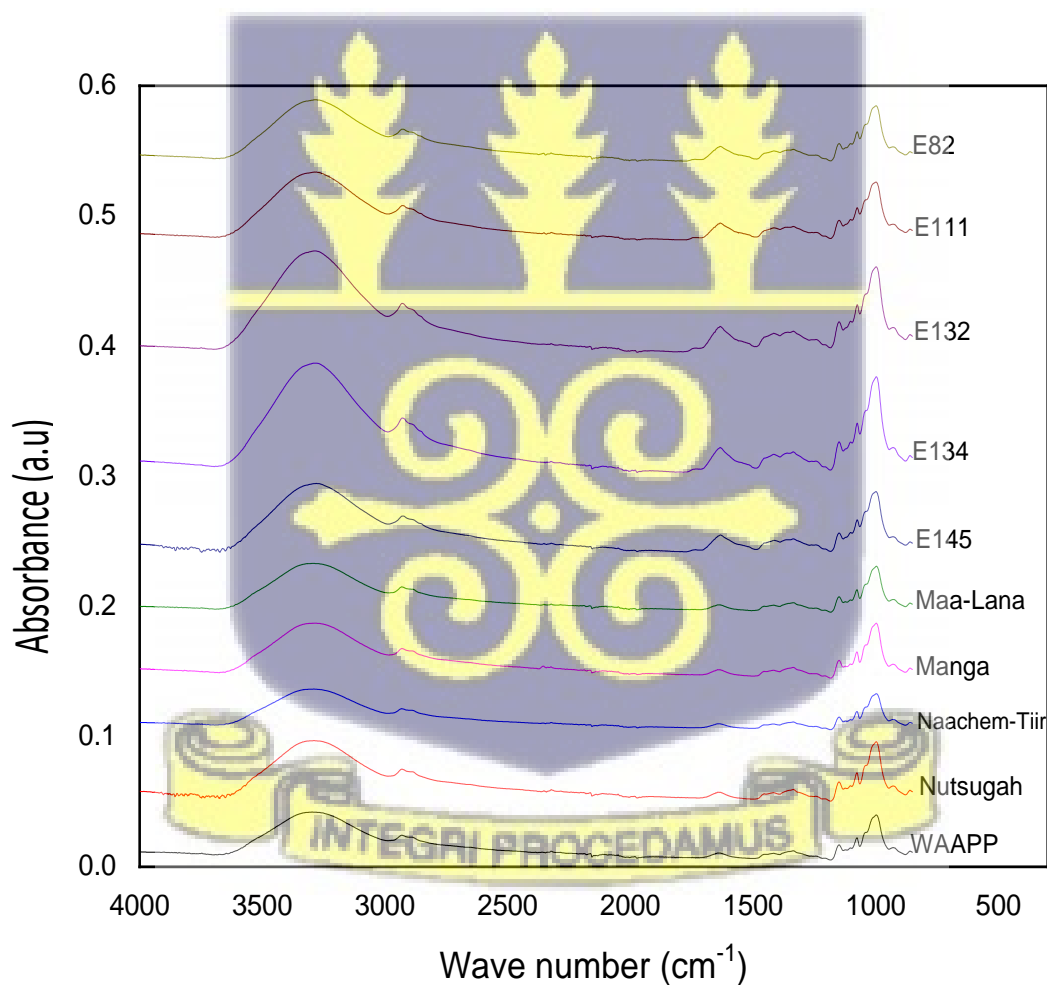


Figure 3.3: FTIR spectra of FPS

Based on figure 3.3, all FPS cultivars showed similar ordered structures in the outer granule region. Even though FTIR is not able to differentiate starch crystal type, native starches with the same crystal type always show a similar FTIR spectrum, the band at 1000/1022 cm^{-1} is more pronounced in A-type starch than in B-type or C-type starches (Kowsik *et al.*, 2018; Gayin *et al.*, 2016; Zhu *et al.*, 2011; Peroni *et al.*, 2006). This result confirms that FPS has an A-type crystallinity.



3.3.8 Scanning Electron Microscopy (SEM)

Micrographs of FPS were captured using a scanning electron microscope. This was done to determine the microstructure of FPS. Figure 3.4 shows the micrographs of FPS.

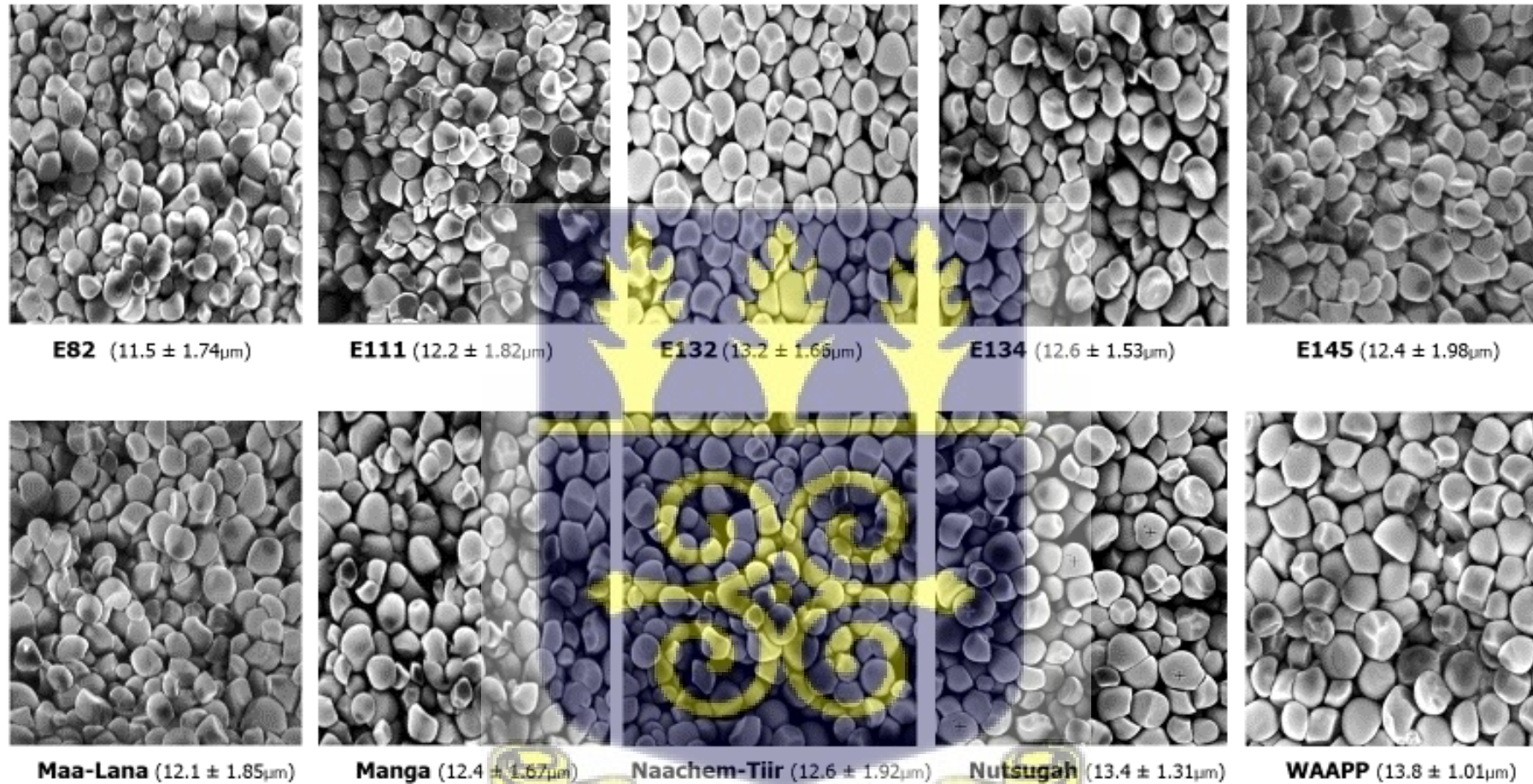
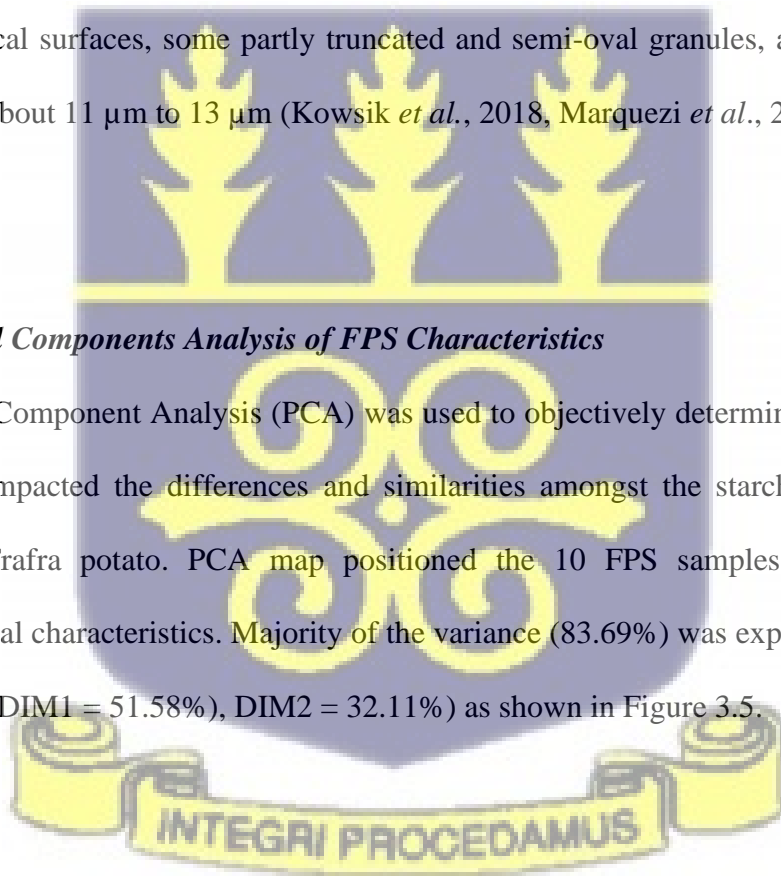


Figure 3.4: SEM structure of starch granules of FPS cultivars (x2300 @ 15 kv)

Micrographs of FPS granules (Figure 3.4) show that all the cultivars had loosely and densely packed small and large granules. FPS cultivars primarily consisted of spherical granules with smooth surfaces. Some oval shape types measuring nearly 14 μm on their central axis and damaged granules (WAAPP) and a few with faceted sides were observed in FPS. Other partly truncated and semi-oval granules were also observed. Generally, the starch granules were within the size range of small and medium (Seetharaman *et al.*, 2010; Lindeboom *et al.*, 2004). Granule diameters ranging between about 12 to 14 μm (mean 12.9 μm) and 11 to 13 μm (mean 12.4 μm) were, respectively, recorded for FPS from the released cultivars and unreleased cultivars. The sizes, shapes, and surface features of FPS were typical of starches from tropical tubers such as sweet potato, cassava, and cocoyam, which are characterised by smooth spherical surfaces, some partly truncated and semi-oval granules, and granule sizes ranging from about 11 μm to 13 μm (Kowsik *et al.*, 2018, Marquezi *et al.*, 2016; Noda *et al.*, 2002).

3.3.9 Principal Components Analysis of FPS Characteristics

The Principal Component Analysis (PCA) was used to objectively determine which features significantly impacted the differences and similarities amongst the starches from the ten cultivars of Frafra potato. PCA map positioned the 10 FPS samples based on their physicochemical characteristics. Majority of the variance (83.69%) was explained in the first 2 dimensions (DIM1 = 51.58%), DIM2 = 32.11%) as shown in Figure 3.5.



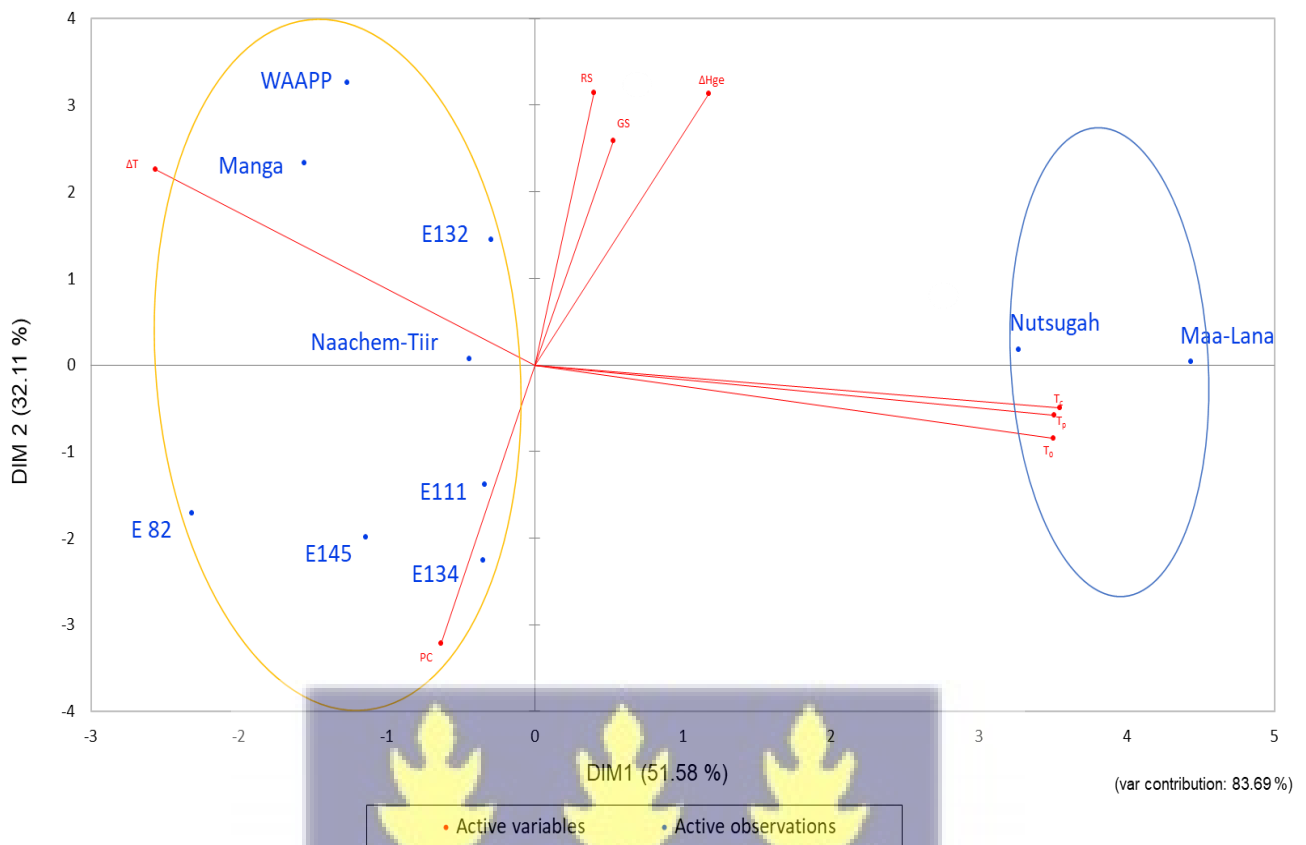


Figure 3.5: PCA Plot for FPS Characteristics

PCA clustered starches from the ten cultivars into two main groups, with Nutsugah and Maa-Lana in one group and the others (E82, E111, E132, E134, E145, Manga, Naachem-Tiir, and WAAPP) in the other group (Figure 3.5). Nutsugah and Maa-Lana starches were mainly characterised by their onset, peak and concluding temperatures. In contrast, the second group was characterised by their paste clarity and change in temperature. According to Gayin *et al.* (2016), Ezekiel and Singh (2011), and Ikegwu *et al.* (2010), these characteristics significantly influence the differences in starches. They are also the critical factors considered in starch modification programs for culinary and pharmaceutical applications.

3.4 Conclusion

Frafra potato starch was of the A-type, with similar amylose content and degree of crystallinity. Starch granules from all ten cultivars were mainly spherical or oval-shaped, with smooth surfaces. PCA grouped starches from the ten cultivars into two clusters defined by their thermal properties and paste clarity. These characteristics provide essential information for practical applications of the starches. They may also influence the advanced selection of accessions of interest for developing suitable cultivars with higher starch yield and cooking quality and specific end-use (baked foods, jellies, powder desserts, thickeners).

3.5 References

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CHAPTER FOUR

4.0 PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERISTICS OF FRAFRA POTATO FLOUR

Abstract

The increasing effects of climate change on food security in developing countries such as Ghana have necessitated evaluating hitherto underutilised climate-resilient crops for food applications. This study processed ten (10) accessions of Frafra potato (FP) into flour (FPF), and the flours were compared based on their physicochemical, functional, and rheological properties to assess their suitability for food applications. Flour yield from the processing of Frafra potato tubers into flour ranged from 59.3 (E134) to 69.3 % (E111). FPF had similar colour but different ($p < 0.05$) paste clarity, ranging from 5.2 (E145 and Maa-Lana) to 8.6% (WAAPP). Least gelation concentration ranged from 8 (WAAPP) to 12% (E 82, 111, 132, 134, 145). The released accessions had significantly higher ($p < 0.05$) protein and ash, ranging from 5.1 to 8.7% and 5.1 to 6.5%, respectively, relative to the unreleased accessions. FPF had significantly different ($p < 0.05$) mineral composition ranging from 6.2 to 8.7 mg with bioavailability of 13.5 to 14.6% for iron, 34.4 to 58.8 mg with bioavailability of 13.1 to 13.7% for calcium, 1.1 to 5.6 mg with bioavailability of 19.1 to 19.9% for zinc, and 334.4 to 451.9 mg with bioavailability of 20.1 to 20.6% for phosphorus. Vitamin A content was also significantly different ($p < 0.05$), ranging from 736.9 to 4948.4 μg . Four proteins commonly found in tuber crops (lipoxygenase, patatin, sporamin and tarin) and protease inhibitors (Bowman-Birk PIs) were identified (SDS-PAGE) in FPF based on their molecular weight. Nineteen (19) amino acids were identified and quantified (LC/MS) in FPF, and out of these, seven (7) were essential amino acids (Valine, Tryptophan, Threonine, Methionine, Lysine, Isoleucine, and Histidine). FPF had similar bulk density (about 0.9 g/ml), oil and water binding capacities (about 3 g/g and about 1.3 g/g respectively), pH (about 6.4) and titratable

acidity (about 0.5). Flow behaviour of FPF indicated a standard non-Newtonian behaviour, which is a pseudoplastic property of gels characterised by changes in viscosity owing to changes in shear rate/stress. Sorption studies revealed that FPF had a maximum allowable moisture content for safekeeping at 10% EMC at about 0.5 water activity. FPF has characteristics that make it suitable for food applications and can promote efforts to address food and nutrition security in Ghana and Sub-Sahara Africa by promoting its utilisation.

4.1 Introduction

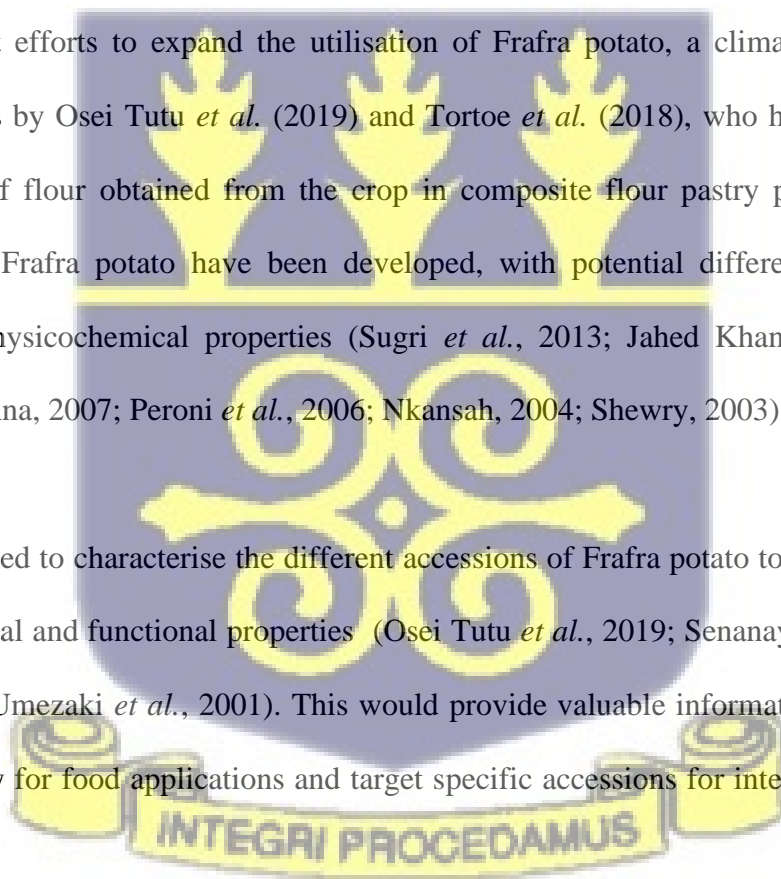
Frafra potato (*Solenostemon rotundifolius*) is a root tuber crop grown in the tropics such as Ghana. In Northern Ghana, Frafra potato is known by common names such as Piesa or Piera (Sugri *et al.*, 2013; Tetteh and Guo, 1997). It is an important food crop grown for domestic consumption and contributes to about 20% of household food, usually from October to December (Sugri *et al.*, 2013; Akanlu *et al.*, 2005; Nkansah, 2004). The tubers are a rich source of carbohydrates, vitamin A precursor (B-carotene) and minerals such as iron and calcium (Akanlu *et al.*, 2005; Nkansah, 2004).

There is evidence that Frafra potato can withstand a wide range of environmental stress conditions, yet it is underutilised in Ghana and Sub-Saharan Africa. The low utilisation of Frafra potato as a staple is due to the relative abundance of close substitutes such as yam (*Dioscorea* spp.) and sweet potato (*Ipomoea batatas*) (Sugri *et al.*, 2013; Tetteh and Guo, 1997). Other reasons for its low utilisation are the high post-harvest losses of about 40%, the lack of appropriate preservation methods, smaller tuber sizes relative to the other more abundant tuber crops, and limited options for promoting utilisation (Sugri *et al.*, 2013; Nkansah, 2004; 2013; Tetteh and Guo, 1997). Frafra potato, if properly harnessed, can contribute to local food and nutrition security (Sugri *et al.*, 2013; Ezekiel and Singh, 2011;

Nkansah, 2004). Considering, for example, that its short shelf life is a barrier to its utilisation, a method for enhancing its uptake could be by processing the tubers into more staple intermediary products such as flour which can then be used as an ingredient in a wide range of food applications (Osei Tutu *et al.*, 2019; Akanlu *et al.*, 2005).

Several efforts are being made to combat the effects of climate change and achieve food security. Prominent among them is recognition of the need to promote climate-resilient, lesser-known crops through extensive characterisation to further expand the portfolio of staple crops. Especially in developing countries (Alencar *et al.*, 2017 and 2015; Marquezi *et al.*, 2016; Ohimain, 2014; Aprianita *et al.*, 2013; Ikegwu *et al.*, 2010; Maeshima *et al.*, 1985). Modest efforts to expand the utilisation of Frafra potato, a climate-resilient crop, include studies by Osei Tutu *et al.* (2019) and Tortoe *et al.* (2018), who have explored the performance of flour obtained from the crop in composite flour pastry products. Several accessions of Frafra potato have been developed, with potential differences in cooking quality and physicochemical properties (Sugri *et al.*, 2013; Jahed Khaniki *et al.*, 2007; Oladele and Aina, 2007; Peroni *et al.*, 2006; Nkansah, 2004; Shewry, 2003).

There is the need to characterise the different accessions of Frafra potato to understand their physicochemical and functional properties (Osei Tutu *et al.*, 2019; Senanayake *et al.*, 2013; Ugwu, 2009; Umezaki *et al.*, 2001). This would provide valuable information to determine their suitability for food applications and target specific accessions for integration into food systems.



4.2 Materials and Methods

4.2.1 Source of Raw Materials

Twenty-five (25) kg each of ten (10) accessions of Frafra potato (FP) were obtained from the Council for Scientific and Industrial Research (CSIR), Savanna Agricultural Research Institute (SARI), Manga-Bawku in the Upper East Region of Ghana. The accessions obtained include WAAPP Piesa, Maa-Lana Piesa, Naachem-Tiir Piesa, Nutsugah Piesa, Manga Moya Piesa (released accessions from Ghana), E82, E111, E132, E134, E145 (unreleased accessions from Burkina Faso). The tubers were transported (about 8 hours) to the food processing laboratory at Food Research Institute, Accra, packed in perforated paper cartons (aeration) for processing.

4.2.2 Frafra Potato Flour Preparation

The procedure described by Tortoe *et al.* (2018) was followed. Frafra potatoes were washed with potable water, weighed, hand-peeled, washed, weighed, and cut into thin slices (five millimetres thick) using a mechanical slicer (SLP45HC, Foshan Sorumpor Electrical, Guangdong, China) into a basin containing a solution of 0.5% sodium metabisulphite to control browning. The slices of tubers were removed after 10 min, and adhering water was allowed to drain before spreading thinly on trays. The sliced tubers were dried at 60 °C in a thermostat-controlled mechanical dryer (TM1006, NEUE HERBOLD, Sinsheim-Reihen, Germany) for 12 h. The dried slices were milled with a hammermill (Full Circle Pulverator, Jacobson Machine Works, Inc., Minneapolis, USA) to pass through a 425 µm sieve (American Standard Test Sieve Mesh No. 40) to obtain Frafra potato flours (FPF). The flours were packed into high-density polyethylene (HDPE) bags, sealed airtight with an impulse sealer (“Oalink, QNS-3200HI, Accra, Ghana”) and kept for further tests.

4.2.3 Physical Characteristics of FPF

4.2.3.1 Flour yield of Frafra Potato

The yield of FPF and the proportion of peels and moisture removed during processing of the ten (10) accessions of FP was determined as a percentage of the unpeeled FP weight.

4.2.3.2 Determination of Colour Parameters of FPF

Using the method of Andrés-Bello *et al.* (2013), the colour parameters of FPF were characterised by their paste clarity and colour. The Colour of FPF was measured using a Minolta Chromameter (CR-400 Chroma Meter, Konica Minolta, Tokyo, Japan). The instrument was calibrated against a standard white tile ($L^* = 98.93$, $a^* = 0.31$, and $b^* = 4.63$) before use. FPF samples were contained in a transparent petri dish and covered with the same. FPF colour was also described using C^* (Chroma) and h^* (Hue angle) notations. Paste Clarity of FPF was determined as follows. Five millilitres (5 mL) of 1% flour suspension in 15 mL screw-capped centrifuge tubes were incubated in a boiling water bath for 30 minutes, with continual shaking. The flour solutions were cooled to 25 ± 0.53 °C, and their transmittance (%) were determined against water blanks at 650 nm on a UV-VIS spectrophotometer (Shimadzu 1800, Tokyo, Japan).

4.2.3.3 Determination of Particle Size Distribution of FPF

According to Aprianita *et al.* (2013), the particle size distribution of FPF was determined using a Meinzer II mechanical shaker (Advantech Manufacturing, Inc., New Berlin, USA) fitted with 100 μm - 500 μm screen sieves. One hundred grams (100 g) of each flour was shaken for about 20 min, with each fitted sieve, at a frequency of 50 Hz. After 20 min, the particles kept by all the sieves were weighed. The weight obtained was calculated as a proportion of the total sample used to determine the particle sizes distribution of the flours.

4.2.4 Chemical Characteristics of FPF

4.2.4.1 Least Gelation Concentration (LGC)

Samples suspension of 2 to 12 % (w/v) were prepared in five millilitres of distilled water. Test tubes containing the suspensions were heated for 1 h in boiling water (100 °C). They were cooled afterwards under running water and then followed by rapid cooling in a refrigerator (4 °C) overnight with tubes inverted. The least gelation concentration (LGC) was measured when the samples in the inverted test tubes slipped along the tubes' walls. The least gelation concentration of the flours was estimated following Adebowale *et al.* (2005).

4.2.4.2 Proximate Composition of FPF Samples

The proximate composition of the flours was characterised using AOAC methods (AOAC, 2005). Components such as moisture (method 925.40), fat (method 948.22), protein concentration (method 955.52), ash (method 935.52), and dietary fibre (method 985.29) were determined, and carbohydrate was estimated as the difference between the sum of the other components and 100%. Energy was determined by calculation, $E = (4 \times \text{protein}) + (4 \times \text{carbohydrate}) + (9 \times \text{fat})$.

Using the methods of Hernández *et al.* (2005), 5 g of flour samples were placed in a previously weighed porcelain crucible and heated for 2 h in a furnace at 350 °C. In a 25 ml calibrated flask, the resulting ash was weighed, dissolved in 3 ml of concentrated nitric acid, and diluted with de-ionized water. The solution was tested for iron, zinc, phosphorus, and calcium using an Atomic Absorption Spectrometer (Shimadzu AA-7000 Atomic Absorption Spectrophotometer, Tokyo, Japan).

Vitamin A composition was also determined according to Garai (2017) using a High-Performance Liquid Chromatography (Shimadzu HPLC-2010HT, Tokyo, Japan). Five grams (5 g) of samples were weighed into a 250 ml flask. Then 300 mg of ascorbic acid, 50 ml of ethanol (96 %), and 15 ml of 50 % potassium hydroxide were added. The samples were rinsed from the flask with 50 ml ethanol into a 250 ml funnel, and then 120 ml water was added, then extraction was made with 3×50 ml hexane. The hexane phase was washed with water three times and was rotary evaporated at 50 °C and 260 mbar. The residue was dissolved in 10 ml of methanol (HPLC purity). A reversed-phase liquid chromatography was used with a diode array detector, pump degasser, Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm), 95:5 methanol/water eluent, and a flow rate of 1 ml/min.

4.2.4.3 Bioavailability of Minerals

Following the method of Skibniewska *et al.* (2002), 2 g of finely pulverised FPF was weighed in conical flasks and treated with deionized water and homogenized. The solution pH was brought to 2.0 with 1 M HCl to produce an appropriate condition for pepsin activity. The enzyme was added in the form of a solution containing 0.5 g enzyme per 100 grams. The samples were then incubated with shaking in a water bath at thirty-seven degrees Celcius. The pH was periodically monitored at this stage and, where necessary, adjusted by adding 6 M HCl. After 2 h, the pH of the solution was raised to 6.8 – 7.0 by adding 6% NaHCO₃ solution, then a 0.4% solution of pancreatin in 0.1 M NaHCO₃ was added at the rate of 10 mL per 40 mL of the sample solution. The samples were incubated for 4 hours. After which, the mineralised sample was centrifuged at 4000 × g for 30 min and decanted by draining through medium-hardness filters. The filtrate was transferred into a borosilicate glass flask and evaporated on an aluminium electric heating block. The samples were ashed in a mixture of acids, as explained earlier. Bioavailability was expressed as a proportion of

the total reference mineral in the samples. Bioavailability = $X/Y \times 100\%$. Where Y is the total content of the reference mineral and X is the reference mineral in the filtrate.

4.2.4.4 Protein profile of FPF

Protein profile was identified using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). One gram (1 g) of FPF was used in sample preparation. Sample buffer and sodium dodecyl sulfate (SDS) were added to the samples and homogenized in a vortex blender; Dithiothreitol (DTT) was added to the homogenized samples and then centrifuged at 5000 g for 10 min. Two hundred microliters (200 μ L) each of the supernatant was pipetted into Eppendorf tubes. Fifty microliters (50 μ L) sample loading buffer was added to the protein sample buffer and boiled at 95 °C for 10 minutes. Fifteen microliters (15 μ L) each of the samples were pipetted into the gel wells with five microlitres of molecular protein ladder. The gel was fixed, after which a staining solution (Coomassie Brilliant Blue) was added. A de-staining solution was then added until the background of the gel was fully de-stained, and the bands were clear. The gel image was captured using an imager (Amersham Imager 600, Xuri, United Kingdom). The molecular weight, indicated by the bands, was used to identify the proteins present.

4.2.4.5 Amino Acids Profile of FPF

An improved analytical method by Ozcan and Senyuva (2006) that offers rapid, accurate determination and identification of amino acids in various foods were used to quantify amino acid concentration in FPF. Amino acids concentration in FPF was determined using Liquid Chromatography/Mass Spectrometry (LC/MS).

A stock solution of 1000 μ g/ml amino acid was prepared by dissolving 25 mg of FPF in 25 ml distilled water. Working standards were prepared by diluting the stock solution of amino

acids to concentrations of 0.05 – 5.00 µg/ml with 0.2mM acetic acid, kept at 4 °C. FPF samples were milled using a blender (IKA T 25 Digital Ultra-Turrax™ Immersion Blender, Thermo Fisher Scientific, USA). The pH of each homogenized sample was measured before sample preparation. Subsamples of the homogenate were stored at –20 °C in high-density polyethylene bottles with plastic screw-capped lids. A finely homogenized sample (1 g) was weighed [fresh weight (FW)] into a 10ml glass centrifuge tube with a cap. Ten millilitres (10 mL) of 0.2 mM acetic acid was added to the samples. After mixing in a vortex mixer for 2 min, the mixture was centrifuged at 5000 rpm for 10 min at 5 °C. The clear supernatant was quantitatively transferred into a vial. It was filtered through a 0.45 µm nylon syringe filter before LC/MS analysis. Quality assurance measures were employed for amino acids, which involved inclusion in each batch of 10 samples, duplicate samples spiked at 5, 10, 50 mg/100 g and reagent blank. Batches of samples were deemed acceptable if spiked samples indicated better than 80% recovery.

An Agilent 1100 Series (Waldbronn, Germany) system comprised of a binary pump, an autosampler, and a temperature-controlled column oven was used for the LC/MS experiments, which were coupled to an Agilent 1100 MS detector equipped with an atmospheric pressure chemical ionization (APCI) interface. The chromatographic separations were carried out on a ZORBAX Bonus-RP narrow-bore (100 mm x 2.1 mm, 3.5 m) column at 400 °C using an isocratic mixture of 0.01 mM acetic acid in a 0.2 % aqueous solution of formic acid at a flow rate of 0.2 mL/min. Data was collected in SIM mode with the following interface parameters: 4 L/min drying gas flow, 55 psig nebulizer pressure, 320 °C drying gas temperature, 425 °C vaporizer temperature, 3 kV capillary voltage, 8 A corona current, 55 V fragmentor voltage, and 27 msec dwell time.

4.2.5 Functional Characteristics of FPF

4.2.5.1 Bulk Density

The flour bulk density was determined using the method described by Oladele and Aina (2007). 50 g each of the flour samples were measured into one hundred millilitres measuring cylinder. The measuring was tapped continuously on a laboratory table until a constant volume was obtained. The bulk density was then calculated using the formula:

$$\text{Bulk density} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}}$$

4.2.5.2 Water and Oil Absorption Capacity

The water and oil absorption capacities of FPF was determined by the method described by Klunklin and Savage (2018) with modification. Ten grams (10 g) of each flour were mixed with 100 ml of distilled water/oil (Frytol vegetable oil, Wilmar Africa Limited, Ghana) in a centrifuge tube and allowed to stand at room temperature (26 ± 1.3 °C) for 1 hour. The mixture was centrifuged (Remi R23 Research Centrifuge, REMI Instruments, Mumbai, India) at 2500 rpm for 30 minutes. Water/oil was drained, and the paste was weighed. Water and oil absorption capacities were expressed as a gram of water/oil absorbed per gram of sample.

4.2.5.3 Swelling Power and Solubility (Soluble matter)

The flour samples' swelling power and soluble matter were determined using the procedure described by Chinma *et al.* (2012). 1 g of each sample was weighed into a 50ml graduated centrifuge tube. Distilled water was added to obtain a total volume of 40ml. The suspension will then be stirred. The samples were heated at 85 °C in a thermostatically controlled bath (Grant OLS 200, Grant Equipment, England) for 30 minutes while shaking continuously. The tube was removed from the bath, wiped dry, cooled to room temperature (25 °C \pm 0.53),

and centrifuged (Remi R23 Research Centrifuge, REMI Instruments, Mumbai, India) for 15 minutes at 2200 revolutions per minute. The supernatant was decanted and evaporated in a hot air oven. The dried residue was weighed to determine the solubility (soluble matter) using the formula:

$$\text{Solubility \%} = \frac{\text{Weight of dried sample in supernatant}}{\text{Weight of original sample}} \times 100$$

The pastes obtained from decanting the supernatant were weighed to determine the swelling power. The swelling power was calculated using the formula:

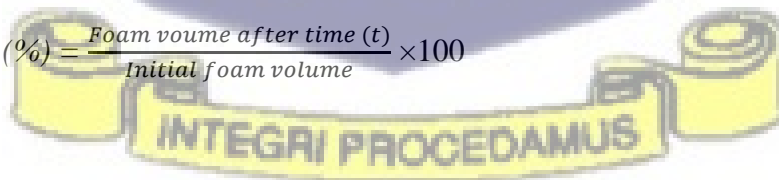
$$\text{Swelling power} = \frac{\text{weight of paste}}{\text{weight of dry flour}}$$

4.2.5.4 Foaming Volume and Stability

The foaming volume and stability of FPF were determined using the method described by Chinma *et al.* (2012). 10 g of each sample were whipped with 100 ml distilled water for 5 minutes in a blender (to foam) and poured into a 250 ml graduated cylinder. Foam stability was determined at 0, 10, 20, 30, 40, 50 and 60 min after whipping. The foam volume at 30 seconds after whipping was expressed as the foam capacity and the foam volume after 60 minutes as the stability for the respective periods. Foaming capacity and Foam stability are given by the following:

$$\text{Foaming capacity (\%)} = \frac{\text{Volume after blending} - \text{Volume before blending}}{\text{Volume before blending}} \times 100$$

$$\text{Foam stability (\%)} = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume}} \times 100$$



4.2.5.5 pH of FPF Samples

The pH of FPF was determined using the method described by Oladele and Aina (2007). Ten per cent (10%) suspension of each flour was prepared, and the pH of 10 ml of each

solution was determined using a pH meter (Mettler Toledo S220-Kit, Benchtop pH/ISE Meter, USA).

4.2.5.6 TTA

TTA of FPF was determined using the method described by Oladele and Aina (2007). The samples used for determining pH were also used to determine total titratable acidity. A few drops of phenolphthalein indicator were added to the solution. Titration was carried out by adding 0.1 M NaOH from a burette until a colour change to pink indicated the endpoint. The volume of NaOH added was read from the burette and multiplied by 0.09 to obtain the % titratable acidity as lactic acid (being the predominant acid).

4.2.5.7 Pasting properties of FPF

The pasting properties of FPF was determined by the method described by Klunklin and Savage (2018). FPF pasting characteristics were determined on a 12% slurry, using a Brabender (Viscograph – E, Brabender GmbH & Co. KG. 803301, 803301E000-02, Germany). This was done to record the following indices quantitatively: peak viscosity, pasting temperature, hot paste viscosity (peak), cool paste viscosity (final), breakdown, and setback.

4.2.5.8 Flow Behaviour of FPF Slurry

Using the method of Klunklin and Savage (2018), the viscosity of FPF gels [12% flour heated at 95 °C for 30 min with continual shaking and cooled to room temperature (26 ± 1.3 °C)] was determined using a viscometer (Brookfield Engineering Labs Inc., Stoughton MA, USA) at different spindle speeds (5-30 rpm) using an RV spindle 2. Measurements were taken in a 250 mL beaker, 2 min after the spindle was immersed in FPF gels. Viscosity data

was recorded at 2 min intervals. This was done to allow thermal equilibrium between the test sample and spindle and to eliminate the effect of immediate time dependence, as directed by the manufacturer of the viscometer. The flow behaviour (n) and apparent viscosity (k) were determined using the Herschel Buckley model.

4.2.5.9 Moisture Sorption Studies

The standard gravimetric method, as explained by Andrade *et al.* (2011) was used for the equilibrium moisture studies. It consisted of moisture sorption measurements using six (6) different concentrations of sulfuric acid (H_2SO_4) solution ranging through five, fifteen, thirty-five, forty-five, fifty-five and sixty-five per cent to produce water activities (aw) ranging from 0.1 to 0.9. These saturated solutions were carefully poured into glass containers. The flours were placed in plastic containers, and a thread was used to support the samples and inserted into the containers to rest just above the acid solution and kept in an oven preset at 30 °C. The samples were removed and weighed every twenty-four hours using an electronic weighing balance until consecutive readings were less than 0.05% of the sample weight. The moisture sorption isotherms were obtained by plotting the equilibrium moisture content (EMC, expressed as a gram of water per gram of dry solids) against aw . The EMC were calculated as averages of triplicates from which the moisture sorption isotherms were determined.

4.2.5.10 Statistical Analysis

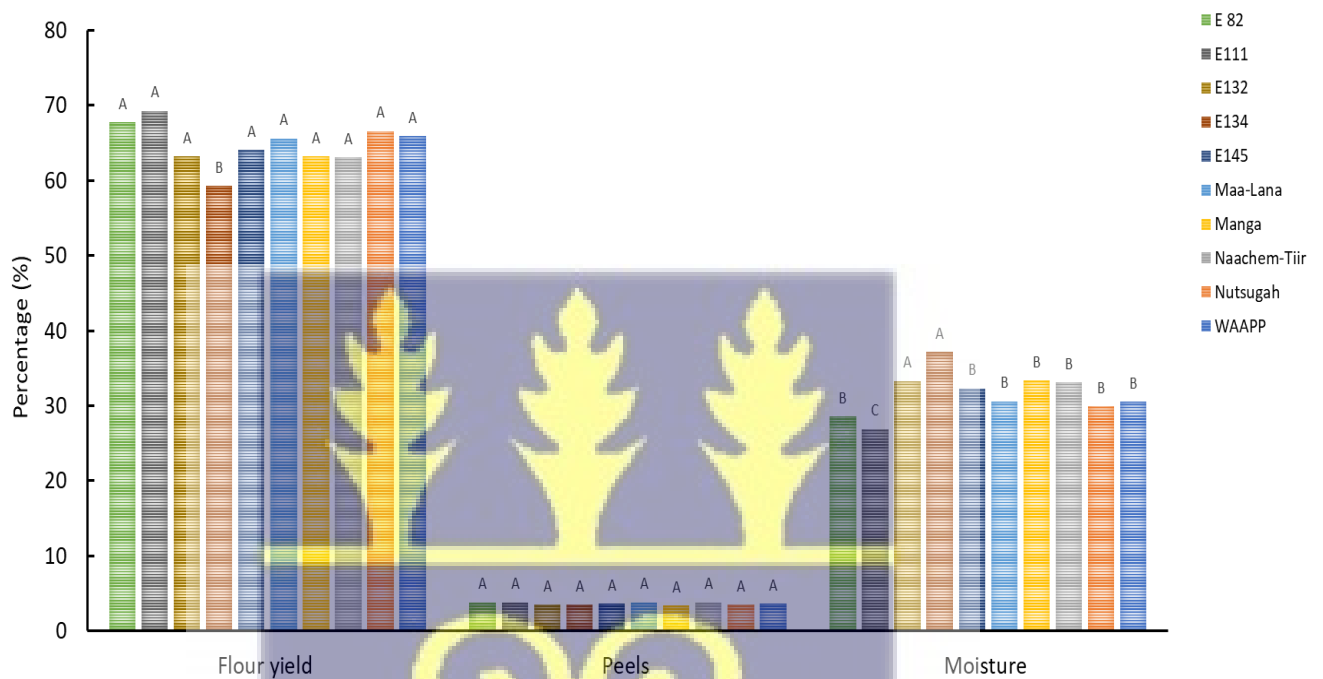
The data obtained were subjected to one-factor Analysis of Variance (ANOVA) using “R” statistical software for Windows pc version 4.1.1 (R Project, Bell Laboratories, USA) and Duncan Multiple Range Test (DMRT) was performed to identify varieties with significantly different ($p < 0.05$) means. Principal component analysis (PCA), in XLSTAT 2018 for

windows pc, was used to cluster samples with close associations based on their physicochemical properties.

4.3 Results and Discussion

4.3.1 Flour Yield of Frafra Potato (FPF)

The flour yield of the ten accessions of Frafra potato tubers are summarized in Figure 4.1



Bars, within attributes, with different letters are significantly different ($p \leq 0.05$)

Figure 4.1: Flour yield, moisture content and proportion of peels from FP

The flour Yield of the Frafra Potato tubers ranged from 59-69% (Figure 4.1). There was a significant difference ($p \leq 0.05$) between the flour yield and moisture among the FPF cultivars. However there were significant differences between peels ranging from 27% (E111) - 38% (E134) and fibre ranging from 25% (Manga) - 31% (E111). The differences observed in the flour yield and moisture content can be attributed to varietal differences reported in other tuber crops flour studies (Tortoe *et al.*, 2017; Ezekiel and Sing, 2011).

However, the flour yield in these FPF cultivars is higher than flours from some potato varieties, ranging from 37 to 49% (Ezekiel and Sing, 2011), and some varieties of cassava, which ranged from 18 to 20 % (Dziedzoave *et al.*, 2006). The yield of FPF provides important information for the selection of indigenous tubers for flour production. It may also influence the advanced selection of accessions of interest for developing suitable cultivars for specific end-use.

4.3.2 Colour of FPF

The colour and optical properties of FPF are summarized in table 4.1

Table 4.1: Color parameters of FPF

Cultivar	L*	a*	b*	h*	C*	PC (%)
E 82	83.62 ± 0.06 ^a	1.10 ± 0.09 ^a	11.87 ± 0.12 ^a	84.71 ± 0.06 ^a	11.92 ± 0.11 ^a	6.27 ± 0.31 ^c
E111	83.82 ± 0.12 ^a	1.12 ± 0.02 ^a	12.05 ± 0.14 ^a	84.69 ± 0.17 ^a	11.99 ± 0.08 ^a	6.20 ± 0.26 ^c
E132	82.59 ± 0.09 ^a	1.10 ± 0.13 ^a	11.79 ± 0.10 ^a	84.67 ± 0.09 ^a	11.99 ± 0.15 ^a	5.43 ± 0.12 ^d
E134	81.90 ± 0.12 ^a	1.08 ± 0.11 ^a	11.95 ± 0.09 ^a	84.84 ± 0.13 ^a	11.99 ± 0.02 ^a	6.47 ± 0.06 ^c
E145	82.86 ± 0.11 ^a	1.12 ± 0.03 ^a	12.03 ± 0.14 ^a	84.68 ± 0.01 ^a	12.08 ± 0.16 ^a	5.20 ± 0.26 ^d
Maa-Lana	83.51 ± 0.05 ^a	1.13 ± 0.05 ^a	11.65 ± 0.11 ^a	84.46 ± 0.03 ^a	11.71 ± 0.05 ^a	5.20 ± 1.07 ^d
Manga	82.49 ± 0.07 ^a	1.11 ± 0.15 ^a	11.82 ± 0.07 ^a	84.64 ± 0.10 ^a	11.87 ± 0.17 ^a	7.73 ± 0.06 ^b
Naachem-Tiir	82.48 ± 0.10 ^a	1.12 ± 0.12 ^a	12.05 ± 0.12 ^a	84.69 ± 0.08 ^a	12.10 ± 0.05 ^a	8.43 ± 0.12 ^a
Nutsugah	83.54 ± 0.04 ^a	1.09 ± 0.06 ^a	11.91 ± 0.13 ^a	84.77 ± 0.12 ^a	11.96 ± 0.01 ^a	7.47 ± 0.06 ^b
WAAPP	83.53 ± 0.08 ^a	1.11 ± 0.10 ^a	12.09 ± 0.08 ^a	84.75 ± 0.05 ^a	12.14 ± 0.12 ^a	8.60 ± 1.40 ^a

“L* - lightness from dark, a* / -a* = redness / greenness, b* / -b* = yellowness / blueness, h* = Hue angle (dominant colour), C* = Chroma (colour intensity), PC = Paste clarity. Values are means of triplicates with standard deviation. Means in the same column with different superscript are significantly different (p ≤ 0.05).”

Table 4.1 shows that the L* values for the flours ranged between about 82 to 84 and indicate that the flours from the different cultivars were darker than the standard white (L* ~ 98).

Except for PC, the flours had similar (p ≤ 0.05) L*, a*, b*, h*, and C*. PC ranged from about 5 to 9 %, with E145 and Maa-Lana having the least and WAAPP the highest. The differences observed in the PC properties of the FPF cultivars may be attributed to variations

in their carotenoid concentrations and varietal differences (Tortoe *et al.*, 2017; Peroni *et al.*, 2006). PC is an essential property of flour gels, especially for culinary applications, and differs amongst various botanic sources or cultivars (Tortoe *et al.*, 2017). The results showed that the flours formed opaque gels, which is not desirable in food systems that require high transparency, such as jellies (Tortoe *et al.*, 2017; Peroni *et al.*, 2006). However, FPF could be useful in food systems that do not require transparency, such as pastry products, soups and stews (Peroni *et al.*, 2006).

4.3.3 Particle Size Distribution of FPF

The particle size distribution of the flours from the ten (10) cultivars of Frafra potato is summarized in figure 4.2.

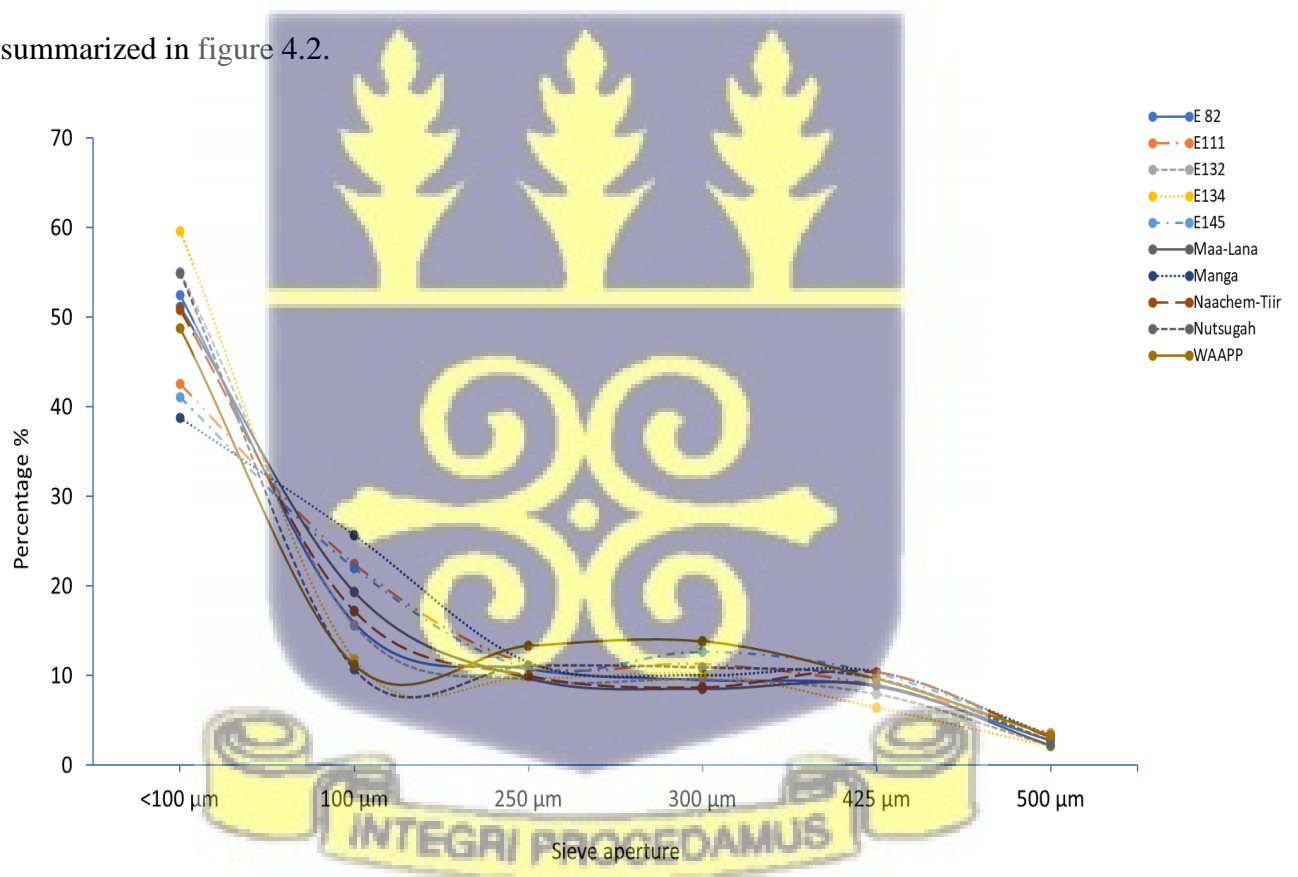


Figure 4.2: Particle size distribution of FPF

Particle size is an important physical property of flour products. It is crucial because it influences the texture and feel of food ingredients, appearance, viscosity, and bulk density (Aprianita *et al.*, 2013). The particles retained by each sieve differed significantly ($p \leq 0.05$) (Figure 4.2). The flours had most of their particles below 100 μm , where E134 had the highest distribution (60 %) and Manga had the least distribution (39 %). A similar particle size distribution was reported for regular wheat flour, with most of the particles below 100 μm , by Hareland (1994). There were fewer particles retained by sieve sizes 100 to 425 μm , respectively. In contrast, sieve size 500 μm retained weights that ranged from 10.7 - 25.7%, with Nutsugah having the least and Manga having the highest, respectively. The results indicate that the flours have smaller particle sizes than larger sizes. Smaller particle sizes suggest that more of the flours have very fine particle distribution, similar to typical wheat flour. Flour with very fine particle size distribution can fill a smaller volume (Marquezi *et al.*, 2016; Aprianita *et al.*, 2013). This would be a great advantage in terms of packaging and transportation. Also, smaller particle sizes have implications for functional properties and rate of chemical reactions in food systems. Flours with smaller particles sizes absorb more water, have lower gelatinization temperature and higher viscosity (Marquezi *et al.*, 2016).

4.3.4 Least Gelation of FPF

The least solids concentration of the flours from the ten (10) cultivars of Frafra potato required to form a stable gel is presented in table 4.2.

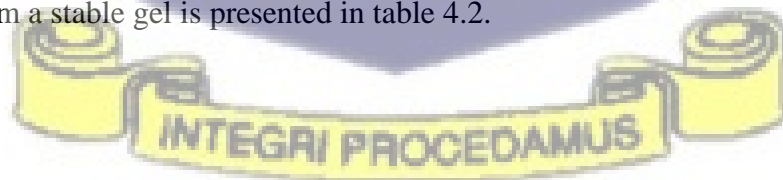


Table 4.2: Least Gelation Concentration of FPF

Cultivar	% w/v					
	2	4	6	8	10	12
E 82	-	-	-	-	-	+
E111	-	-	-	-	-	+
E132	-	-	-	-	-	+
E134	-	-	-	-	-	+
E145	-	-	-	-	-	+
Maa-Lana	-	-	-	-	+	+
Manga	-	-	-	-	+	+
Naachem-Tiir	-	-	-	-	+	+
Nutsugah	-	-	-	-	+	+
WAAPP	-	-	-	+	+	+

% w/v = percent solids, + = gel suspension did not slip along walls of inverted test tubes (for 24 hours, at 4 °C), - = gel suspension slipped along walls of inverted test tubes (for 24 hours, at 4 °C)

The results in Table 4.2 indicate that the released FP cultivars had the least gelation concentration from 8 – 10% w/v, with WAPP having the least concentration (value). The unreleased E-group cultivars required 12% w/v to form a stable gel. In a predominantly starchy matrix such as FPF, Gelation properties are influenced by starch concentration, water absorption capacity, and particle size distribution (Chinma *et al.*, 2012; Ikegwu *et al.*, 2010). Variations in the protein concentration and particle size distribution of FPF could explain the slight differences in their gel formation capacity. Based on the results in table 4.3, it can be suggested that the released cultivars had the least gelation concentration because they had a higher protein concentration. This will impact the use of these varieties in food systems where viscosity is desired.

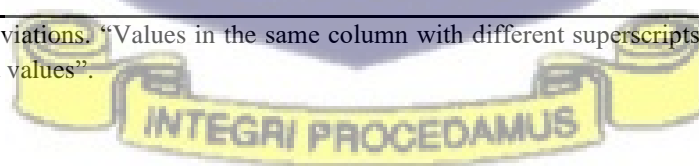
4.3.5 Proximate Composition of FPF

The proximate composition of the flours from the ten (10) cultivars of Frafra potato is summarized in table 4.3.

Table 4.3: Proximate Composition of FPF (per 100 g)

Cultivar	Moisture	Fat	Protein	Ash	Carbohydrate	Dietary Fibre	Energy (kcal)
E 82	7.91 ± 0.12 ^a	1.69 ± 0.07 ^a	6.19 ± 0.43 ^c	5.62 ± 0.08 ^b	81.26 ± 0.26 ^a	3.24 ± 0.05 ^a	380.97 ± 0.09 ^a
E111	7.20 ± 0.09 ^a	1.46 ± 0.04 ^a	6.88 ± 0.08 ^c	5.82 ± 0.01 ^b	80.84 ± 0.33 ^a	3.07 ± 0.08 ^a	379.02 ± 0.23 ^a
E132	7.88 ± 0.22 ^a	1.40 ± 0.02 ^a	5.08 ± 0.12 ^d	5.11 ± 0.13 ^b	82.46 ± 0.15 ^a	3.95 ± 0.11 ^a	381.56 ± 0.03 ^a
E134	7.20 ± 0.09 ^a	1.36 ± 0.01 ^a	5.89 ± 0.11 ^d	5.12 ± 0.03 ^b	83.08 ± 0.21 ^a	2.55 ± 0.65 ^a	381.32 ± 0.12 ^a
E145	7.53 ± 0.19 ^a	1.57 ± 0.01 ^a	5.22 ± 0.23 ^d	5.23 ± 0.17 ^b	82.72 ± 0.09 ^a	3.26 ± 0.16 ^a	381.93 ± 0.22 ^a
Maa-Lana	5.53 ± 0.28 ^b	1.43 ± 0.02 ^a	8.72 ± 0.13 ^a	6.42 ± 0.29 ^a	79.38 ± 0.61 ^b	3.05 ± 0.05 ^a	376.47 ± 0.25 ^a
Manga	5.69 ± 0.28 ^b	1.42 ± 0.05 ^a	7.98 ± 0.13 ^a	6.42 ± 0.29 ^a	79.75 ± 0.61 ^b	3.43 ± 0.05 ^a	376.42 ± 0.32 ^a
Naachem-Tiir	5.48 ± 0.16 ^b	1.45 ± 0.03 ^a	8.53 ± 0.03 ^a	6.37 ± 0.09 ^a	80.23 ± 0.54 ^a	2.59 ± 0.78 ^a	374.17 ± 0.01 ^a
Nutsugah	5.61 ± 0.15 ^b	1.48 ± 0.09 ^a	8.41 ± 0.06 ^a	6.47 ± 0.34 ^a	79.15 ± 0.43 ^b	3.46 ± 0.05 ^a	376.52 ± 0.17 ^a
WAAPP	5.77 ± 0.36 ^b	1.44 ± 0.07 ^a	8.73 ± 0.11 ^a	6.41 ± 0.18 ^a	78.93 ± 0.52 ^b	3.49 ± 0.13 ^a	376.56 ± 0.14 ^a

Mean values (g/100g) of triplicates with standard deviations. *Values in the same column with different superscripts are significantly different ($p \leq 0.05$). The values were reported as % dry matter, except for moisture content values".



It is observed in Table 4.3 that there were significant differences ($p \leq 0.05$) in the moisture, protein, and ash composition of the flours; however, there was no significant difference ($p \leq 0.05$) in the fat, dietary fibre, carbohydrate, and energy content of the flours. Moisture content ranged from 6 to 8 %, and ash content ranged from 5 to 7 %. The released cultivars recorded higher protein content from about 8 – 9%, while the unreleased E-group cultivars had the least, ranging from 5 – 7%. This trend was observed for ash content as well. The higher protein content of the released cultivars indicates relatively better performance in food applications such as pastry (Senanayake *et al.*, 2013; Ugwu, 2009; Maeshima and Ashi, 1985). Also, variations in the proximate composition have implications for functional properties of the flours, such as pasting, least gelation concentration and viscosity (Aprianita *et al.*, 2013; Ezekiel and Singh, 2011). Flours with higher protein content could be useful in baking applications (Alencer *et al.*, 2017 and 2015; Ezekiel and Singh, 2011).

4.3.6 Mineral and Vitamin A Composition

The minerals and vitamin A composition of the flours from the ten (10) cultivars of Frafra potato are summarized in table 4.4.

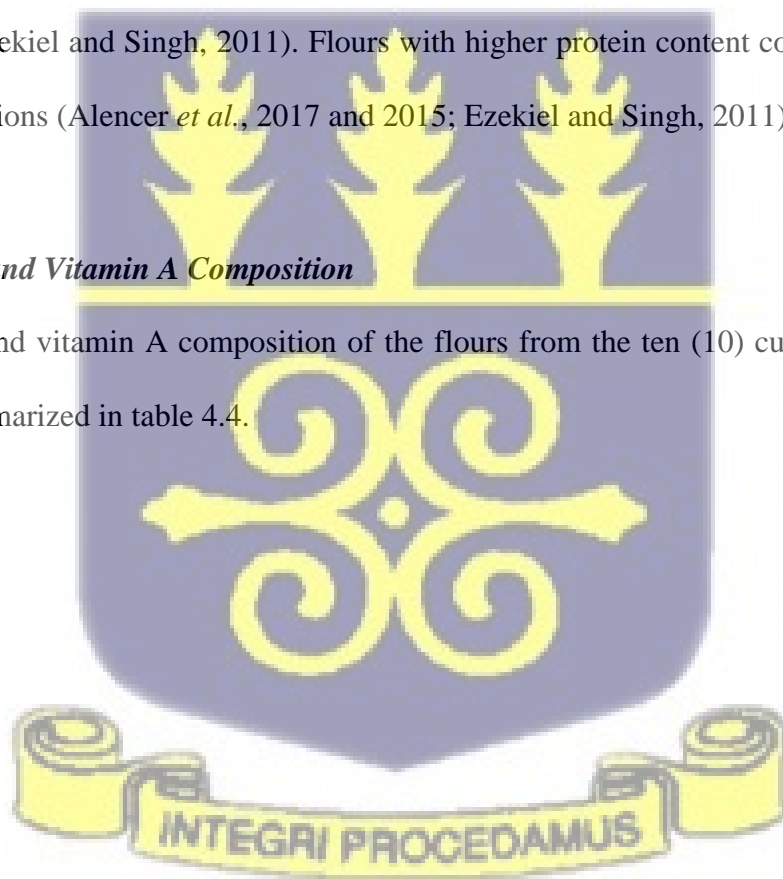


Table 4.4: Mineral and Vitamin A Composition of FPF (mg)

Cultivar	Iron	Calcium	Zinc	Phosphorus	Vitamin A (μg)
E 82	7.04 ± 0.12^b	45.36 ± 0.01^b	2.19 ± 0.03^b	451.90 ± 0.08^a	3080.80 ± 0.06^c
E111	6.50 ± 0.89^b	43.86 ± 0.01^b	1.73 ± 0.88^b	408.82 ± 0.01^b	3181.90 ± 0.03^c
E132	7.10 ± 0.09^b	48.70 ± 0.02^b	2.08 ± 0.02^b	393.11 ± 0.01^b	4948.43 ± 0.05^a
E134	7.20 ± 0.06^b	47.83 ± 0.04^b	4.89 ± 0.51^a	424.12 ± 0.01^b	3969.47 ± 0.01^b
E145	7.15 ± 0.04^b	34.41 ± 0.01^c	2.22 ± 0.13^b	424.23 ± 0.02^b	4117.90 ± 0.09^b
Maa-Lana	8.70 ± 0.10^a	58.84 ± 0.02^a	4.62 ± 0.13^a	413.42 ± 0.01^b	1403.53 ± 0.01^d
Manga	7.05 ± 0.18^b	35.93 ± 0.01^c	2.94 ± 0.03^b	366.42 ± 0.71^b	808.63 ± 0.06^f
Naachem-Tiir	6.78 ± 0.16^b	46.66 ± 0.02^b	2.78 ± 0.03^b	334.37 ± 0.81^b	736.86 ± 0.04^g
Nutsugah	6.76 ± 0.15^b	48.39 ± 0.01^b	5.55 ± 0.06^a	334.47 ± 0.94^b	1100.77 ± 0.03^d
WAAPP	7.70 ± 0.19^b	56.94 ± 0.01^a	1.88 ± 0.71^b	401.50 ± 0.02^b	895.13 ± 0.02^e

Values are means (mg or $\mu\text{g}/100\text{g}$) and standard deviations of triplicates. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

The flours obtained from the ten cultivars showed significant differences ($p \leq 0.05$) in vitamin A and mineral composition (Table 4.4). The unreleased E-group cultivars had significantly higher vitamin A ranging from about 3081 to 4118 μg , whereas the released cultivars were lower, ranging from 737 to 1404 μg . Iron content was similar except for Maa-Lana, which had a significantly higher value, about 9 mg, than the other cultivars. Calcium content ranged from 34 mg to 59 mg, with Maa-Lana and WAAPP having the highest, and E145 and Manga having the least. Phosphorus content was also similar, ranging from 334 to 451 mg, except for E 82, which recorded a significantly higher value. According to Nkansah (2004) and Tortoe *et al.* (2017), differences in mineral and vitamin A composition could be due to varietal differences.

4.3.7 Bioavailability of Minerals

Micronutrient deficiencies, especially in vulnerable groups, such as children and women of reproductive age, are of great concern in developing countries (Sugri *et al.*, 2013). This makes the determination of the bioavailability of minerals in foods essential. Bioavailability is a critical link between food ingredients and the nutritional effects of their proximate composition (Skibniewska *et al.*, 2002). Per cent bioavailability of the minerals in the flours from the ten (10) cultivars of Frafra potato is summarized in table 4.5.

Table 4.5: Percent bioavailability of minerals

Cultivar	Fe	Ca	Zn	P
E 82	13.5 ± 1.03 ^a	13.1 ± 0.24 ^a	19.1 ± 0.35 ^a	20.6 ± 0.14 ^a
E111	13.6 ± 1.04 ^a	13.2 ± 0.05 ^a	19.2 ± 0.26 ^a	20.2 ± 0.09 ^a
E132	14.1 ± 0.05 ^a	13.1 ± 0.20 ^a	19.4 ± 0.17 ^a	20.1 ± 0.23 ^a
E134	14.3 ± 0.06 ^a	13.3 ± 0.17 ^a	19.3 ± 0.28 ^a	20.1 ± 0.18 ^a
E145	14.2 ± 0.07 ^a	13.1 ± 0.28 ^a	19.1 ± 0.19 ^a	20.4 ± 0.40 ^a
Maa-Lana	14.4 ± 0.08 ^a	13.7 ± 0.19 ^a	19.6 ± 0.30 ^a	20.2 ± 0.41 ^a
Manga	14.6 ± 0.09 ^a	13.4 ± 0.30 ^a	19.7 ± 0.21 ^a	20.3 ± 0.42 ^a
Naachem-Tiir	14.5 ± 0.32 ^a	13.7 ± 0.11 ^a	19.5 ± 0.32 ^a	20.6 ± 0.43 ^a
Nutsugah	14.6 ± 0.11 ^a	13.3 ± 0.22 ^a	19.8 ± 0.13 ^a	20.4 ± 0.44 ^a
WAAPP	14.2 ± 0.21 ^a	13.5 ± 0.33 ^a	19.9 ± 0.24 ^a	20.5 ± 0.45 ^a

“Values are means and standard deviations of triplicates. Means in the same column with the same superscripts are not significantly different ($p \leq 0.05$)”.

There were no significant differences ($p \leq 0.05$) between the per cent bioavailability of minerals in the ten (10) FPF samples (Table 4.5). The bioavailability of iron was about 15%, calcium was about 14%, zinc and phosphorus were about 20% and 21%, respectively. Since these flours were made from a common tuber crop variety, it is practical for the flours to exhibit similar chemical characteristics (Dziedzoave *et al.*, 2006), including the bioavailability of their minerals. The low bioavailability values for the minerals in FPF

suggests that it can not be the only source of minerals in food applications; other mineral-rich food ingredients should be added to ensure a greater likelihood of mineral bioavailability.

4.3.8 Protein Profile of FPF

Protein profiling provides useful information on the types of proteins present in food ingredients and their implications for their functionality and food applications. SDS-PAGE was used for protein profiling of FPF. In SDS-PAGE, visible bands indicate different proteins of different sizes. Even though the data is qualitative, the intensities of the bands are indicative of the concentrations of the proteins present. The protein profile of FPF is presented in figure 4.3.

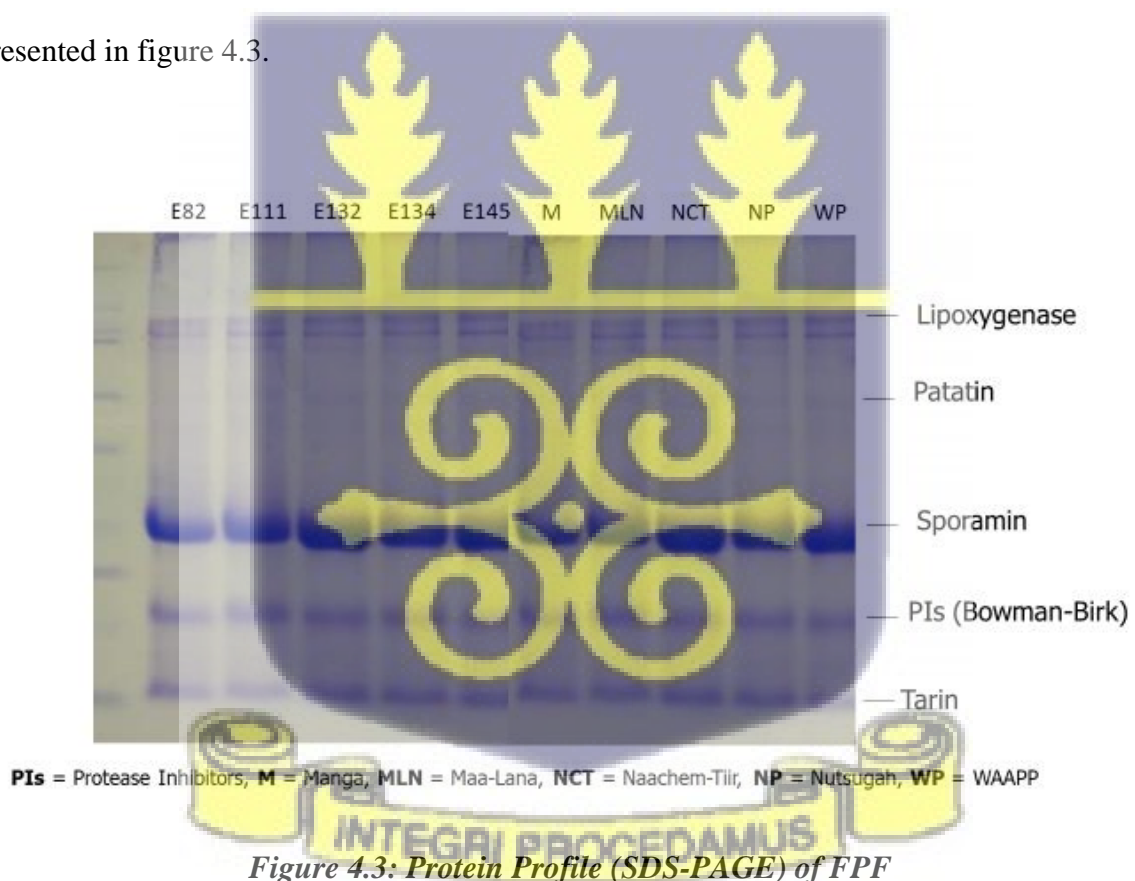
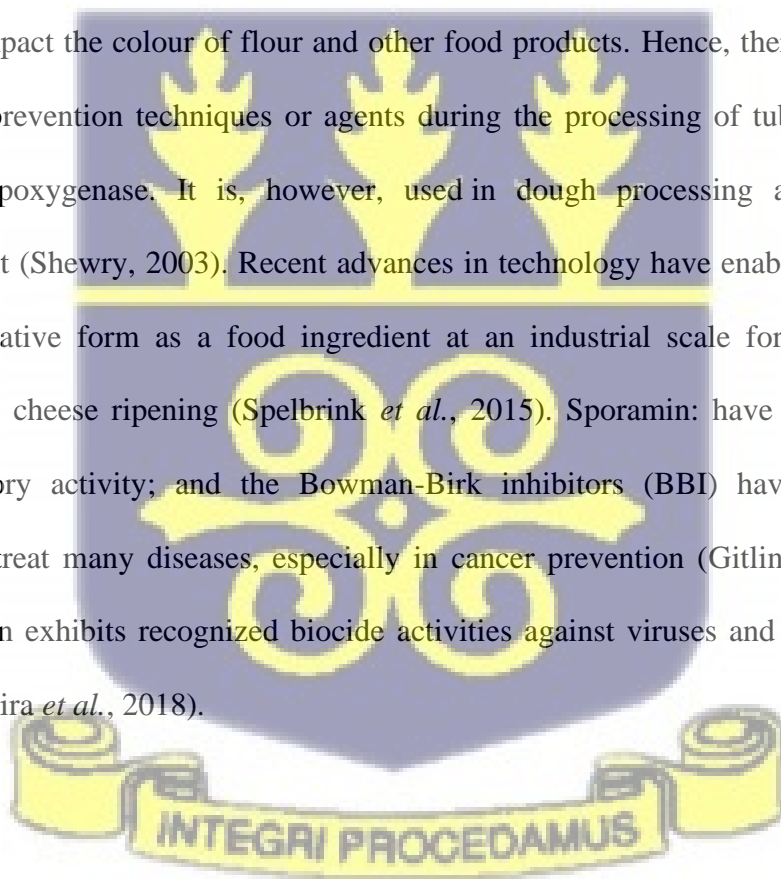


Figure 4.3: Protein Profile (SDS-PAGE) of FPF

Five visible bands with varying intensities were observed for FPF (figure 4.3). The bands were similar in terms of their visibility and intensity, suggesting that all the FPF cultivars

had similar proteins (Shewry, 2003; Maeshima *et al.*, 1985). Based on the molecular weight of these proteins, the corresponding proteins were identified. The proteins are Lipoxygenase, Patatin, Sporamin, Bowman-Birk Inhibitors, and Tarin (Gitlin-Domagalska *et al.*, 2020; Pereira *et al.*, 2018; Spelbrink *et al.*, 2015; Shewry, 2003; Maeshima *et al.*, 1985). The bands for Sporamin were the deepest, and Patatin was the faintest. Amongst all the proteins, Sporamin was observed to be the most abundant for all the cultivars. It is reported that Lipoxygenase, Patatin, Sporamin and Tarin are globular proteins, hence are soluble in water and will influence certain functional properties such as water solubility index, foaming capacity, and gelation (Senanayake *et al.*, 2013; Shewry, 2003; Maeshima *et al.*, 1985). Lipoxygenase is a protein known to cause enzymatic browning in foods; this can significantly impact the colour of flour and other food products. Hence, there is the need to use browning prevention techniques or agents during the processing of tubers such as FP that contain lipoxygenase. It is, however, used in dough processing as an oxidative improving agent (Shewry, 2003). Recent advances in technology have enabled the isolation of Patatin in native form as a food ingredient at an industrial scale for use in flavour development in cheese ripening (Spelbrink *et al.*, 2015). Sporamin: have antioxidant and trypsin inhibitory activity; and the Bowman-Birk inhibitors (BBI) have been studied extensively to treat many diseases, especially in cancer prevention (Gitlin-Domagalska *et al.*, 2020). Tarin exhibits recognized biocide activities against viruses and has antitumoral properties (Pereira *et al.*, 2018).



4.3.9 Amino Acid Profile of FPF

Amino acids concentration was determined to identify the amino acids profile of FPF. The amino acid profile of the flours from the ten (10) cultivars of Frafra potato is presented in Tables 4.6A and 4.6B.

Table 4.6A: Amino acid profile of FPF ($\mu\text{g}/100\text{g}$)

Cultivar	ala	asn	asp	cys	gln	gly	his	ile	lys	met
E 82	2.1 \pm 0.01 ^f	0.4 \pm 0.01 ^e	0.1 \pm 0.01 ^g	0.4 \pm 0.01 ^d	1.1 \pm 0.01 ^e	1.6 \pm 0.01 ^f	ND	1.0 \pm 0.02 ^a	1.0 \pm 0.01 ^d	0.5 \pm 0.01 ^e
E111	18.4 \pm 0.02 ^d	10.9 \pm 0.03 ^b	15.8 \pm 0.02 ^f	1.1 \pm 0.02 ^b	3.0 \pm 0.03 ^d	5.8 \pm 0.03 ^c	4.7 \pm 0.02 ^e	2.1 \pm 0.01 ^a	4.1 \pm 0.02 ^c	2.6 \pm 0.03 ^c
E132	19.7 \pm 0.03 ^d	9.5 \pm 0.01 ^c	18.4 \pm 0.01 ^d	2.1 \pm 0.03 ^a	4.7 \pm 0.01 ^c	3.0 \pm 0.02 ^d	6.7 \pm 0.01 ^c	1.8 \pm 0.02 ^a	4.8 \pm 0.03 ^c	6.1 \pm 0.02 ^a
E134	38.6 \pm 0.03 ^b	8.7 \pm 0.03 ^d	41.1 \pm 0.02 ^a	2.7 \pm 0.02 ^a	4.3 \pm 0.01 ^c	2.2 \pm 0.03 ^e	7.7 \pm 0.02 ^b	1.7 \pm 0.03 ^a	4.3 \pm 0.02 ^c	1.9 \pm 0.03 ^d
E145	14.1 \pm 0.02 ^e	10.3 \pm 0.03 ^b	36.7 \pm 0.02 ^b	0.9 \pm 0.01 ^c	4.5 \pm 0.02 ^c	1.7 \pm 0.01 ^f	3.6 \pm 0.02 ^f	1.2 \pm 0.03 ^b	4.0 \pm 0.01 ^c	2.1 \pm 0.03 ^c
Maa-Lana	28.1 \pm 0.03 ^c	12.3 \pm 0.01 ^a	20.5 \pm 0.03 ^c	1.4 \pm 0.03 ^b	6.8 \pm 0.01 ^a	5.5 \pm 0.02 ^c	6.7 \pm 0.03 ^c	1.4 \pm 0.01 ^b	6.6 \pm 0.03 ^a	3.4 \pm 0.02 ^b
Manga	34.0 \pm 0.01 ^b	12.4 \pm 0.02 ^a	16.0 \pm 0.03 ^e	0.5 \pm 0.02 ^d	4.9 \pm 0.01 ^c	10.3 \pm 0.03 ^a	4.7 \pm 0.01 ^e	1.7 \pm 0.02 ^a	4.8 \pm 0.01 ^c	2.6 \pm 0.03 ^c
Naachem-Tiir	33.1 \pm 0.02 ^b	8.2 \pm 0.02 ^d	15.5 \pm 0.03 ^f	1.1 \pm 0.03 ^b	5.1 \pm 0.02 ^b	3.6 \pm 0.02 ^d	7.0 \pm 0.03 ^b	1.8 \pm 0.01 ^a	4.9 \pm 0.03 ^c	3.3 \pm 0.01 ^b
Nutsugah	28.8 \pm 0.01 ^c	11.8 \pm 0.02 ^a	22.9 \pm 0.01 ^c	1.1 \pm 0.02 ^b	5.2 \pm 0.03 ^b	6.6 \pm 0.01 ^b	5.9 \pm 0.02 ^d	1.6 \pm 0.03 ^a	5.1 \pm 0.02 ^b	2.3 \pm 0.01 ^c
WAAPP	44.1 \pm 0.01 ^a	8.1 \pm 0.01	36.2 \pm 0.01 ^b	1.9 \pm 0.01 ^b	6.6 \pm 0.03 ^a	2.2 \pm 0.01 ^e	8.1 \pm 0.03 ^a	2.2 \pm 0.01 ^a	6.2 \pm 0.03 ^a	6.2 \pm 0.02 ^a

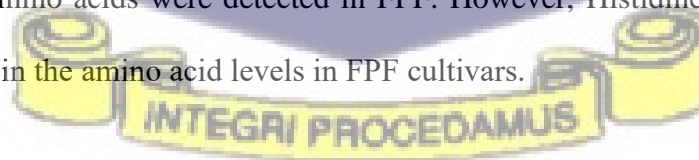
“ala = DL-Alpha-Alanine, asn = Asparagine, asp = Aspartic acid, cys = Cysteine, gln = Glutamine, gly = Glycine, his = Histidine, ile = Isoleucine, lys = Lysine, met = Methionine, ND = Not detected. Essential amino acids detected = 7 (Histidine, Isoleucine, Lysine, Methionine, Threonine, Tryptophan, Valine). Values are means ($\mu\text{g}/100\text{g}$) of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$)”.

Table 4.6B: Amino acid profile of FPF

Cultivar	DL-β-P	pro	trx4-hy-pro	ser	D-thr	L-thr	tyr	trp	val
E 82	0.5 ± 0.01 ^c	0.9 ± 0.01 ^c	1.1 ± 0.01 ^c	1.1 ± 0.01 ^f	0.9 ± 0.01 ^c	0.6 ± 0.01 ^a	ND	330.7 ± 0.01 ^c	0.8 ± 0.01 ^c
E111	464.3 ± 0.02 ^b	1.7 ± 0.03 ^b	112.1 ± 0.01 ^b	6.1 ± 0.03 ^d	4.2 ± 0.02 ^b	1.0 ± 0.01 ^a	18.0 ± 0.03 ^e	3594.5 ± 0.02 ^a	28.1 ± 0.01 ^b
E132	467.5 ± 0.01 ^b	2.5 ± 0.02 ^a	148.8 ± 0.03 ^a	6.5 ± 0.01 ^d	4.5 ± 0.01 ^b	1.0 ± 0.03 ^a	303.7 ± 0.02 ^b	4012.2 ± 0.03 ^a	30.4 ± 0.03 ^a
E134	458.7 ± 0.01 ^b	1.7 ± 0.02 ^b	110.7 ± 0.01 ^b	8.5 ± 0.02 ^c	4.8 ± 0.01 ^b	0.9 ± 0.01 ^a	16.7 ± 0.03 ^e	3297.8 ± 0.03 ^b	28.4 ± 0.02 ^b
E145	464.4 ± 0.03 ^b	1.8 ± 0.01 ^b	111.8 ± 0.02 ^b	5.3 ± 0.02 ^e	5.6 ± 0.03 ^a	0.8 ± 0.01 ^a	273.7 ± 0.01 ^c	3351.1 ± 0.03 ^b	26.3 ± 0.01 ^b
Maa-Lana	635.7 ± 0.01 ^a	1.8 ± 0.03 ^b	124.5 ± 0.01 ^b	6.4 ± 0.03 ^d	5.1 ± 0.01 ^a	1.1 ± 0.03 ^a	500.0 ± 0.03 ^a	3860.2 ± 0.02 ^a	32.4 ± 0.03 ^a
Manga	484.2 ± 0.02 ^b	1.7 ± 0.02 ^b	115.1 ± 0.03 ^b	10.3 ± 0.02 ^a	5.0 ± 0.01 ^a	0.9 ± 0.03 ^a	17.6 ± 0.02 ^e	3580.1 ± 0.01 ^a	28.0 ± 0.03 ^b
Naachem-Tiir	624.1 ± 0.03 ^a	1.6 ± 0.01 ^b	124.1 ± 0.03 ^b	7.3 ± 0.03 ^d	5.3 ± 0.01 ^a	1.3 ± 0.03 ^a	29.6 ± 0.01 ^d	3762.4 ± 0.02 ^a	32.6 ± 0.03 ^a
Nutsugah	499.5 ± 0.03 ^b	1.7 ± 0.02 ^b	112.7 ± 0.03 ^b	1.2 ± 0.02 ^f	4.6 ± 0.02 ^b	0.7 ± 0.01 ^a	297.8 ± 0.02 ^c	3649.5 ± 0.01 ^a	26.1 ± 0.02 ^b
WAAPP	496.7 ± 0.03 ^b	2.3 ± 0.01 ^a	151.1 ± 0.02 ^a	9.0 ± 0.03 ^b	5.4 ± 0.03 ^a	0.8 ± 0.02 ^a	20.3 ± 0.01 ^d	4000.5 ± 0.03 ^a	31.0 ± 0.01 ^a

DL-β-P = DL-β-Phenylalanine, **pro** = D-Proline, **trx4-hy-pro** = Trans-4-Hydroxy-L-Proline, **ser** = Serine, **D-thr** = D-Threonine, **L-thr** = Threonine, **tyr** = Tyrosine, **trp** = Tryptophan, **val** = Valine, **ND** = Not detected. “Essential amino acids detected = 7 (Histidine, Isoleucine, Lysine, Methionine, Threonine, Tryptophan, Valine). Values are means (µg/100g) of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$)”.

Table 4.6A and 4.6B show that about 19 amino acids were detected in FPF. However, Histidine and Tyrosine were not detected in E82, and there were significant differences ($p \leq 0.05$) in the amino acid levels in FPF cultivars.



Seven (7) out of the nineteen (19) amino acids detected were essential amino acids. They are Histidine (with concentrations ranging from about 0 to 8 $\mu\text{g}/100\text{g}$), Isoleucine (with concentrations ranging from about 1 to 2.2 $\mu\text{g}/100\text{g}$), Lysine (with concentrations ranging from about 1 to 7 $\mu\text{g}/100\text{g}$), Methionine (with concentrations ranging from about 1 to 6 $\mu\text{g}/100\text{g}$), Threonine (about 1 $\mu\text{g}/100\text{g}$), Tryptophan (with concentrations ranging from about 331 to 4012 $\mu\text{g}/100\text{g}$), and Valine (with concentrations ranging from about 1 to 33 $\mu\text{g}/100\text{g}$). Quantification of amino acids in foods such as FPF is vital for food applications because it plays a role in the nutritional value of foods and products for which it may be used. Variations in amino acid concentration might be due to differences in protein concentration and varietal differences. This is because protein composition and varietal differences can give rise to differences in the free amino acids in foods (Ozcan, and Senyuva, 2006; Shewry, 2003; Maeshima et al., 1985). The released cultivars generally had higher amino acids concentration, hence may have better performance in pastry applications and other food systems that require high protein/amino acids concentrations (Alencar *et al.*, 2015) compared to the unreleased E-group cultivars. The profile of essential amino acids in FP is not typical of tuber proteins; hence, this makes FP unique with great potential as a non-conventional food ingredient in applications such as buttered and breaded foods.

4.3.10 Functional Characteristics of FPF

The functional characteristics of FPF were characterised to understand how the flours would be suitable for and perform in food systems. The functional characteristics of FPF are summarized in table 4.7.

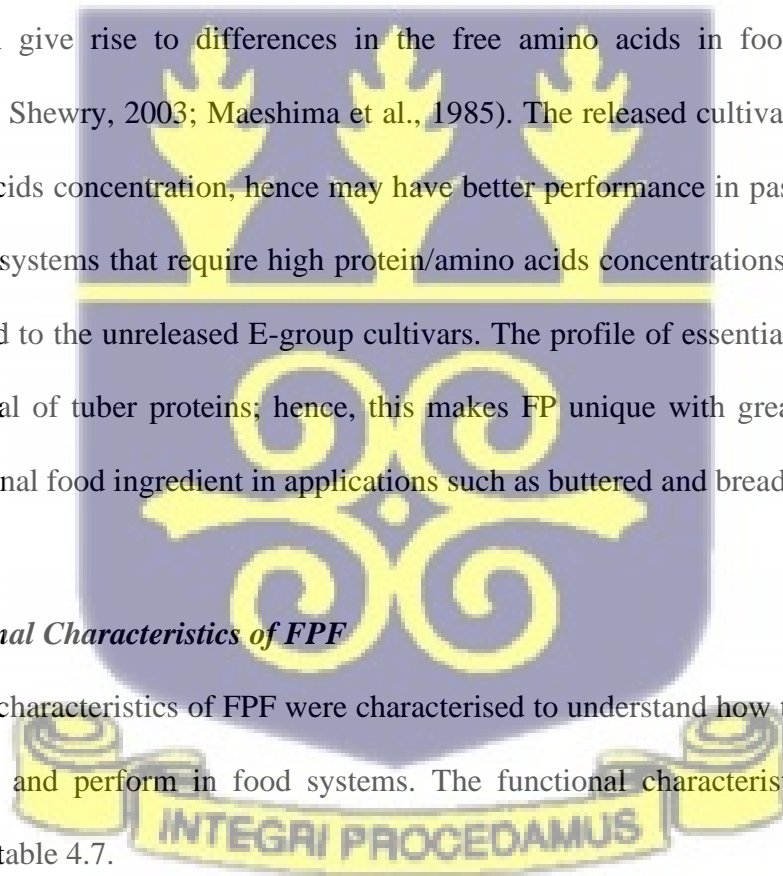


Table 4.7: Functional characteristics of FPF

Cultivar	BD (g/ml)	SP (g/g)	SI (g/g)	WB (g/g)	OB (g/g)	FC (ml)	FS (ml)	pH	TTA (%)
E 82	0.9 ± 0.1 ^a	19.2 ± 1.3 ^a	8.7 ± 0.4 ^a	2.8 ± 1.8 ^a	1.2 ± 0.6 ^a	9.4 ± 1.4 ^d	4.7 ± 1.2 ^d	6.4 ± 0.1 ^a	0.5 ± 0.6 ^a
E111	0.9 ± 0.2 ^a	19.5 ± 1.1 ^a	8.8 ± 0.7 ^a	2.5 ± 0.6 ^a	1.3 ± 0.5 ^a	8.9 ± 0.8 ^d	4.3 ± 1.1 ^d	6.4 ± 0.1 ^a	0.4 ± 1.6 ^a
E132	0.9 ± 0.2 ^a	19.3 ± 1.6 ^a	8.6 ± 0.4 ^a	2.6 ± 1.3 ^a	1.4 ± 1.4 ^a	8.7 ± 0.8 ^d	4.3 ± 1.3 ^d	6.3 ± 0.1 ^a	0.4 ± 0.6 ^a
E134	0.9 ± 0.4 ^a	21.4 ± 0.1 ^a	8.5 ± 0.5 ^a	3.0 ± 0.6 ^a	1.2 ± 0.7 ^a	9.1 ± 1.3 ^d	4.5 ± 0.7 ^d	6.4 ± 0.1 ^a	0.4 ± 0.6 ^a
E145	0.9 ± 0.2 ^a	21.0 ± 0.1 ^a	8.7 ± 0.1 ^a	3.0 ± 1.9 ^a	1.1 ± 1.8 ^a	9.2 ± 1.4 ^d	4.6 ± 1.5 ^d	6.4 ± 0.1 ^a	0.5 ± 0.5 ^a
Maa-Lana	0.9 ± 0.1 ^a	22.2 ± 0.1 ^a	6.5 ± 0.1 ^b	2.5 ± 1.4 ^a	1.4 ± 0.3 ^a	15.7 ± 1.6 ^a	7.8 ± 1.1 ^b	6.4 ± 0.1 ^a	0.5 ± 1.6 ^a
Manga	0.9 ± 0.1 ^a	22.0 ± 0.1 ^a	6.7 ± 0.9 ^b	2.6 ± 0.6 ^a	1.2 ± 0.1 ^a	14.1 ± 1.0 ^b	6.6 ± 1.3 ^c	6.3 ± 0.1 ^a	0.5 ± 0.5 ^a
Naachem-Tiir	0.9 ± 0.1 ^a	22.3 ± 0.5 ^a	7.1 ± 1.2 ^b	2.9 ± 0.1 ^a	1.2 ± 1.1 ^a	14.7 ± 1.3 ^b	7.7 ± 1.0 ^b	6.4 ± 0.1 ^a	0.5 ± 0.6 ^a
Nutsugah	0.9 ± 0.9 ^a	22.0 ± 0.1 ^a	7.0 ± 0.3 ^b	3.0 ± 1.3 ^a	1.3 ± 0.6 ^a	14.5 ± 1.3 ^b	7.5 ± 1.4 ^b	6.4 ± 0.1 ^a	0.5 ± 1.6 ^a
WAAPP	0.8 ± 0.2 ^a	22.8 ± 0.1 ^a	7.3 ± 0.6 ^b	3.0 ± 0.6 ^a	1.2 ± 0.4 ^a	16.7 ± 1.2 ^a	10.1 ± 1.1 ^a	6.4 ± 0.1 ^a	0.5 ± 0.6 ^a

BD = Bulk density, **SP** = Swelling power, **SI** = Solubility index, **WB** = Water binding capacity, **OB** = Oil binding capacity, **FC** = Foaming capacity, **FS** = Foam stability, **TTA** = Total titratable acidity. Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.7 shows no significant differences ($p \leq 0.05$) between the bulk density, swelling power, water and oil binding capacities, pH, and total titratable acidity of all the FPF cultivars. However, significant differences ($p \leq 0.05$) existed for the solubility, foam volume and stability indices.



Solubility index is the per cent soluble solids, and it ranged from 6.7 – 8.5% for all FPF cultivars (Table 4.7). According to Apea-Bah *et al.* (2011), solubility is essential, especially in food systems, such as pastry, since flour with high solubility may give a soggy and less cohesive dough. It is observed (Table 4.7) that the unreleased E-group cultivars had a significantly higher solubility index compared to the released cultivars. This implies that in baking applications such as bread making, the flours from the unreleased E-group cultivars are more likely to perform poorly during the dough making stage. Variations in the functional properties, in general, could be associated with the proximate composition of FPF. WB capacities ranged from 2.5 to 3 g/g; while OB capacities ranged from 1.1 – 1.4 g/g for all FPF cultivars. Water and oil binding capacities are essential in food products' textural and flavour characteristics (Alencar *et al.*, 2015; Andrés-Bello *et al.*, 2013; Apea-Bah *et al.*, 2011). Hence flours with high W/OB could be a good ingredient in baking applications, such as bread, since higher water absorption enables bakers to add water to the dough, thus improving the handling characteristics and maintaining freshness in bread (Alencar *et al.*, 2015; Andrés-Bello *et al.*, 2013; Apea-Bah *et al.*, 2011). Also, flours with high WB capacities are considered critical in viscous foods such as porridge, soups, and gravies (Chinma *et al.*, 2012). Hence, FPF may be useful as a functional ingredient in porridges, soups, gravies, and pastry products. FC and FS are dependent on the protein and carbohydrate content in the flour (Adebowale *et al.*, 2005). This could be the reason why the released cultivars recorded higher values (14.1 – 16.7%) relative to that of the unreleased E-group cultivars (8.7 – 9.4%) ($p \leq 0.05$). Functional characteristics of flours are influenced by different resistant starch, amylose/amylopectin ratio and protein concentrations, their degree of interaction with water and their conformational characteristics (Aprianita *et al.*, 2013; Chinma *et al.*, 2012; Apea-Bah *et al.*, 2011). Hence the differences ($p \leq 0.05$) in the functional properties of FPF could be due to their different resistant starch and protein

concentrations. Based on the results (Table 4.7), FPF may be helpful in pastry applications, and a thickener in liquid and semi-liquid foods since the flours can absorb water and swell for improved consistency in these foods.

4.3.11 Pasting Properties of FPF

The pasting characteristics of Frafra potato flours were determined to record the indices presented in Table 4.8 quantitatively.

Table 4.8: Pasting Characteristics of FPF

Cultivar	P _{Time} (min)	P _{Temp} (°C)	PV (BU)	BD (BU)	SB (BU)	FV (BU)
E 82	20.40 ± 0.10 ^a	78.30 ± 0.10 ^a	93.20 ± 0.20 ^a	6.01 ± 0.01 ^a	19.10 ± 0.01 ^b	92.13 ± 0.08 ^a
E111	19.55 ± 0.21 ^{ab}	78.90 ± 0.14 ^a	89.50 ± 0.03 ^{ab}	5.40 ± 0.91 ^b	21.59 ± 0.05 ^a	98.40 ± 0.45 ^a
E132	18.40 ± 0.03 ^c	76.80 ± 0.02 ^a	79.10 ± 0.01 ^c	3.10 ± 0.06 ^d	16.33 ± 0.06 ^e	84.20 ± 0.24 ^b
E134	19.20 ± 0.50 ^b	78.10 ± 0.40 ^a	82.20 ± 0.05 ^b	5.20 ± 0.12 ^b	17.14 ± 0.91 ^d	89.60 ± 0.07 ^b
E145	18.50 ± 0.41 ^{bc}	77.10 ± 0.08 ^a	77.60 ± 0.91 ^c	4.10 ± 0.04 ^c	18.23 ± 0.23 ^c	84.50 ± 0.16 ^b
Maa-Lana	17.55 ± 1.12 ^d	75.60 ± 0.05 ^a	85.20 ± 3.51 ^b	2.70 ± 0.13 ^e	18.62 ± 3.10 ^c	94.70 ± 1.85 ^a
Manga	20.35 ± 0.91 ^a	79.90 ± 0.23 ^a	68.10 ± 0.04 ^d	2.03 ± 0.01 ^e	18.20 ± 0.32 ^c	95.03 ± 0.14 ^a
Naachem-Tiir	20.15 ± 0.27 ^a	79.10 ± 0.02 ^a	84.30 ± 0.01 ^b	3.10 ± 0.09 ^d	15.14 ± 0.09 ^f	93.21 ± 0.06 ^a
Nutsugah	18.20 ± 1.01 ^c	75.90 ± 0.09 ^a	85.40 ± 3.19 ^b	3.40 ± 2.25 ^d	17.83 ± 2.94 ^d	84.90 ± 1.91 ^b
WAAPP	19.10 ± 0.10 ^b	77.40 ± 0.21 ^a	78.10 ± 0.03 ^c	3.10 ± 0.06 ^d	22.13 ± 0.71 ^a	85.05 ± 0.02 ^b

P_{Temp} = Pasting temperature; P_{Time} = Pasting time (min); PV = Peak Viscosity; FV = Final Viscosity; BD = Break Down; SB = Set Back. Values are means of triplicates with standard deviation. Means in the same column with different superscript are significantly different ($p \leq 0.05$).

From table 4.8, it is observed that there were significant differences ($p \leq 0.05$) in all the pasting property indices recorded, except for the pasting temperature of the FPF cultivars. Differences in pasting indices are influenced by the amylose/amylopectin ratio and chemical composition of the flours (Andrés-Bello *et al.*, 2013; Apea-Bah *et al.*, 2011. Variations in most of the indices could be associated with the proximate composition of FPF (Chinma *et al.*, 2012; Ikegwu *et al.*, 2010). All the FPF cultivars had higher paste stability relative to close alternative tuber crops such as cassava and sweet potato (Tortoe *et al.*, 2017; Ikegwu *et*

al., 2010). The released cultivars and E132 had a relatively lower breakdown. The released cultivars formed more stable gels than the unreleased E-group cultivars (Table 4.2); this could explain why they have a somewhat lower breakdown. Higher setback values show a higher tendency to retrograde and vice versa (Aprianita *et al.*, 2013; Chinma *et al.*, 2012). The tendency of flour pastes to retrograde was highest in WAAPP, followed by E111. Therefore, pastry, such as bread, made from these cultivars is likely to stale faster than bread made from the other cultivars such as Naachem-Tiir and E132.

4.3.12 Flow Behaviour of Cooked FPF Slurry

The flow behaviour of FPF was determined to understand how cooked slurries from flour made using the different cultivars differed. Figure 4.4 and Table 4.9 summarize the flow behaviour and the apparent viscosity of FPF slurries.

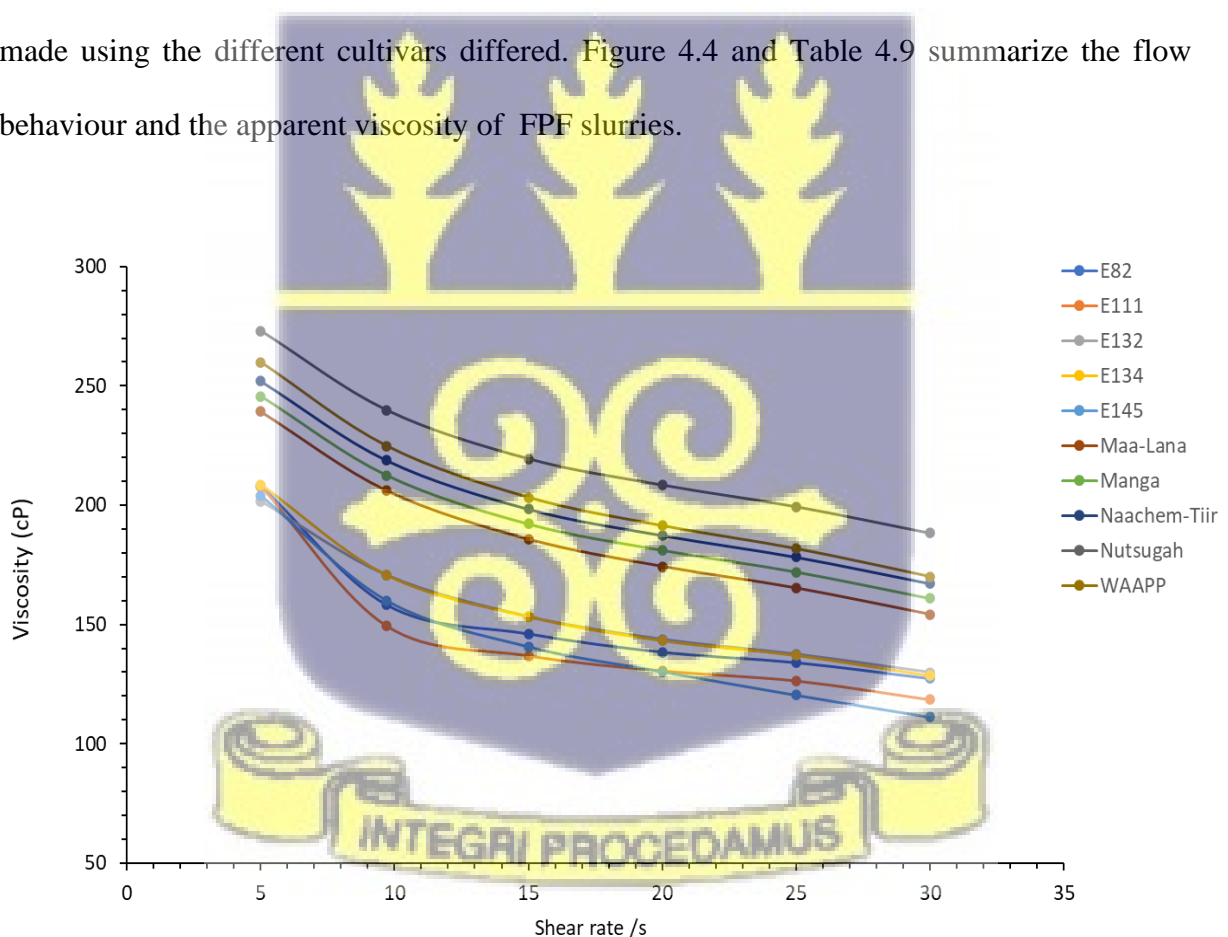


Figure 4.4: Viscosity profile of gels from FPF

From figure 4.4, the gels showed a typical non-Newtonian, shear-thinning (or pseudoplastic) behaviour, characterised by a reduction in apparent viscosity with increasing shear rate (Mrokowska and Krztoń-Maziopa, 2019). Gels made from the released cultivars were significantly ($p < 0.05$) more viscous than the unreleased E-group cultivars. For instance, at the lowest shear rate, a viscosity range of 239 - 273 cP and 24 - 109 cP was recorded correspondingly to gels from the released and unreleased E-group cultivars, respectively. The proximate composition and least gel concentration of flours impact the flow behaviour of slurries made from them (Chinma *et al.*, 2012; Ikegwu *et al.*, 2010; Peroni *et al.*, 2006). Hence the variations in the apparent viscosity of FPF gels could be due to the variations observed in their proximate composition and least gelation concentrations of the FPF cultivars.

Table 4.9: Viscosity and the degree of non-Newtonian of FPF

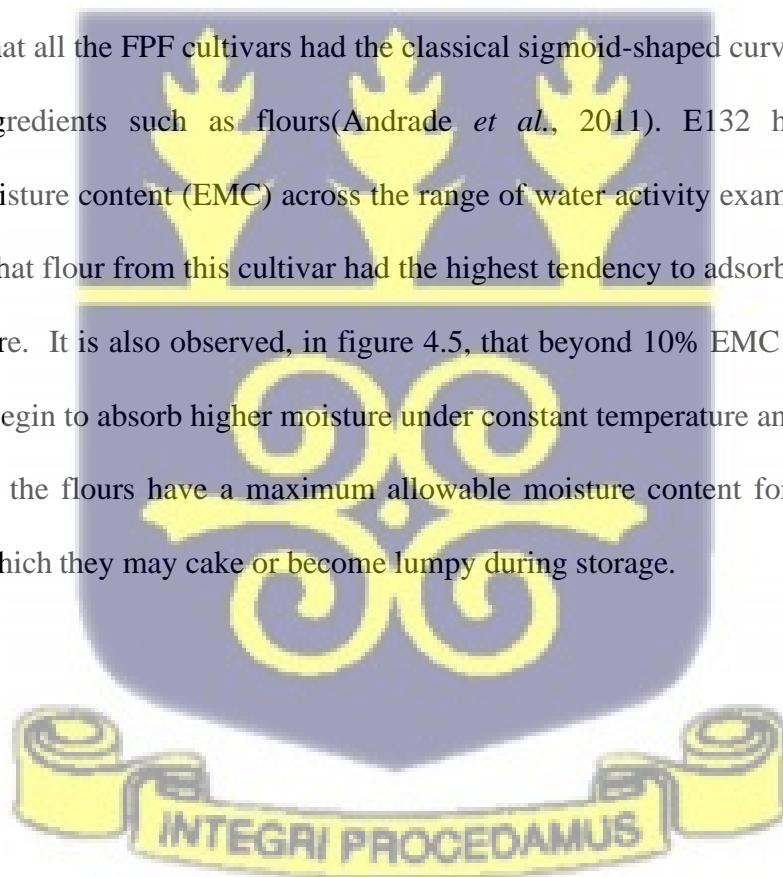
Cultivars	K	n	R ²
E82	2.28 ± 0.01 ^a	0.53 ± 0.00	0.987
E111	2.28 ± 0.00 ^a	0.61 ± 0.00	0.988
E132	2.44 ± 0.01 ^a	0.61 ± 0.00	0.998
E134	2.50 ± 0.01 ^a	0.66 ± 0.00	0.996
E145	2.54 ± 0.01 ^a	0.43 ± 0.00	0.997
Maa-Lana	2.55 ± 0.01 ^a	0.24 ± 0.00	0.996
Manga	2.55 ± 0.00 ^a	0.23 ± 0.00	0.996
Naachem-Tiir	2.56 ± 0.01 ^a	0.22 ± 0.00	0.996
Nutsugah	2.58 ± 0.00 ^a	0.20 ± 0.00	0.997
WAAPP	2.58 ± 0.00 ^a	0.23 ± 0.00	0.996

n (power-law) = flow behaviour index, K = consistency index, R² = Coefficient. "Means with the same superscripts in the column are statistically indifferent ($p \leq 0.05$)".

The 'n' values suggest whether fluids have a Newtonian or non-Newtonian behaviour. Newtonian fluids have been shown to have $n = 1$, while non-Newtonian fluids have values below 1 (George and Qureshi, 2013). The results (Table 4.9) implies that all the FPF cultivars had 'n' values less than 1, confirming that they exhibited a non-Newtonian shear-thinning fluid characteristic. Table 4.9 shows that all the FPF gels had a similar consistency index (K) ($p \leq 0.05$). This also indicates the mixing tolerance (Alencar *et al.*, 2015; George and Qureshi, 2013) of FPF flours in culinary applications such as pastry and porridge.

4.3.13 Moisture Sorption Behaviour of FPF (Isotherms)

The sorption behaviour of FPF is summarized in figure 4.5 and table 4.10. From the figure, it is observed that all the FPF cultivars had the classical sigmoid-shaped curve as expected of dried food ingredients such as flours (Andrade *et al.*, 2011). E132 had the highest equilibrium moisture content (EMC) across the range of water activity examined ($p < 0.05$). This indicates that flour from this cultivar had the highest tendency to adsorb moisture under constant pressure. It is also observed, in figure 4.5, that beyond 10% EMC and a_w of about 0.5, the flours begin to absorb higher moisture under constant temperature and pressure. This implies that all the flours have a maximum allowable moisture content for safekeeping at 10%, beyond which they may cake or become lumpy during storage.



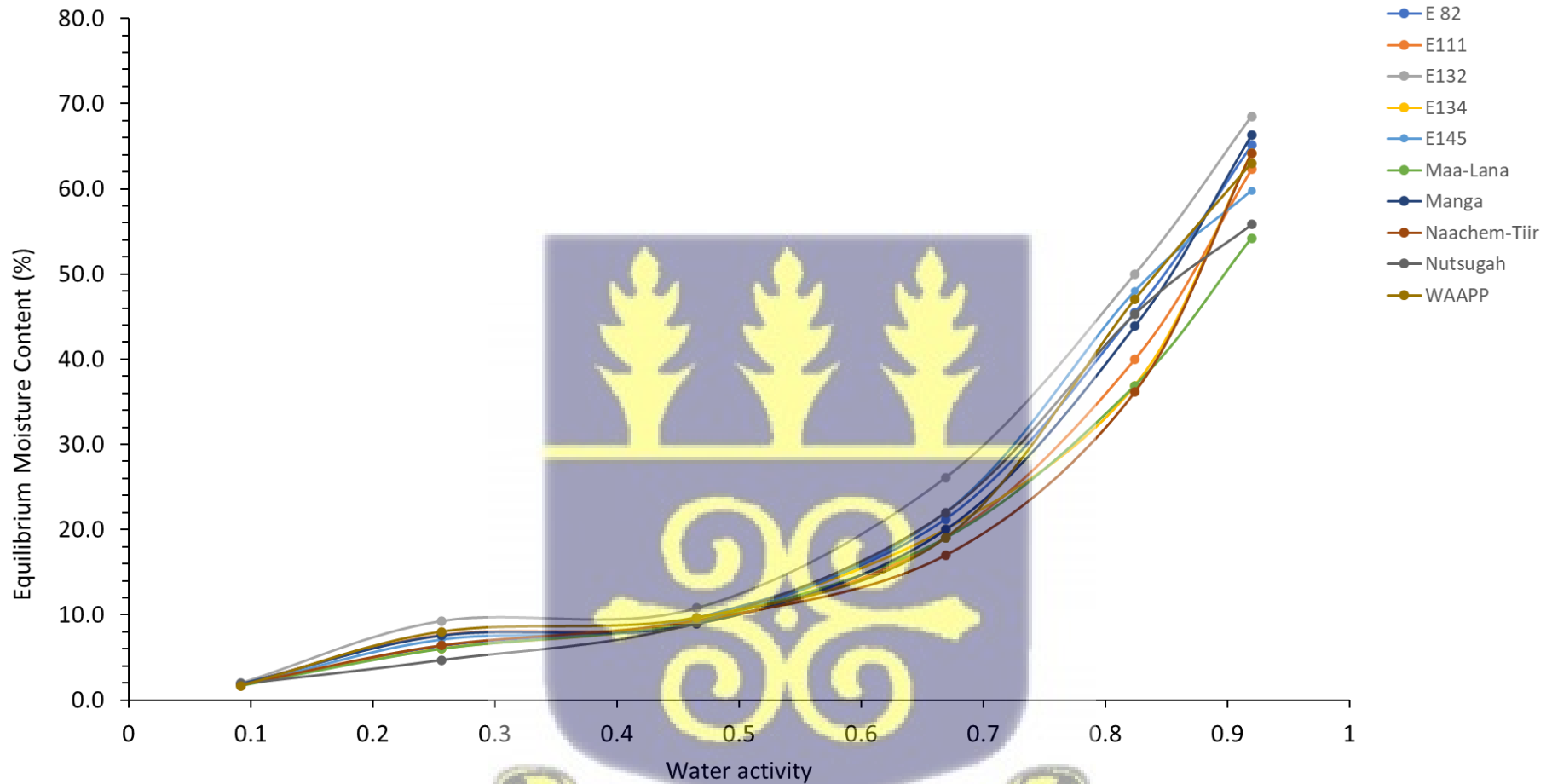
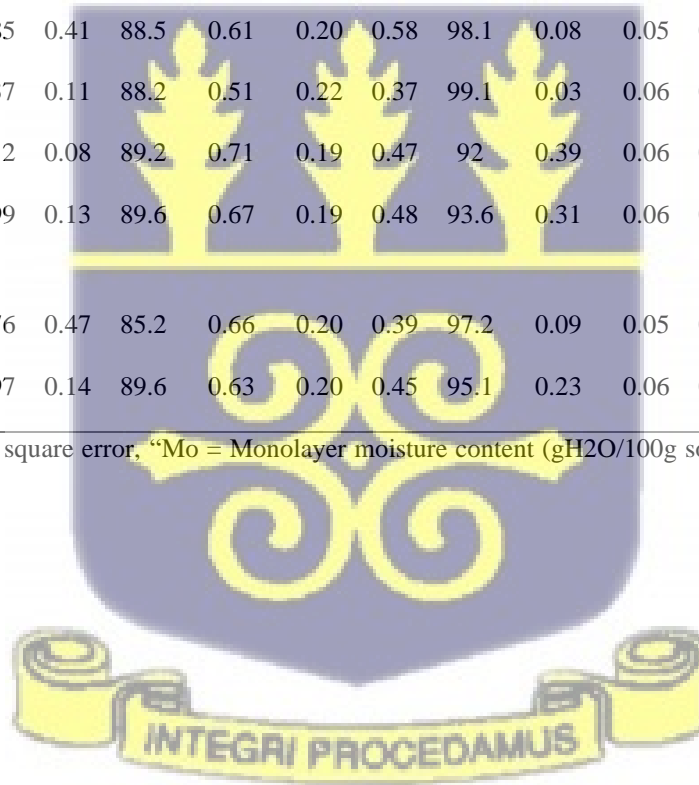


Figure 4.5: Moisture sorption behaviour of FPF

Table 4.10: Constants and results obtained from isotherm models for FPF

Cultivar	BET				GAB					Oswin				Henderson				Smith				
	Mo	C	R ²	RMSE	Mo	K	C	R ²	RMSE	A	B	R ²	RMSE	A	B	T	R ²	RMSE	A	B	R ²	RMSE
E82	4.31	0.19	88.7	0.57	3.26	0.82	0.14	89.6	0.51	0.1	0.48	94.7	0.30	0.05	0.16	0.16	83.6	0.75	0.78	3.02	90.8	0.67
E111	3.08	0.07	87.5	0.59	2.82	0.15	0.18	86.2	0.72	0.18	0.47	93.0	0.40	0.05	0.16	0.16	83.9	0.74	0.69	2.10	98.8	0.03
E132	4.35	0.08	90.2	0.43	2.01	0.91	0.13	87.4	0.76	0.20	0.45	96.2	0.21	0.06	0.16	0.16	84.8	0.58	0.83	2.02	91.2	0.36
E134	6.67	0.05	93.1	0.22	7.31	0.47	0.15	85.2	0.80	0.20	0.57	97.0	0.10	0.06	0.16	0.16	87.4	0.60	0.91	2.14	98.2	0.04
E145	5.40	0.43	91.2	0.31	8.27	0.85	0.41	88.5	0.61	0.20	0.58	98.1	0.08	0.05	0.16	0.16	86.8	0.62	0.81	2.33	96.7	0.22
Maa-Lana	10.6	0.04	93.3	0.22	8.37	0.37	0.11	88.2	0.51	0.22	0.37	99.1	0.03	0.06	0.16	0.16	84.4	0.67	0.90	1.72	99.1	0.03
Manga	3.96	0.09	87.9	0.59	3.62	0.12	0.08	89.2	0.71	0.19	0.47	92	0.39	0.06	0.15	0.15	82.6	1.14	0.73	2.01	88.8	0.55
Naachem-	3.71	0.09	89.9	0.48	2.16	0.99	0.13	89.6	0.67	0.19	0.48	93.6	0.31	0.06	0.16	0.15	85.5	0.93	0.66	2.01	90.8	0.44
Tiir																						
Nutsugah	6.60	0.56	90.4	0.32	7.67	0.76	0.47	85.2	0.66	0.20	0.39	97.2	0.09	0.05	0.16	0.16	84.3	0.70	0.85	1.73	97.1	0.10
WAAPP	4.15	0.08	90.8	0.42	2.12	0.97	0.14	89.6	0.63	0.20	0.45	95.1	0.23	0.06	0.16	0.16	86.2	0.57	0.72	1.98	92.8	0.33

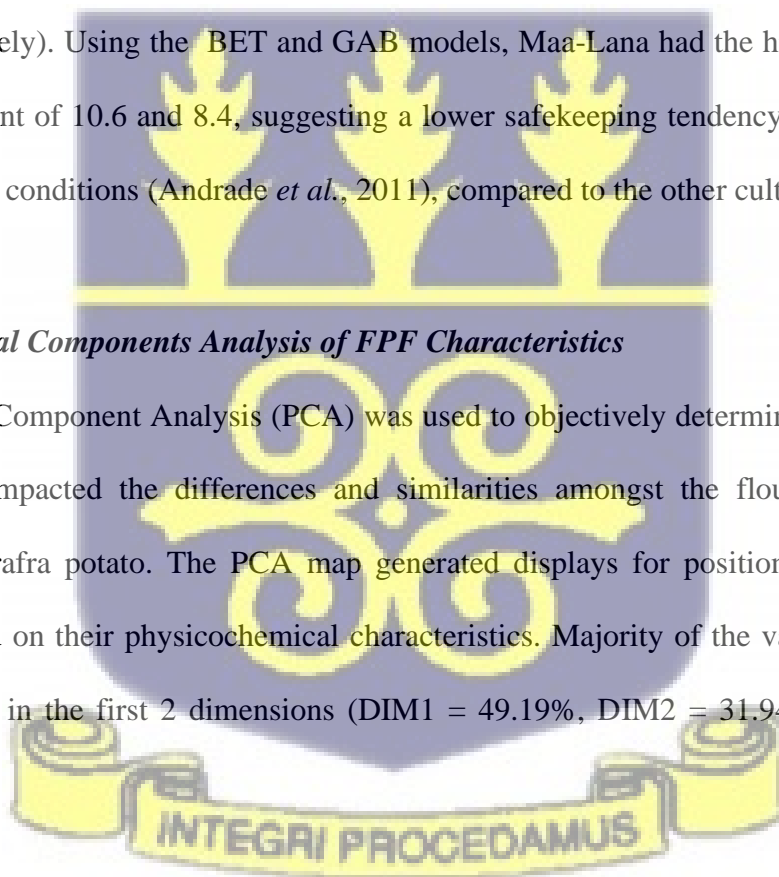
R² = Coefficient of regression, RMSE = Root mean square error, “Mo = Monolayer moisture content (gH₂O/100g solid), C = BET constant, G and K = GAB constants, A and B” = Henderson and Smith constants.



Several models are used to explain the moisture sorption characteristics of foods. The popular models are the BET, GAB, Oswin, Henderson, and Smith models (Andrade *et al.*, 2011). This study fitted the data into the five models, as shown in Table 4.10. The goodness of fit of the models (as indicated by the R^2 and RMSE values) varied for all the FPF cultivars. The Oswin model was the best fit for the majority of FPF cultivars (WAAPP, Maa-Lana, Naachem-Tiir, Nutsugah, Manga, E82, E132 and E145), this is because the model had higher R^2 values (93.6 – 99.1%) with lower residuals (RMSE ranged between 0.03 – 0.39), which implies it can explain about 94 to 99% of the variability in the sorption behaviour of those flours. However, the Smith model was the best fit for E111 and E134. It had higher R^2 values (98.8% and 98.2%, respectively) with very low residuals (0.03 and 0.04, respectively). Using the BET and GAB models, Maa-Lana had the highest monolayer moisture content of 10.6 and 8.4, suggesting a lower safekeeping tendency while in storage under standard conditions (Andrade *et al.*, 2011), compared to the other cultivars.

4.3.13 Principal Components Analysis of FPF Characteristics

The Principal Component Analysis (PCA) was used to objectively determine which features significantly impacted the differences and similarities amongst the flours from the ten cultivars of Frafra potato. The PCA map generated displays for positioning the ten FPF cultivars based on their physicochemical characteristics. Majority of the variance (81.13%) was explained in the first 2 dimensions (DIM1 = 49.19%, DIM2 = 31.94%) as shown in Figure 4.6.



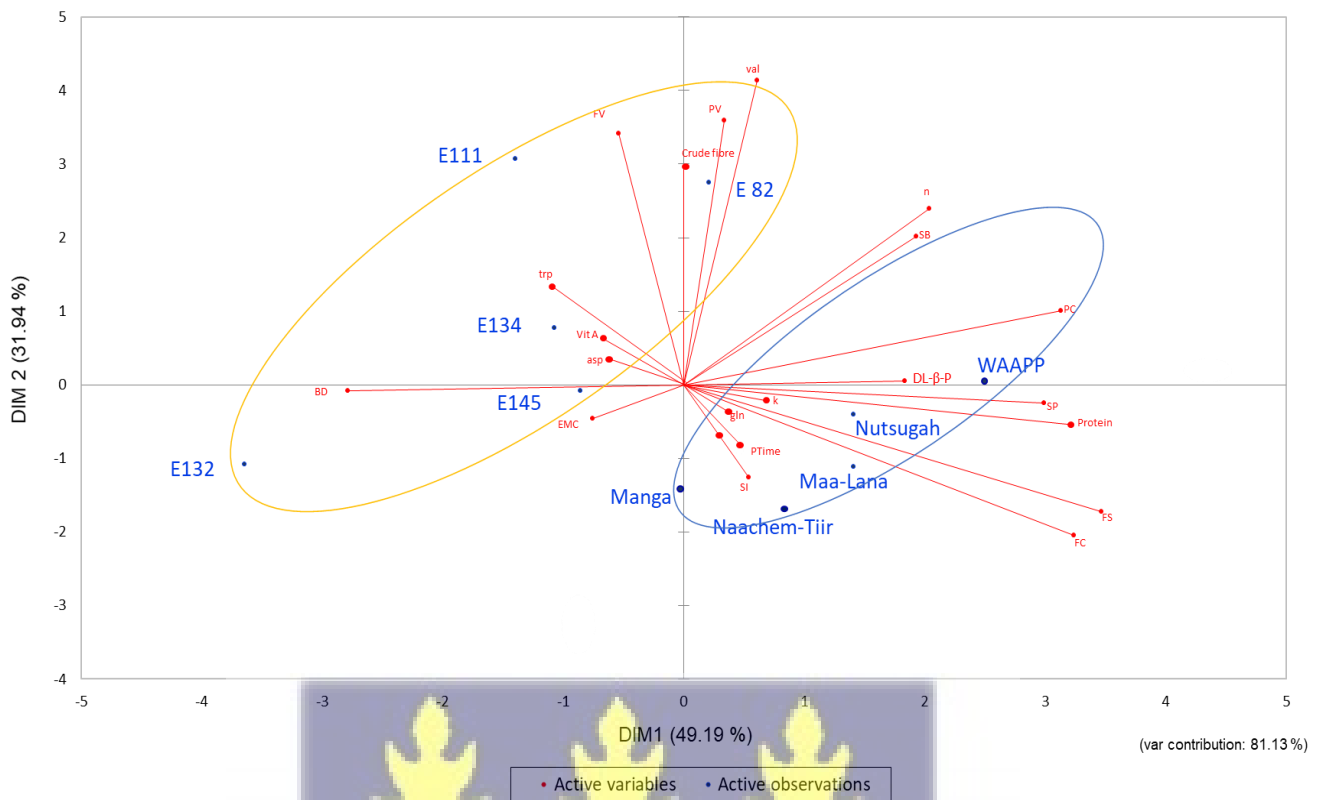


Figure 4.6: PCA plot for FPF cultivars

From figure 4.6, PCA clustered the flours from the ten cultivars of FPF into two main groups, with the released cultivars in one group and the unreleased E-group cultivars in the other group. The released FPF cultivars were mainly characterised by their protein, lysine, glutamine and DL-β-phenylalanine concentrations, foaming capacity, foam stability, solubility, pasting time, and paste clarity. The second group was characterised by their vitamin A, tryptophan, aspartic acid valine and crude fibre concentrations, paste break down, and equilibrium moisture content. According to Ezekiel and Singh (2011), Klunklin and Savage (2018) and Dziedzoave *et al.* (2006), these characteristics significantly influence the differences in flours. They are also important factors considered in flour selection for food applications.

4.4 Conclusion

Flour yield from the Frafra potatoes ranged between 59 – 70%, with no apparent difference between the released and most of the unreleased E group cultivars, except E134. Compositional analyses showed differences in FP flours' protein, ash, fibre, and elemental composition from the ten cultivars. The released FP had higher protein and ash concentrations than the unreleased E group cultivars. Protein profile analysis revealed the dominance of three major storage proteins (patatin, sporamin, and tarin) and two others (Lipoxygenase and BBIs). Amino acid analyses showed nineteen (19) amino acids in the FP flours, out of which seven (7) were essential. Marked differences were observed in the rheological properties of slurries made from the flours, with Manga being the most stable and WAAPP exhibiting the highest tendency to retrograde. Pastes from the released cultivars relatively were more viscous than the E group. PCA distinguished flours from the released and the unreleased E group cultivars. The characteristics of the flours from the ten cultivars of Frafra potato provide essential data for practical culinary applications of the flours. They can also be used for the advanced selection of accessions of interest to develop suitable cultivars for specific end-use (baked foods, noodles, thickener).

4.5 References

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CHAPTER FIVE

5.0 PHYSICOCHEMICAL AND MICROSTRUCTURAL CHARACTERISTICS OF GLUTEN-FREE FRAFRA POTATO BREAD

Abstract

The rising incidences of celiac disease, an intolerance to gluten in wheat and related products have created the need to explore ingredients and processes for developing gluten-free flour and products. Frafra potato flour has been characterised to have fine particle size distribution and relatively high amounts of protein and could serve as a candidate for gluten-free ingredients. This study examined the performance of Frafra potato flour in the production of bread with the aid of dough conditioners (egg, gelatin, and egg-gelatin powder combination) followed by Transglutaminase. The results showed that FPB made with a single conditioner had weak dough, very high development time, low stability, mixing tolerance and cooking loss ($p < 0.05$). A combination of egg and gelatin as dough conditioners followed by transglutaminase as a protein crosslinking agent effectively improved the dough and bread structure towards that from wheat flour. SEM revealed the improved network matrix and well-embedded starch granules in FPB (B11 – B15), comparable to WTB. The use of Frafra potato flour for gluten-free bread is achievable with combined dough conditioners (egg and gelatin powder) followed by Transglutaminase.

Keywords: Frafra potato, bread, gluten-free, transglutaminase, dough conditioner.

5.1 Introduction

Consumption of baked products keeps growing steadily (Aguilar *et al.*, 2015; Alencar *et al.*, 2017; Jahed Khaniki *et al.*, 2007). Among bakery products, bread is the most consumed, and it is traditionally prepared with hard wheat flour as the major ingredient (Aguilar *et al.*, 2017; Komlaga *et al.*, 2012; Laureati *et al.*, 2012). Wheat is essential in bread production because

of its exceptional characteristics that impact the sensory attributes of bread, which is greatly appreciated by consumers (Pertuzatti *et al.*, 2015; Nindjin *et al.*, 2011; Aboaba and Obakpolor, 2010). However, wheat is an expensive ingredient, especially in the tropics where it is imported (Dankwa and Peprah, 2019; Pasha *et al.*, 2011; Quainoo and Bayorbor, 2002). Furthermore, a rising number of consumers are beginning to show conditions of celiac disease, an intolerance to gluten, which is a major protein formed from flour of many kinds of cereal, especially wheat, rye and related cereals, during dough formation. This situation has created the need to explore ingredients and processes for the development of gluten-free flour and products (Ohimain, 2014; Nkansah, 2004).

Gluten-free pastry products have been developed using flour from tuber crops with the help of dough conditioners to improve dough processing and the overall quality of the baked products (Cauvain, 2015; Scaman, 2003). Dough conditioners help to improve dough handling properties, enabling a gluten-like matrix for gas retention and improving the volume of bread, crumb structure and texture, and shelf-life by delaying the onset of staling (Cauvain, 2015; Popov-Raljić *et al.*, 2013; Shittu, 2009). There are different types of dough conditioners for gluten-free products, with different characteristics. Some such as transglutaminase serve technological functions and are used in minute concentrations. Others, such as egg and gelatin powder, add protein to the product (USFDA, 2014; Williams and Pullen, 2007).

Frafra potato is a climate-resilient and underutilised root crop grown for domestic consumption. Frafra potato flour has been explored as a local alternative to wheat flour (Osei Tutu *et al.*, 2019; Sugri *et al.*, 2013; Akanlu *et al.*, 2005). This study explored the potential of Frafra potato flour as an ingredient for gluten-free flour for the production of consumer acceptable bread with the aid of dough conditioners.

5.2 Materials and Methods

5.2.1 Source of Raw Materials

Five accessions of flour (FPF) were selected, based on their availability, to produce gluten-free bread FPB. The released accessions are available to consumers; however, the unreleased accessions are only for evaluation purposes. The selected accessions include Maa-Lana Piesa, Manga Moya Piesa, Naachem-Tiir Piesa, Nutsugah Piesa and WAAPP Piesa are released accessions of Frafra potato. Commercially produced pastry dough conditioners, Egg and Gelatin powders, and Transglutaminase (TGase, 94 100 U/g) (Yiming Biologicals Company Limited, Jiangsu, China) were obtained from local suppliers. Analytical grade chemicals and distilled water were also used.

5.2.2 Bread Preparation

Based on the design in Figure 5.1 and ingredients in table 5.1, gluten-free bread (GFB) from each of the 5 FPF accessions were made using equal amounts of the three dough conditioners and the enzyme Transglutaminase (TGase) as a protein cross-linking catalyst to mimic the characteristics (gluten-like matrix) and compared with typical wheat bread. The recipe used followed that of typical wheat bread, with the other ingredients being margarine, baker's yeast, and portable water, as shown in Table 5.1.

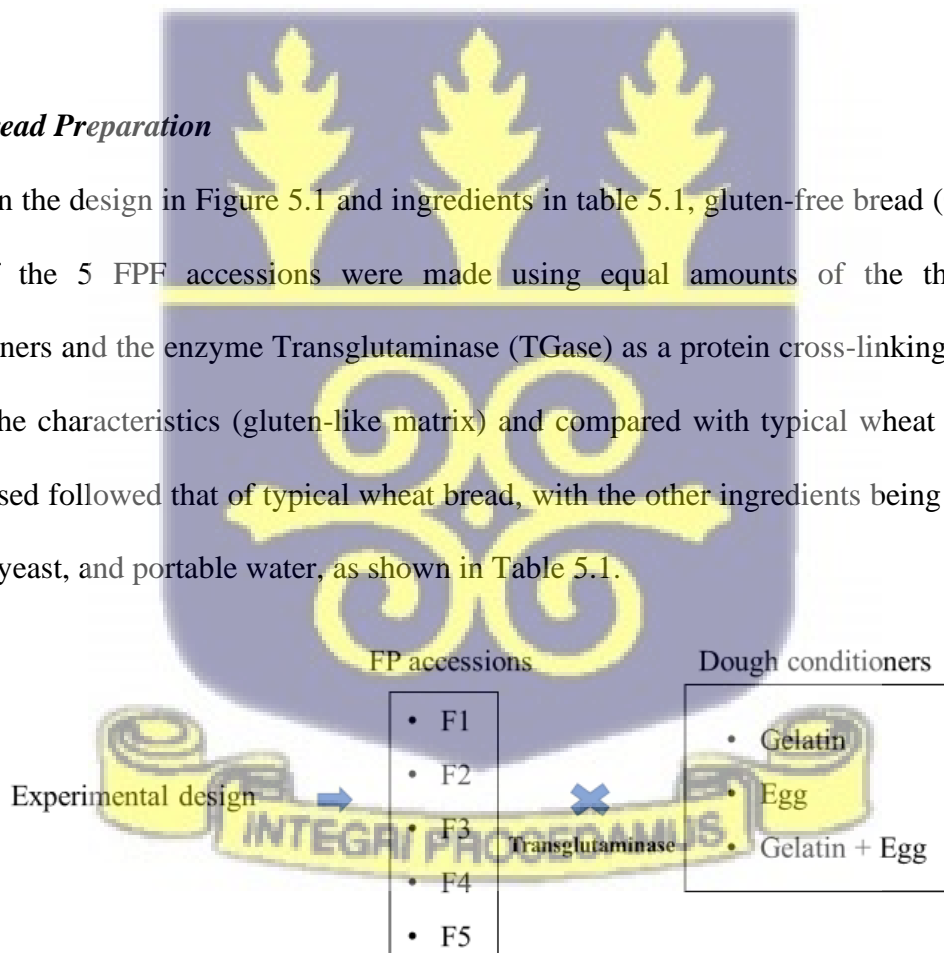


Figure 5.1: Experimental design for gluten-free bread production

Table 5.1: Proportion by weight of ingredients for GFB

Ingredients	Baker's Percentage
Flour	100
Water	43.6
Margarine	16.6
Yeast	0.6
Egg powder	8
Gelatin powder	8
Transglutaminase	1.2

Bread samples were produced based on figure 5.1 and table 5.1, and designated based on the type of dough conditioner used as follows: Samples made using egg powder only were designated as B1 to B5, gelatin powder only samples were B6 to B10, and samples made using egg-gelatin powder were B11 – B15. These were compared to wheat bread.

5.2.2.1 Characteristics of Bread Dough

According to Han *et al.* (2021), dough properties were characterised employing a Mixolab rheometer (Chopin Technologies, France) and the Chopin⁺ protocol (Chopin Applications Laboratory, July 2009). Bread dough was made following the manufacturer's procedure with modifications using the proportion of ingredients in Table 5.2. The proportion of ingredients in Table 5.2, except for TGase, were poured into the rheometer and premixed for 1 min. Thereafter, a prescribed amount of TGase (1.2%) was dispersed in forty microlitres of distilled water and then added to the ingredients. The water distribution unit of the rheometer was filled with distilled water, after which 25% was dispensed to remove air pockets and then topped up to mark before the commencement of the test. Parameters such as the amount of water absorbed during dough development, development time (the time required for all the compounds to be hydrated to form a dough), dough stability (time for which dough rheology remains constant during mixing after development and before breaking down), and mixing tolerance (rate of breakdown after development) were recorded.

5.2.2.2 Bread Baking Capacity

Baking capacity was determined according to Cauvain's (2015) method. To form a dough, a prescribed amount of TGase (1.2%) was dispersed in 40 ml of water and then mixed with the ingredients for about 10 min. The proportion of ingredients in Table 5.1 were mixed for 5 min in a mixer. The doughs were rounded, covered with wrap film, and allowed to rest at forty degrees Celsius for two hours. This allowed the dough to undergo adequate protein crosslinking to mimic the gluten-like matrix in wheat dough. The rested dough was cut into 100 g pieces, rounded, and proofed at 30 °C for 60 min. The bread was baked in an electric oven at 180 °C for 30 min, thereafter, allowed to cool to about 25 ± 0.46 °C, then wrapped and stored in plastic bags.

5.2.3 Weight of Bread

The weight of bread was determined using a digital scale (Escali Digital Scale, Burnsville, USA).

5.2.4 Baking Loss

The baking loss was determined as loss in weight, according to Cauvain (2015). Loss in weight of bread after baking was estimated using the change in weight of dough before and after baking. This was expressed as per cent weight loss (LW).

5.2.5 Volume of Bread

Bread volume (VB) was determined by modifying the rapeseed replacement method according to the procedure of AACC (2000). Millet was used in place of rapeseeds. The loaves were put in a container of known volume (VN). The basin was filled to the top with millet, the bread was removed, and the volume of the millet (VM) was measured with a



measuring cylinder. The bread volume was calculated as $VB (cm^3) = VN - VM$; Specific volume (SV) was expressed as $SV (cm^3/g) = V/LW$

5.2.6 Density of Bread

The density of bread (DB) was calculated using the weight (WB) and volume of bread with the formula:

$$DB (g/cm^3) = WB/VB$$

5.2.7 Colour Analysis of Bread

The Colour of the bread crumb and the crust was determined according to Popov-Raljić (2013) using a Chromameter (CR-400 Chroma Meter, Konica Minolta, Tokyo, Japan). The Chromameter was calibrated against a standard white tile ($L^*0 = 98.93$, $a^*0 = 0.31$, and $b^*0 = 4.63$) before use. The samples were held in a clear petri dish and covered. Bread crust and crumb colour were also described using C^* (Chroma), h^* (Hue angle), and Total change in colour (ΔE^*) notations.

5.2.8 Proximate Composition of Bread

The proximate composition of the bread samples was determined according to AOAC methods (AOAC, 2005). Properties such as moisture (method 925.40), fat (method 948.22), protein concentration (method 955.52), ash (method 935.52), and fibre (method 985.29) were determined, and carbohydrate was estimated as the difference between the sum of the other components and 100%. The energy was determined by calculation, $E = (9 \times \text{fat}) + (4 \times \text{carbohydrate}) + (4 \times \text{protein})$.

5.2.9 Texture of Bread

The texture profile of the bread crumb was determined according to Alencar *et al.* (2015) using three slices taken from the centre of each loaf of bread using a texture analyser (TA.XT2 Texture Analyser, Stable MacroSystem, UK). The bread samples (3 x 3 x 3 cm) from the centre of each slice were compressed two times using a pre-set speed of 1.5 ms⁻¹, the contact force of 5 g, a distance of 8 millimetres and a data acquisition rate of 100 pps. The parameters assessed include hardness, adhesiveness, cohesiveness and chewiness, and resilience.

5.2.10 Microstructure of Bread

According to Aguilar *et al.* (2015), the morphology of bread samples was characterised by SEM (Phenom ProX World Desktop SEM + EDS, Thermo Fisher Scientific, USA) following the procedure established by the manufacturer. Samples were first coated with gold dust (15nm thickness) using Emitech K550X Sputter Coater (Quorum Technologies Limited, Kent, UK). Imaging at x2300 magnification was done at an accelerating voltage of 15 kV.

5.2.11 Statistical Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using “R” statistical software for Windows pc version 4.1.1 (R Project, Bell Laboratories, USA) and Duncan Multiple Range Test (DMRT) was performed to identify varieties with significantly different ($p < 0.05$) means. Principal component analysis (PCA) in XLSTAT 2018 for windows pc was used to cluster cultivars with similar characteristics.

5.3 Results and Discussion

5.3.1 Characteristics of Dough

Bread dough characteristics of FPF was determined to assess the performance of the dough conditioners used on the dough characteristics of gluten-free flours. Dough characteristics of FPF are presented in table 5.2.

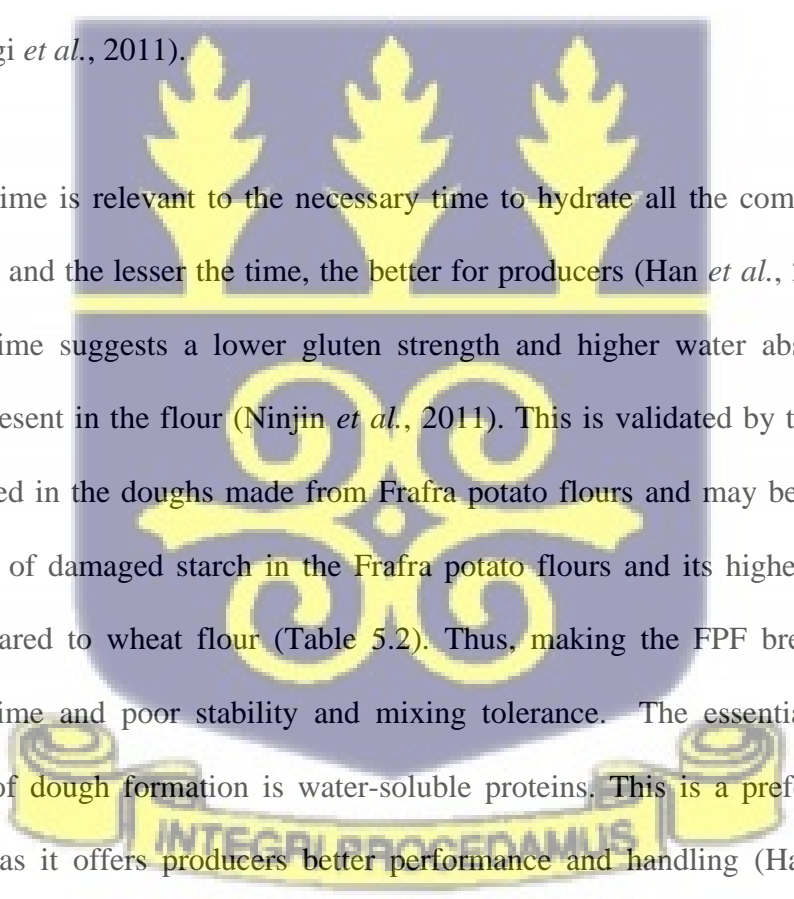
Table 5.2: Characteristics of FPF Dough

Dough	WA (%)	DDT (min)	Dough Stability (min)	MT (BU)
D1	154.5 ± 0.03 ^a	10.56 ± 1.29 ^a	5.29 ± 2.08 ^d	12.67 ± 1.55 ^d
D2	159.6 ± 0.10 ^a	11.12 ± 0.96 ^a	4.89 ± 2.01 ^d	12.33 ± 1.53 ^d
D3	160.9 ± 0.07 ^a	10.67 ± 1.11 ^a	5.11 ± 2.43 ^d	11.67 ± 0.58 ^d
D4	164.5 ± 0.12 ^a	10.85 ± 1.34 ^a	4.99 ± 1.55 ^d	10.67 ± 1.55 ^d
D5	168.1 ± 0.09 ^a	11.09 ± 1.87 ^a	5.05 ± 1.34 ^d	12.33 ± 2.08 ^d
D6	116.8 ± 0.14 ^b	8.22 ± 1.53 ^b	6.19 ± 1.53 ^c	17.37 ± 0.58 ^c
D7	118.7 ± 0.05 ^b	8.83 ± 1.66 ^b	6.07 ± 0.98 ^c	16.33 ± 1.55 ^c
D8	120.2 ± 0.11 ^b	8.91 ± 1.72 ^b	6.01 ± 1.21 ^c	17.33 ± 2.08 ^c
D9	119.6 ± 0.12 ^b	8.65 ± 0.59 ^b	6.13 ± 1.62 ^c	16.67 ± 0.58 ^c
D10	119.8 ± 0.10 ^b	8.77 ± 0.96 ^b	6.04 ± 1.83 ^c	15.53 ± 0.58 ^c
D11	99.2 ± 0.07 ^c	6.92 ± 1.53 ^c	7.98 ± 0.58 ^b	21.33 ± 2.08 ^b
D12	100.3 ± 0.11 ^c	7.14 ± 1.72 ^c	7.26 ± 1.87 ^b	19.67 ± 0.58 ^b
D13	99.5 ± 0.13 ^c	7.05 ± 1.11 ^c	7.32 ± 1.55 ^b	20.33 ± 1.53 ^b
D14	100.2 ± 0.12 ^c	6.98 ± 1.87 ^c	7.19 ± 2.43 ^b	19.67 ± 0.58 ^b
D15	99.9 ± 0.03 ^c	7.01 ± 1.55 ^c	7.25 ± 0.96 ^b	19.53 ± 2.08 ^b
WTD	58.6 ± 0.01 ^d	1.73 ± 0.05 ^d	10.95 ± 0.20 ^a	32.67 ± 0.58 ^a

D1-D5 = Doughs with Egg conditioner only, **D6-D10** = Doughs with Gelatin conditioner only, **D11-D15** = Doughs with Egg-Gelatin conditioner, **WTD** = hard wheat flour dough, **WA** = Water absorption, **DDT** = Dough development time, **MT** = Mixing tolerance. "Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$)".

Significant differences ($p \leq 0.05$) were observed for water absorption (WA), dough development time (DDT), dough stability, and mixing tolerance (MT) of the doughs (Table

5.2). Doughs made with egg powder conditioner only (D1 - D5) had the highest ($p \leq 0.05$) WA, DDT, but the least ($p \leq 0.05$) stability and MT followed by doughs with gelatin powder conditioner (D6 - D10). They had the least stability due to their higher rate of breakdown (Han *et al.*, 2021; Cauvain, 2015). Doughs with egg-gelatin powder conditioner (D11 – D15) had the least WA and DDT but the highest stability relative to the other gluten-free doughs (D1 – D10). Overall, wheat flour dough had superior ($p \leq 0.05$) characteristics compared to the FPF gluten-free doughs. Gluten is responsible for pastry dough elasticity. It allows the dough to expand during proofing and baking in an oven by preventing the carbon dioxide produced by the yeast from escaping. At higher temperatures during baking, gluten coagulates and forms a rigid skeleton that gives the bread loaf a structure that does not collapse (Mongi *et al.*, 2011).

The logo of the University of Ghana is a watermark in the center of the page. It features a shield with three golden wheat stalks at the top, a central golden emblem with a cross and four scrolls, and a banner at the bottom with the Latin motto 'INTEGRUM PROCEDEMUS'.

Development time is relevant to the necessary time to hydrate all the compounds in flour during mixing, and the lesser the time, the better for producers (Han *et al.*, 2021). A longer development time suggests a lower gluten strength and higher water absorption by the components present in the flour (Ninjin *et al.*, 2011). This is validated by the higher water absorption noted in the doughs made from Frafra potato flours and may be correlated to a higher amount of damaged starch in the Frafra potato flours and its higher water-binding capacity compared to wheat flour (Table 5.2). Thus, making the FPF bread took longer development time and poor stability and mixing tolerance. The essential factor in the mixing stage of dough formation is water-soluble proteins. This is a preferred quality in bread doughs as it offers producers better performance and handling (Han *et al.*, 2021; Cauvain, 2015; Nindjin *et al.*, 2011). The relatively better performance from the egg-gelatin doughs, relative to the other FPF doughs, could be attributed to a better protein crosslinking when hydrated and during proofing, probably due to a relatively superior protein mix that

the egg-gelatin mixture provides for Transglutaminase activity (Han *et al.*, 2021; Shittu *et al.*, 2009). The results suggest that a combination of several pastry dough conditioners can produce bread with similar characteristics as conventional wheat bread, as observed in doughs made with egg-gelatin conditioners (D11 – D15).

5.3.2 Baking Capacity of Bread

Baking capacity is a useful measure to evaluate the quality of alternative bread to conventional ones. Gluten-free FPF bread was made to compare their baking characteristics to conventional wheat flour bread. The baking capacity of the bread is presented in table 5.3.

Table 5.3: Baking capacity of bread

Loaf	WB (g)	VB (cm ³)	SV (cm ³ /g)	DB (g/cm ³)	LW (%)
B1	91.97 ± 0.53 ^a	362.40 ± 1.01 ^c	3.94 ± 1.55 ^b	0.25 ± 0.02 ^a	8.03 ± 0.86 ^c
B2	91.99 ± 1.22 ^a	359.63 ± 0.93 ^c	3.91 ± 1.62 ^b	0.26 ± 0.05 ^a	8.01 ± 1.58 ^c
B3	91.93 ± 0.58 ^a	359.10 ± 1.58 ^c	3.91 ± 1.05 ^b	0.26 ± 0.85 ^a	8.07 ± 0.97 ^c
B4	91.96 ± 1.34 ^a	361.15 ± 1.05 ^c	3.93 ± 2.08 ^b	0.25 ± 0.55 ^a	8.04 ± 1.10 ^c
B5	91.94 ± 0.42 ^a	361.60 ± 2.05 ^c	3.93 ± 0.55 ^b	0.25 ± 0.41 ^a	8.06 ± 0.55 ^c
B6	91.16 ± 0.63 ^a	379.10 ± 2.58 ^c	4.16 ± 1.53 ^b	0.24 ± 0.56 ^a	8.84 ± 1.63 ^c
B7	91.24 ± 1.19 ^a	372.35 ± 1.08 ^c	4.08 ± 1.78 ^b	0.25 ± 0.63 ^a	8.76 ± 1.55 ^c
B8	91.09 ± 0.62 ^a	372.67 ± 1.58 ^c	4.09 ± 0.95 ^b	0.24 ± 0.57 ^a	8.91 ± 1.13 ^c
B9	91.35 ± 0.53 ^a	374.55 ± 2.08 ^c	4.10 ± 1.55 ^b	0.24 ± 0.48 ^a	8.65 ± 0.75 ^c
B10	91.21 ± 1.21 ^a	376.47 ± 1.55 ^c	4.13 ± 0.87 ^b	0.24 ± 0.29 ^a	8.79 ± 2.15 ^c
B11	88.66 ± 0.53 ^b	405.72 ± 0.93 ^b	4.58 ± 1.53 ^b	0.22 ± 0.21 ^a	11.34 ± 0.58 ^b
B12	89.38 ± 0.86 ^b	403.24 ± 0.62 ^b	4.51 ± 0.78 ^b	0.22 ± 0.18 ^a	10.62 ± 1.86 ^b
B13	89.46 ± 0.42 ^b	402.86 ± 0.67 ^b	4.50 ± 2.08 ^b	0.22 ± 0.09 ^a	10.54 ± 1.44 ^b
B14	89.29 ± 0.12 ^b	402.95 ± 0.54 ^b	4.51 ± 1.05 ^b	0.22 ± 0.23 ^a	10.71 ± 0.21 ^b
B15	89.15 ± 0.52 ^b	404.25 ± 0.71 ^b	4.53 ± 1.10 ^b	0.22 ± 0.17 ^a	10.85 ± 1.05 ^b
WTB	79.36 ± 0.58 ^c	458.52 ± 0.63 ^a	5.78 ± 1.53 ^a	0.17 ± 0.05 ^b	20.64 ± 2.08 ^a

B1-B5 = Loafs containing Egg only, **B6-B10** = Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg+Gelatin, **WTB** = wheat flour loaf, **WB** = Weight of loaf, **VB** = Volume of loaf, **SV** = Specific volume, **DB** = Specific density, **LW** = Weight loss. Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

From table 5.3, all the bread made from Frafra potato flours (FPF) were significantly ($p \leq 0.05$) heavier (about 89 to 92 g) but smaller in volume (about 359 to 404 cm³) than the loaf made from wheat flour (WTB) which had an average weight and volume of about 79 g and 459 cm³ respectively (Table 5.4). Loaves produced with either egg or gelatin were significantly ($p \leq 0.05$) heavier but smaller than those containing egg and gelatin. FPF loaves were significantly ($p \leq 0.05$) denser than the wheat flour bread. The differences observed in the baking capacities of the FPF loaves may be ascribed to the lack of adequate gluten-like matrix giving rise to differences in crumb structure, which is better in WTB samples because of the gluten content of wheat flour (Mongi *et al.*, 2011; Ninjin *et al.*, 2011). Thus, the absence of adequate levels of gluten, as in wheat flour, would give a weaker and less elastic dough and a reduction in the leavening ability, resulting in bread with lower loaf volume and higher density. The weight loss (LW) in the WTB loaf was at least twice ($p \leq 0.05$) the loss observed in FPF bread samples. The lowest LW (about 9%) was recorded in loaves containing either egg or gelatin only. The difference in LW could be attributed to lower water absorption in the WTB dough relative to FPF doughs (Nindjin *et al.*, 2011; Shittu *et al.*, 2009). It is possible that the higher protein concentration in FPF resulted in a relatively higher water binding ability in bread made from them (Han *et al.*, 2021; Cauvain, 2015).

5.3.3 Colour of Bread

Colour plays a critical role in pastry products such as bread because it impacts consumer acceptance and overall acceptability (Pertuzattiet *et al.*, 2015; Popov-Raljić, 2013). The colour of FPF bread crust and the crumb was measured and compared to wheat flour bread. The colour characteristics of the samples are presented in Tables 5.4 and 5.5.

Table 5.4: Colour of bread crust

Loaf	L*	a*	b*	h*	C*	ΔE*
B1	50.42 ± 1.14 ^b	12.28 ± 0.21 ^a	23.53 ± 0.06 ^a	62.44 ± 0.13 ^b	26.54 ± 0.09 ^a	26.54 ± 0.15 ^a
B2	51.13 ± 0.08 ^b	12.87 ± 0.13 ^a	24.43 ± 0.11 ^a	62.57 ± 0.10 ^b	27.94 ± 0.07 ^a	27.95 ± 0.06 ^a
B3	50.65 ± 0.07 ^b	12.84 ± 0.05 ^a	23.05 ± 0.15 ^a	60.88 ± 0.14 ^b	26.39 ± 0.08 ^a	26.38 ± 0.12 ^a
B4	51.29 ± 0.23 ^b	11.91 ± 0.32 ^a	22.67 ± 0.24 ^a	62.28 ± 0.10 ^b	25.61 ± 0.09 ^a	25.61 ± 0.14 ^a
B5	51.58 ± 0.15 ^b	12.22 ± 0.16 ^a	22.48 ± 0.28 ^a	61.47 ± 0.12 ^b	25.59 ± 0.06 ^a	25.59 ± 0.10 ^a
B6	51.12 ± 0.27 ^b	12.28 ± 0.33 ^a	23.43 ± 0.53 ^a	62.34 ± 0.58 ^b	26.45 ± 0.22 ^a	26.54 ± 0.19 ^a
B7	51.13 ± 0.13 ^b	12.37 ± 0.17 ^a	24.16 ± 0.93 ^a	63.49 ± 0.62 ^b	27.71 ± 0.58 ^a	27.71 ± 0.13 ^a
B8	51.15 ± 0.37 ^b	12.24 ± 0.23 ^a	23.05 ± 0.16 ^a	62.03 ± 0.53 ^b	26.10 ± 0.28 ^a	26.10 ± 0.23 ^a
B9	51.18 ± 0.23 ^b	12.31 ± 0.62 ^a	22.65 ± 0.27 ^a	61.48 ± 0.18 ^b	25.78 ± 0.53 ^a	25.80 ± 0.58 ^a
B10	51.18 ± 0.19 ^b	12.22 ± 0.45 ^a	22.48 ± 0.15 ^a	61.47 ± 0.10 ^b	25.59 ± 0.62 ^a	25.59 ± 0.14 ^a
B11	51.22 ± 0.21 ^b	12.28 ± 0.23 ^a	23.61 ± 0.58 ^a	62.52 ± 0.47 ^b	26.61 ± 0.23 ^a	26.54 ± 0.12 ^a
B12	51.13 ± 0.62 ^b	12.27 ± 0.58 ^a	23.70 ± 0.22 ^a	62.63 ± 0.12 ^b	26.69 ± 0.58 ^a	26.69 ± 0.53 ^a
B13	51.15 ± 0.43 ^b	12.24 ± 0.55 ^a	23.05 ± 0.19 ^a	62.03 ± 0.62 ^b	26.10 ± 0.37 ^a	26.10 ± 0.28 ^a
B14	51.29 ± 0.58 ^b	12.22 ± 0.18 ^a	22.78 ± 0.47 ^a	61.79 ± 0.28 ^b	25.85 ± 0.32 ^a	25.75 ± 0.19 ^a
B15	51.28 ± 0.25 ^b	12.23 ± 0.58 ^a	22.48 ± 0.21 ^a	61.45 ± 0.53 ^b	25.59 ± 0.23 ^a	25.59 ± 0.37 ^a
WTB	61.63 ± 0.23 ^a	9.01 ± 0.32 ^b	22.67 ± 0.24 ^a	68.33 ± 0.11 ^a	21.40 ± 0.08 ^b	25.84 ± 0.05 ^a

L* - lightness from dark (0-100), a* / -a* = redness / greenness, b* / -b* = yellowness / blueness, h* = Hue angle, C* = Chroma (colour intensity), ΔE* = Total colour change. **B1-B5** = Loafs containing Egg only, **B6-B10** Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg + Gelatin, **WTB** = Loaf from hard wheat flour. Values are means of triplicates with standard deviation. Means in the same column with different superscript are significantly different ($p \leq 0.05$).

The colour of the FPF loaves (B1 – B15) show that their crusts were darker than the crust of wheat flour bread (WTB) (Table 5.4), and the lower L* indicates this, and higher C* and a* in FPF loaves crust compared to the crust of WTB. Loafs made from wheat flour had a significantly ($p \leq 0.05$) brighter crust colour than those made from FPF, indicating the higher h* in WTB. This indicates the browning, occurring primarily because of dextrinization and partly by caramelization and Maillard reactions, was extensive in FPF loafs compared to wheat bread (Morais *et al.*, 2014; Nindjin *et al.*, 2011). No clear ($p \leq 0.05$)

trend in colour differences (ΔE^*) and b^* between the dough and final baked bread was noticed in the crust.

Table 5.5: Colour of bread crumb

Loaf	L*	a*	b*	h*	C*	ΔE^*
B1	61.39 ± 1.14 ^b	3.28 ± 0.21 ^a	12.53 ± 0.06 ^a	75.33 ± 0.05 ^b	12.95 ± 0.10 ^a	12.95 ± 0.05 ^a
B2	63.58 ± 0.08 ^b	3.87 ± 0.13 ^a	12.80 ± 0.11 ^a	71.84 ± 0.03 ^b	12.42 ± 0.14 ^a	12.42 ± 0.10 ^a
B3	64.32 ± 0.07 ^b	3.84 ± 0.05 ^a	13.05 ± 0.15 ^a	70.84 ± 0.09 ^b	11.70 ± 0.12 ^a	12.01 ± 0.12 ^a
B4	62.29 ± 0.23 ^b	3.01 ± 0.32 ^a	12.67 ± 0.24 ^a	75.54 ± 0.06 ^b	12.05 ± 0.09 ^a	12.05 ± 0.08 ^a
B5	61.58 ± 0.15 ^b	4.22 ± 0.16 ^a	12.98 ± 0.28 ^a	70.60 ± 0.08 ^b	12.70 ± 0.13 ^a	12.64 ± 0.09 ^a
B6	63.58 ± 2.08 ^b	3.77 ± 0.33 ^a	12.80 ± 0.17 ^a	72.28 ± 0.05 ^b	12.39 ± 0.22 ^a	12.39 ± 0.08 ^a
B7	64.21 ± 0.63 ^b	3.64 ± 0.55 ^a	13.05 ± 0.18 ^a	71.78 ± 0.03 ^b	11.63 ± 0.62 ^a	12.63 ± 0.05 ^a
B8	61.34 ± 1.05 ^b	4.01 ± 0.47 ^a	12.87 ± 0.08 ^a	71.33 ± 0.08 ^b	12.53 ± 0.47 ^a	12.63 ± 0.09 ^a
B9	61.35 ± 0.97 ^b	3.68 ± 0.13 ^a	12.61 ± 0.23 ^a	73.73 ± 0.06 ^b	13.14 ± 2.53 ^a	13.06 ± 0.07 ^a
B10	62.23 ± 2.08 ^b	3.91 ± 0.53 ^a	12.65 ± 0.62 ^a	71.45 ± 0.05 ^b	12.29 ± 0.08 ^a	12.31 ± 0.03 ^a
B11	63.51 ± 1.13 ^b	3.87 ± 0.27 ^a	12.21 ± 0.16 ^a	72.41 ± 0.02 ^b	12.81 ± 1.08 ^a	12.42 ± 0.11 ^a
B12	64.14 ± 0.67 ^b	3.93 ± 0.58 ^a	12.05 ± 0.05 ^a	70.42 ± 0.07 ^b	11.73 ± 0.53 ^a	12.73 ± 0.22 ^a
B13	61.62 ± 1.53 ^b	4.12 ± 0.62 ^a	11.99 ± 0.21 ^a	71.04 ± 0.04 ^b	12.68 ± 0.97 ^a	12.67 ± 0.38 ^a
B14	61.63 ± 0.58 ^b	3.88 ± 0.15 ^a	12.53 ± 0.09 ^a	71.40 ± 0.09 ^b	12.17 ± 0.62 ^a	12.17 ± 0.21 ^a
B15	62.05 ± 2.05 ^b	3.65 ± 0.11 ^a	12.66 ± 0.06 ^a	72.62 ± 0.11 ^b	12.22 ± 0.53 ^a	12.23 ± 1.08 ^a
WTB	73.15 ± 0.23 ^a	1.01 ± 0.32 ^b	8.67 ± 0.24 ^b	84.01 ± 0.05 ^a	9.72 ± 0.11 ^b	10.93 ± 0.07 ^b

L* - lightness from dark (0-100), a* / -a* = redness / greenness, b* / -b* = yellowness / blueness, h* = Hue angle, C* = Chroma (colour intensity), ΔE^* = Total colour change. **B1-B5** = Loafs containing Egg only, **B6-B10** Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg + Gelatin, **WTB** = Loaf from hard wheat flour. Values are means of triplicates with standard deviation. Means in the same column with different superscript are significantly different ($p \leq 0.05$).

As expected, the colour of the FPF loaves (B1 – B15) show that their crusts were darker than the crumb, and that of wheat flour bread (WTB) (Tables 5.4 and 5.5), and the higher L* indicates this, and lower C* and a* in FPF loaves crumb compared to the crust. A similar trend was observed when the colour of FPF loaves was compared to WTB loaf. Loaves made from wheat flour had a significantly ($p \leq 0.05$) brighter crumb colour than those made from FPF, as indicated by the higher h* in WTB (Table 5.5). The darker colours observed in FPF loaves crumb also indicates the browning, occurring partly by caramelization and

Maillard reactions, was extensive in FPF loafs compared to wheat bread (Morais *et al.*, 2014; Nindjin *et al.*, 2011). No clear ($p \leq 0.05$) trend in colour differences (ΔE^*) and b^* between the dough, and final baked bread was noticed in the crumb of FPF loaves. However, there was a considerable change in colour in the crumb of WTB.

5.3.4 Proximate Composition of Bread

Compositional analysis of bread made from gluten-free Frafra potato flours, using dough conditioners, was determined and compared to typical wheat flour bread. The proximate composition of the bread is presented in table 5.6.

Table 5.6: Proximate composition of bread (g/100g)

Loaf	Ash	Fat	Protein	Crude fibre	Carbohydrate	Energy (kcal)
B1	4.05 ± 1.08 ^a	12.13 ± 1.63 ^a	19.42 ± 0.32 ^b	2.32 ± 0.09 ^a	61.88 ± 1.15 ^a	435.17 ± 0.65 ^a
B2	3.89 ± 0.62 ^a	12.11 ± 1.62 ^a	19.35 ± 0.58 ^b	2.21 ± 0.21 ^a	62.44 ± 1.52 ^a	459.35 ± 0.22 ^a
B3	3.75 ± 0.28 ^a	12.24 ± 1.13 ^a	19.22 ± 0.04 ^b	2.24 ± 0.37 ^a	62.55 ± 0.63 ^a	437.24 ± 0.58 ^a
B4	4.02 ± 0.34 ^a	13.05 ± 0.53 ^a	18.23 ± 0.23 ^c	2.31 ± 0.53 ^a	62.39 ± 1.18 ^a	439.93 ± 0.62 ^a
B5	4.01 ± 0.26 ^a	13.63 ± 0.14 ^a	18.19 ± 0.15 ^c	2.02 ± 0.02 ^a	62.15 ± 1.27 ^a	444.03 ± 0.33 ^a
B6	4.36 ± 0.54 ^a	12.86 ± 0.12 ^a	20.02 ± 0.58 ^a	2.43 ± 0.32 ^a	60.33 ± 2.58 ^a	446.14 ± 0.54 ^a
B7	3.54 ± 1.08 ^a	12.71 ± 0.11 ^a	19.96 ± 0.62 ^{ab}	2.35 ± 0.06 ^a	61.44 ± 1.37 ^a	439.99 ± 0.58 ^a
B8	3.61 ± 0.58 ^a	12.62 ± 0.32 ^a	20.01 ± 0.37 ^a	2.39 ± 0.13 ^a	61.37 ± 1.09 ^a	439.10 ± 0.63 ^a
B9	4.23 ± 0.63 ^a	13.01 ± 0.62 ^a	19.98 ± 0.10 ^{ab}	2.38 ± 0.09 ^a	60.40 ± 2.30 ^a	438.61 ± 0.21 ^a
B10	4.19 ± 0.09 ^a	13.76 ± 0.43 ^a	20.01 ± 0.32 ^a	2.11 ± 0.12 ^a	59.93 ± 1.24 ^a	443.60 ± 0.52 ^a
B11	4.41 ± 1.18 ^a	12.15 ± 1.04 ^a	24.15 ± 0.01 ^a	2.49 ± 0.13 ^a	63.80 ± 0.74 ^a	465.15 ± 1.40 ^a
B12	3.67 ± 0.34 ^a	12.01 ± 1.01 ^a	22.67 ± 0.23 ^a	2.39 ± 0.05 ^a	61.45 ± 1.66 ^a	444.57 ± 2.17 ^a
B13	3.51 ± 0.09 ^a	12.12 ± 1.04 ^a	23.22 ± 0.21 ^a	2.42 ± 0.22 ^a	62.12 ± 1.10 ^a	450.44 ± 1.12 ^a
B14	4.42 ± 0.29 ^a	13.31 ± 0.23 ^a	20.23 ± 0.04 ^a	2.43 ± 0.05 ^a	60.31 ± 2.20 ^a	441.95 ± 1.45 ^a
B15	4.13 ± 0.15 ^a	13.06 ± 0.23 ^a	20.19 ± 0.04 ^a	2.05 ± 0.05 ^a	60.15 ± 2.14 ^a	447.90 ± 1.08 ^a
WTB	1.49 ± 0.03 ^b	10.08 ± 0.35 ^b	16.86 ± 0.01 ^d	2.17 ± 0.04 ^a	68.51 ± 0.30 ^a	469.70 ± 2.06 ^a

B1-B5 = Loafs containing Egg only, **B6-B10** Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg+Gelatin, **WTB**= Loaf from hard wheat flour. Values are means of triplicates with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 5.6 shows no significant ($p \leq 0.05$) differences between the fibre, carbohydrate, and energy concentrations for all the breads. No significant difference existed in all the FPF loaves; however, they were significantly ($p \leq 0.05$) higher than wheat flour bread (WTB). The protein content of FPF loaves was higher ($p \leq 0.05$) than WTB, and this is attributed to the additional proteins (egg albumin and or gelatin powders) in FPF loaves. Overall, FPF loaves which contained both egg and gelatin (B11 – B15), expectedly, had higher protein compared to the remaining loaf samples (B1 – B10). The fat content of FPF loaves was similar but also higher ($p \leq 0.05$) than WTB. The higher fat content observed in FPF loaves could be attributed to the added egg powder since egg yolk is fatty (Han *et al.*, 2021). Also, the high oil absorption capacity of Frafra potato flours. Pastry made with flours with high water, and oil binding capacities tend to absorb and retain more water and oil in their end products (Mongi *et al.*, 2011; Nindjin *et al.*, 2011).

5.3.5 Texture Profile of Bread

Texture profile analysis of the bread samples was measured to mimic what happens when the bread is bitten into or pressed between the molars. The texture profile of the bread samples is presented in table 5.7.

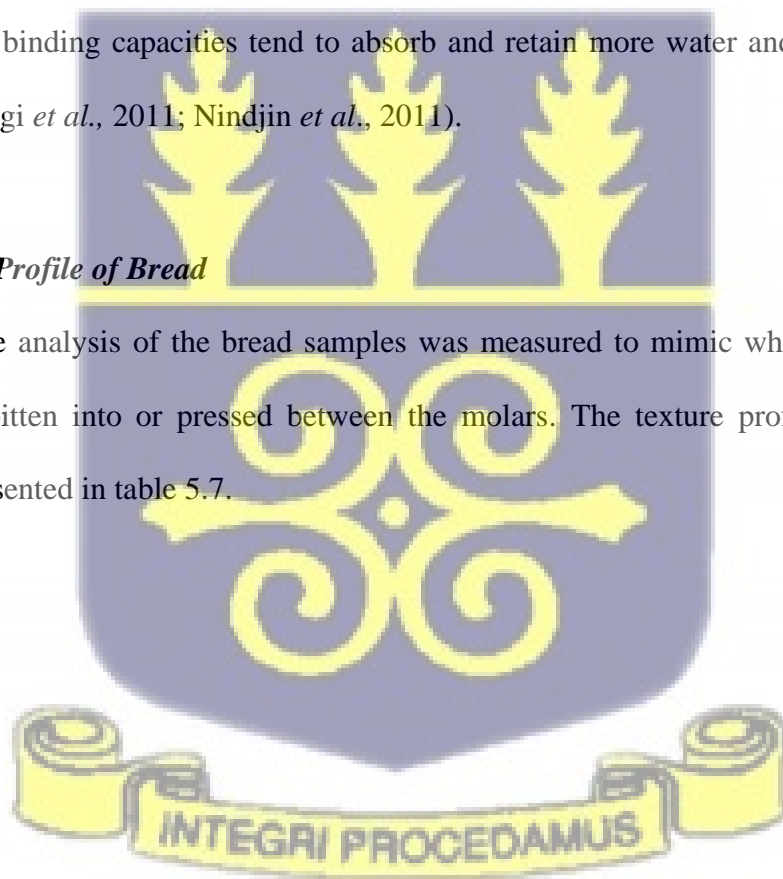


Table 5.7: Texture profile of bread samples

Loaf	Hardness (g)	Adhesiveness (gs)	Cohesiveness	Chewiness	Resilience
B1	2289.30 ± 1.24 ^a	-1.86 ± 2.13 ^a	0.14 ± 1.01 ^b	124.23 ± 2.26 ^c	0.09 ± 0.76 ^b
B2	2356.29 ± 1.67 ^a	-1.73 ± 3.24 ^a	0.15 ± 0.56 ^b	112.14 ± 1.09 ^c	0.09 ± 0.15 ^b
B3	2410.66 ± 1.74 ^a	-1.58 ± 2.67 ^a	0.13 ± 0.83 ^b	123.27 ± 2.01 ^c	0.08 ± 0.32 ^b
B4	2339.71 ± 1.53 ^a	-1.55 ± 4.91 ^a	0.16 ± 1.22 ^b	106.03 ± 0.73 ^c	0.09 ± 0.49 ^b
B5	2339.71 ± 1.92 ^a	-1.62 ± 3.82 ^a	0.14 ± 0.91 ^b	119.15 ± 1.01 ^c	0.08 ± 0.92 ^b
B6	1845.08 ± 1.81 ^b	-5.02 ± 3.48 ^b	0.32 ± 1.15 ^b	179.47 ± 1.05 ^b	0.11 ± 0.53 ^b
B7	1867.19 ± 1.79 ^b	-4.87 ± 4.16 ^b	0.28 ± 0.76 ^b	164.55 ± 0.82 ^b	0.10 ± 0.45 ^b
B8	1828.52 ± 1.55 ^b	-4.98 ± 3.58 ^b	0.21 ± 0.31 ^b	181.26 ± 2.13 ^b	0.12 ± 0.34 ^b
B9	1834.35 ± 1.88 ^b	-5.08 ± 3.55 ^b	0.23 ± 1.01 ^b	168.95 ± 1.19 ^b	0.10 ± 0.66 ^b
B10	1817.44 ± 0.97 ^b	-5.23 ± 2.94 ^b	0.28 ± 0.65 ^b	194.23 ± 1.74 ^b	0.11 ± 0.53 ^b
B11	1201.23 ± 1.16 ^c	-7.12 ± 3.09 ^b	0.61 ± 1.05 ^a	262.13 ± 1.68 ^a	0.15 ± 0.18 ^a
B12	1208.31 ± 1.45 ^c	-6.15 ± 3.11 ^b	0.62 ± 0.85 ^a	254.46 ± 2.13 ^a	0.16 ± 0.13 ^a
B13	1226.25 ± 1.61 ^c	-6.13 ± 4.02 ^b	0.58 ± 0.93 ^a	257.62 ± 1.95 ^a	0.15 ± 0.29 ^a
B14	1214.19 ± 1.94 ^c	-6.09 ± 3.89 ^b	0.61 ± 0.72 ^a	259.93 ± 1.14 ^a	0.17 ± 0.11 ^a
B15	1239.04 ± 1.76 ^c	-7.10 ± 3.63 ^b	0.63 ± 0.90 ^a	261.34 ± 1.08 ^a	0.15 ± 0.13 ^a
WTB	856.06 ± 0.53 ^d	-10.62 ± 1.10 ^c	0.57 ± 0.15 ^a	286.09 ± 0.09 ^a	0.18 ± 0.06 ^a

B1-B5 = Loafs containing Egg only, **B6-B10** Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg + Gelatin, **WTB** = Loaf from hard wheat flour. Values are means of 5 reps with standard deviation. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Frafra potato loaves (FPB) made with egg conditioner only (B1 – B5) were significantly ($p \leq 0.05$) harder (2289 – 2410.7 g force) than those containing only gelatin (B6 – B10) (1817.4 – 1867.2 g force) or a combination of egg and gelatin (B11 – B15) (1201.2 – 1239.0 g force) (Table 5.7). These samples (FPB) were also significantly ($p \leq 0.05$) harder than the wheat bread (WTB), which recorded a mean hardness of 856 g force. However, FPB were less chewy compared to WTB. WTB was ($p \leq 0.05$) more adhesive than B1 – B15, and this could be attributed to a superior gluten matrix formed in WTB, which makes it gummy (Mongi *et al.*, 2011; Nindjin *et al.*, 2011). However, B11 – B15 was more adhesive compared to B1 – B10. The cohesiveness of samples containing both egg and gelatin (B11 – B15) were comparable to WTB. B11 – B15 and WTB had similar ($p \leq 0.05$) resilience, but

significantly ($p \leq 0.05$) differed from B1 – B5 and B6 – B10. This implies B11 – B15 and WTB had a higher ability to regain their height after compression than loaves containing only gelatin or egg (B1 – B10). It is possible that the alveoli structure formed by a combination of egg and gelatin conditioners had comparable stability to that of WTB (Mongi *et al.*, 2011; Nindjin *et al.*, 2011), which was more stable compared to the one formed by loaves containing either only egg or only gelatin.

5.3.6 Microstructure of Bread

The alveoli structure of FPF bread were imaged and compared to that of typical wheat flour bread. Figure 5.2.



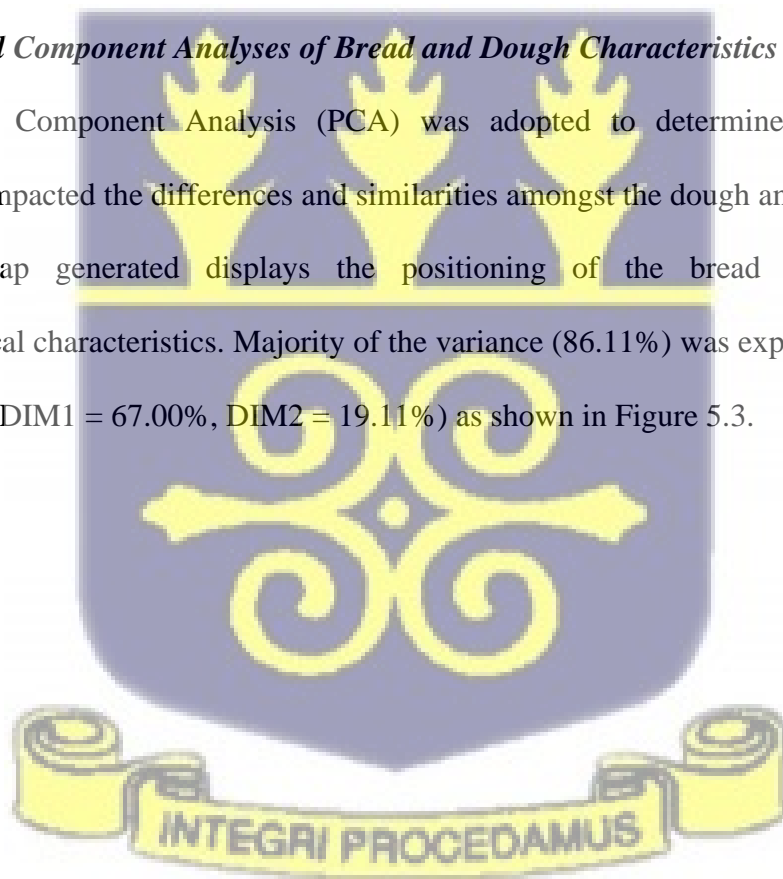
GFBE = Bread containing egg conditioner only (B1 – B5), **GFBG** = Bread containing gelatin conditioner only (B6 – B10), **GFBE**G = Bread containing egg-gelatin conditioner (B11 – B15), **WTB** = Wheat flour bread

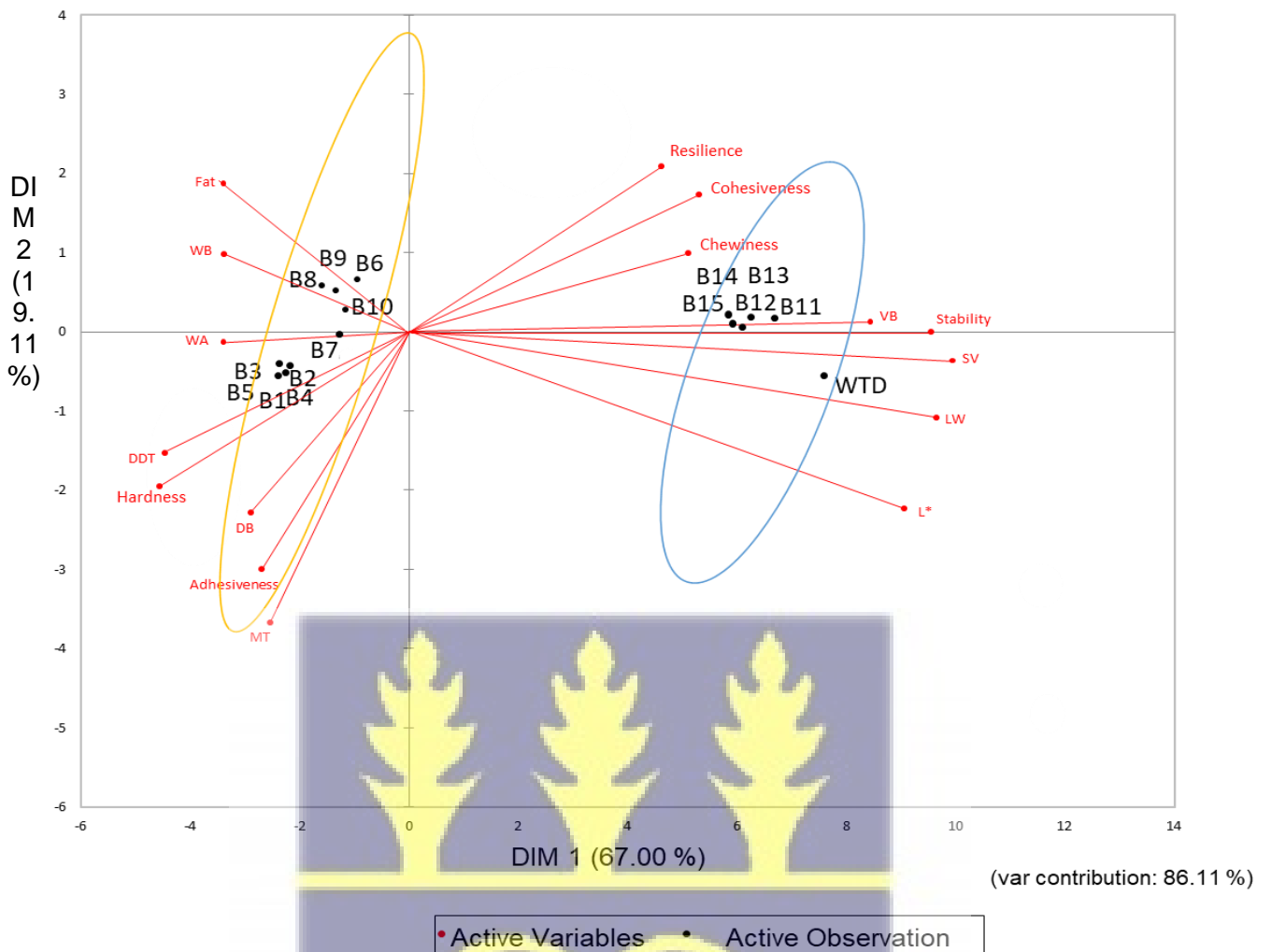
Figure 5.2: Microstructure of Bread Samples (x2300 magnification @ 15 kV)

SEM imaging revealed a more open crumb structure consisting of thinner walls in bread made from wheat flour (WTB) compared to those made with egg or gelatin only (GFBE and GFBG, respectively), which showed a denser network (Figure 5.2). Some almost intact starch granules were noticed in the structure of bread made with a combination of egg-gelatin conditioner (GFBEG). It is possible that the firmer gel network formed by this combination conditioner chelated the starch granules and restricted starch granule swelling during the dough forming stages (Nindjin *et al.*, 2011; Mongi *et al.*, 2011). However, bread with egg-gelatin conditioner had a crumb structure similar to wheat flour bread compared to those made with either egg or gelatin only.

5.3.7 Principal Component Analyses of Bread and Dough Characteristics

The Principal Component Analysis (PCA) was adopted to determine which features significantly impacted the differences and similarities amongst the dough and bread samples. The PCA map generated displays the positioning of the bread based on their physicochemical characteristics. Majority of the variance (86.11%) was explained in the first 2 dimensions (DIM1 = 67.00%, DIM2 = 19.11%) as shown in Figure 5.3.





B1-B5 = Loafs containing Egg only, **B6-B10** Loafs containing Gelatin only, **B11-B15** = Loafs containing Egg + Gelatin, **WTB** = Loaf from hard wheat flour.

Figure 5.3: PCA Plot for Bread Characteristics

From figure 5.3, PCA clustered the loafs of bread into two main groups, with B11 – B15 and WTB in one group and B1 – B10 in another. B11 – B15 and WTB were mainly characterised by their dough stability, bread volume, the colour of bread, loss in weight, cohesiveness, chewiness, and resilience (area of the blue ellipse). In contrast, the second group (B1 – B10) was characterised by their fat concentrations, the weight of bread, water absorption, development time and mixing tolerance of their doughs, density, loaf hardness, and adhesiveness (area of the yellow ellipse). The grouping by PCA suggests that bread

made with egg and gelatin combination (B11 – B15) was more likely to compare with typical bread than the other FPF bread (B1 – B10). Thus, B11 – B15 may be considered for advanced selection for further investigation.

5.4 Conclusion

Bread made with two dough conditioners (B11-B15) and typical wheat bread were characterised by similar properties and grouped (PCA). FPF dough absorbed more water, required a longer time for development, had lower mixing tolerance, and was less stable than wheat flour dough. FPF were darker than WTB in the dough, crust, and crumb colour. Egg and gelatin combination produced dough with characteristics and baking capacity closer to wheat flour. B11-B15 were as cohesive, chewy, and resilient as WTB but were harder. The products were clustered into two groups by PCA, based on similarities. In contrast, bread made with one conditioner only (B1 – B10) were also grouped and differed from wheat flour bread. Thus, in developing bread from FPF, multiple dough conditioners should be considered instead of one, as this will provide a much better substrate for mimicking a gluten-like matrix in gluten-free bread. All the five released accessions performed similarly in gluten-free bread. Hence these five accessions would be desirable to continue further studies for gluten-free bread.

5.5 References

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CHAPTER SIX

6.0 MICROBIAL QUALITY AND SENSORY PROFILE OF GLUTEN-FREE

FRAFRA POTATO BREAD

Abstract

Five gluten-free bread products (B11 – B15) were prepared using Frafra potato flour (FPF) and compared to bread made from wheat flour (WTB). Microbial tests were carried out to ensure the products met the minimum thresholds for microbial quality for human consumption using the Ghana Standards Authority's microbiological limits (GS 955:2018) for bread. All the products had counts far below the limits, hence were safe for human consumption. A panel of nine (9) trained assessors were used to assess six (6) bread samples, five of which were gluten-free, and one was typical wheat bread, using the Quantitative Descriptive Analysis (QDA®) method. All the six bread samples were not significantly different to each other for most of the attributes used to differentiate between them ($p \leq 0.05$). Only 5 out of the total number of 48 attributes significantly differentiated the products. The products were differentiated in appearance by brown crust (top and bottom), smooth bottom crust and homogeneous bottom crust and by a minor bitter flavour note perceived in the gluten-free products. The top and bottom crusts of WTB and B14 had the lowest intensity (lightest) of brown colour in the sample set, but they differed statistically ($p \leq 0.05$) from each other. The top crusts of products B11, B12, B13 and B15 had higher brown colour intensities and were not significantly different from one another ($p \leq 0.05$). The brown colour of the bottom crust of B11 was not significantly different from that of products B12, B13 and B15. B11 and B13 had a significantly higher brown bottom crust colour compared. Bitterness perceived in B11 was significantly intense than in B12 and B15 ($p \leq 0.05$). The intensities of bitterness were, however, very low. All the bread smelled and tasted like typical bread. Sensory profile of the products correlated strongly with the optical

and dough properties of FPF. Thus, in developing bread from FPF, attributes of colour and dough properties should be considered.

6.1 Introduction

Bread stands out as the most most important among bakery products because it is consumed daily and appreciated greatly by consumers globally (Pertuzatti *et al.*, 2015; Williams and Pullen, 2007). Traditionally, bread is made from wheat flour. However, due to the cost of importation and the health considerations for some consumers, because they are gluten intolerant, there has been a great deal of research effort to develop gluten-free flour as a substitute for wheat flour for bakery products, including bread (Popov-Raljić *et al.*, 2013; USFDA, 2014). The use of substitute flour for wheat, such as tuber crop flour in gluten-free bread production, has achieved some modest success technologically (Popov-Raljić *et al.*, 2013; Tenehaus *et al.*, 2005). In gluten-free flour pastry production, pastry dough conditioners have been used to improve the product's crumb structure, texture, and sensory profile (Nindjin *et al.*, 2011; Scaman, 2003). Dough conditioners such as transglutaminase have been applied to serve technological functions. Moreover, they have been used in such minute concentrations that they do not contribute to caloric content in the product (Chlopika *et al.*, 2012; Williams and Pullen, 2007) and have generally been considered safe for human consumption (USFDA, 2014; Laureati *et al.*, 2012).

However, due to differences in the sensory profile of bread made using gluten-free flour, broad consumer acceptance and adoption is slow. It is suggested that further studies are required to investigate modifications that may be necessary for gluten-free flour to achieve similar sensory attributes as wheat flour when used to bake bread (Dankwa and Peprah, 2019). Understanding the factors that affect product quality by using descriptive profiling has been done extensively by researchers and manufacturers as part of research and

development (R&D) processes (Lawless and Heymann, 2010; Stone *et al.*, 1974). The objective of this study was to assess the sensory profile of gluten-free bread made with Frafra potato flour using Quantitative Descriptive Analysis (QDA®).

6.2 Materials and Methods

6.2.1 Source of Raw Materials

Five (5) Frafra potato bread types (B11 to B15) were selected in chapter five. The selection was based on the PCA clustering (Figure 5.3) observed in chapter five. Microbiological media for safety tests were obtained from local chemical suppliers.

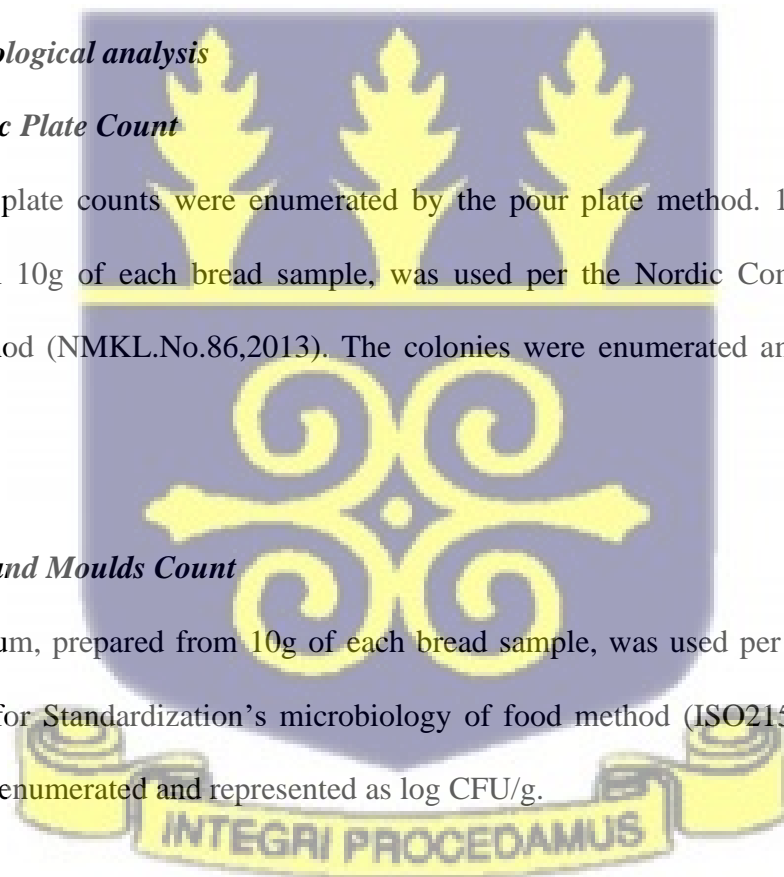
6.2.2 Microbiological analysis

6.2.2.1 Aerobic Plate Count

Total aerobic plate counts were enumerated by the pour plate method. 1ml of inoculum, prepared from 10g of each bread sample, was used per the Nordic Committee on Food Analysis method (NMKL.No.86,2013). The colonies were enumerated and represented as log CFU/g.

6.2.2.2 Yeast and Moulds Count

1ml of inoculum, prepared from 10g of each bread sample, was used per the International Organization for Standardization's microbiology of food method (ISO21527-1:2008). The colonies were enumerated and represented as log CFU/g.



6.2.2.3 Total Coliforms Count

Total coliforms were enumerated by the pour plate method. 1ml of inoculum, prepared from 10g of each bread sample, was used per the Nordic Committee on Food Analysis method (NMKL.No.44,2004). The colonies were enumerated and represented as log CFU/g.

6.2.2.4 Enterobacteriaceae Count

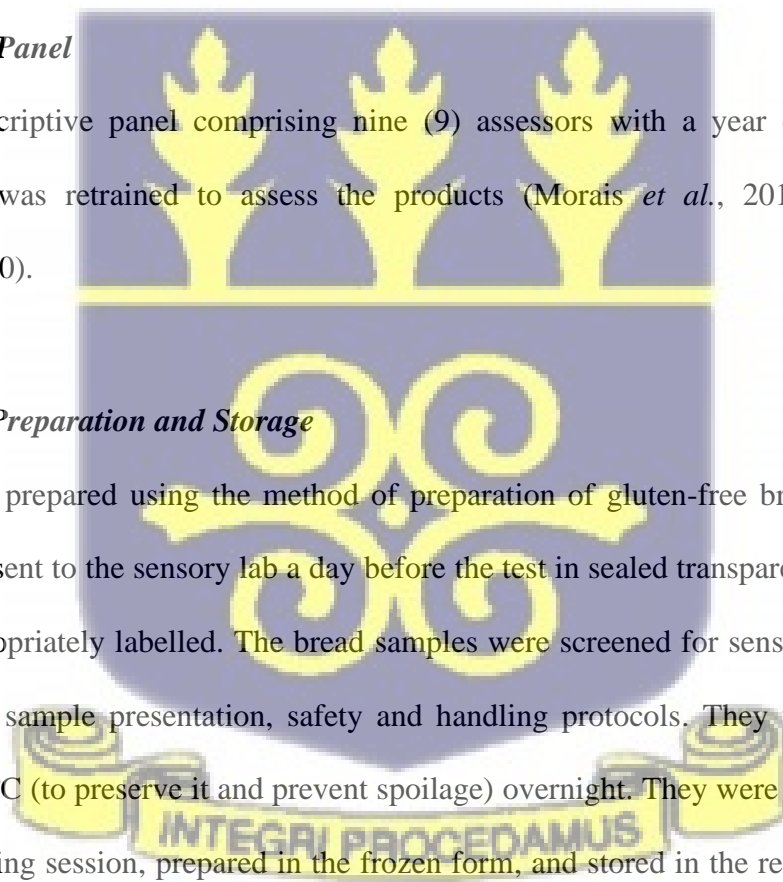
1µl of serial decimal dilutions, prepared from 10g of each bread sample, was used per the Nordic Committee on Food Analysis method (NMKL.No.71,1999). The colonies were enumerated and represented as log CFU/g.

6.2.3 Sensory Panel

A trained descriptive panel comprising nine (9) assessors with a year of experience in QDA® tests was retrained to assess the products (Morais *et al.*, 2014; Lawless and Heymann, 2010).

6.2.4 Sample Preparation and Storage

Samples were prepared using the method of preparation of gluten-free bread described in chapter 5 and sent to the sensory lab a day before the test in sealed transparent Ziploc plastic bags and appropriately labelled. The bread samples were screened for sensory cues (colour, aroma) to aid sample presentation, safety and handling protocols. They were kept in the freezer at -19 °C (to preserve it and prevent spoilage) overnight. They were removed 4 hours before the testing session, prepared in the frozen form, and stored in the refrigerator at 7 °C for distribution to the assessors at the commencement of the test sessions.



6.2.5 Sample Preparation and Serving

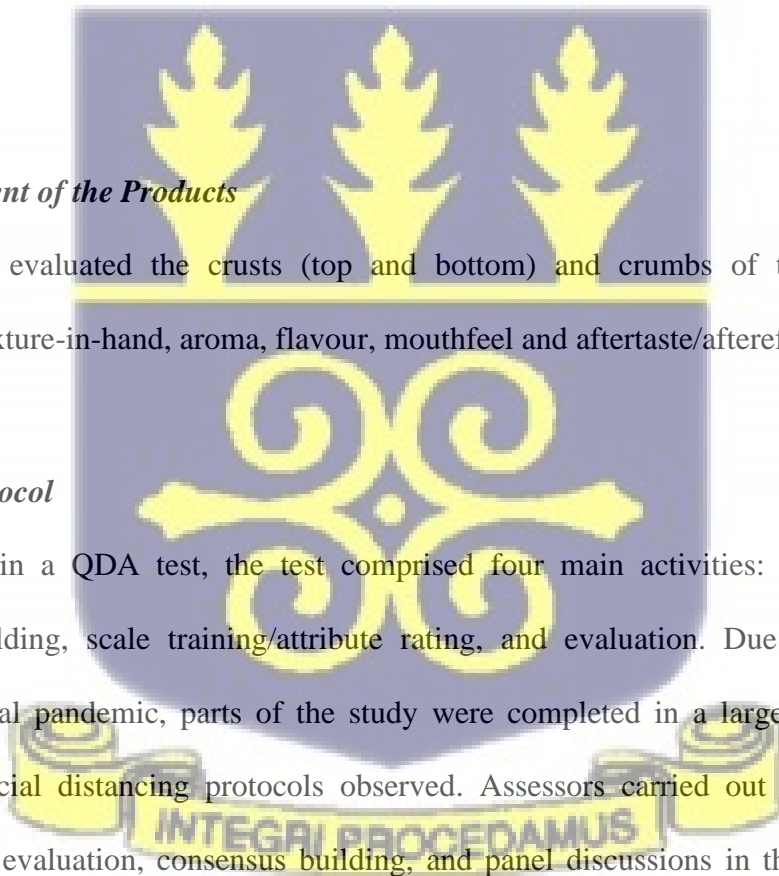
The bread samples were handled in the frozen state to allow easy cutting and avoid deformation because of their soft texture. The sides of the frozen bread were cut off and discarded to obtain a portion of the bread that had a uniform colour. The remaining portion was divided into four equal parts. Each part was served in an 80 mL plastic sauce cup labelled with a random 3-digit code and stored in the fridge. The samples to be assessed were removed from the fridge at least 45 minutes before the start of the testing session to allow them to attain a room temperature of about 25 ± 0.57 °C. They were served to the assessors with 500mL of bottled water as a palate cleanser, disposable forks, tissue, environmental monitoring, sample handling recording sheets, and instructions on sample handling.

6.2.6 Assessment of the Products

The assessors evaluated the crusts (top and bottom) and crumbs of the products for appearance, texture-in-hand, aroma, flavour, mouthfeel and aftertaste/aftereffects.

6.2.7 Test Protocol

As is typical in a QDA test, the test comprised four main activities: term generation, consensus building, scale training/attribute rating, and evaluation. Due to the ongoing Covid-19 global pandemic, parts of the study were completed in a large classroom with appropriate social distancing protocols observed. Assessors carried out term generation, scale training, evaluation, consensus building, and panel discussions in the Oracca Tetteh classroom adjacent to the Sensory Evaluation Laboratory, University of Ghana, Legon Campus.



Each assessor evaluated all six products for term generation and developed attributes that described each product, defined those attributes, and allocated anchors. Assessors also listed references where necessary to provide clarity on ambiguous terms. All the assessors agreed on a final collated list of descriptors that accurately described the product set during consensus building. Reference products were provided by the panel where needed to clarify vague descriptors and align panel agreement. During the scale training session, assessors were trained to score each attribute on a 10cm intensity line scale (Popov-Raljić *et al.*, 2013). They collectively agreed on the ranges of each attribute per product to align scale usage and establish the correct ranking order of the products. Each assessor then individually scored the intensity of each product per attribute on individual 10 cm intensity scales in duplicate for the final evaluation. On the line scale, 0 is not intense or not present, and ten is very intense or very present.

Assessors evaluated the samples one after the other in randomized order. Randomization was achieved using Williams Latin Square design (Williams, 1949). Assessors used room temperature water as a palate cleanser and had a forced 30 seconds break after tasting each product. Assessors evaluated a maximum number of 6 products. The duration of the study was eight days.

6.2.8 Statistical Analysis

Data were collected electronically (online) using Compusense Cloud[®] SaaS (Compusense, Guelph, Ontario, Canada). ANOVA with interactions where the products and replicates were maintained as fixed factors and assessors as a random factor was performed. Tukey's HSD post hoc analysis ($\alpha = 0.05$) was performed to identify significant differences in the product means. Panel analysis was also conducted to determine panel performance based on their repeatability, discrimination, and interactions. Product characterisation using Principal

Component Analysis was also carried out to provide a 2-Dimensional multivariate map of the products in the sensory space. Non-discriminating descriptors were filtered out to reduce the noise in the PCA data presentation. All data analyses were carried out using XLSTAT 2018 (Addinsoft, New York, USA) for Windows pc.

6.3 Results and Discussion

6.3.1 Microbiological Safety of Bread Samples

The results for microbiological tests to ensure the products met the minimum thresholds for microbial quality for human consumption are summarized in table 6.1.

Table 6.1: Microbiological examination of bread samples (cfu/g)

Sample	Aerobic Plate	Yeast/ Mould	Coliform	Enterobacteriaceae
B11	140	20	<10	<10
B12	230	60	<10	<10
B13	110	<10	<10	<10
B14	180	<10	<10	<10
B15	90	<10	<10	<10
WTB	190	40	<10	<10

<10 = No microorganism isolated per that test. Values are means of triplicates. Ghana Standards Authority microbiological limits (GS 955:2018) for bread, biscuits, cake etc: APC = 1×10^4 , Yeast/moulds = 1×10^3 , Coliform = 1×10^2 , Enterobacteriaceae = 1×10^2

No coliforms and Enterobacteria were isolated in the bread samples. APC was higher in B12, followed by WTB and B11. B15 had the least APC count (Table 6.1). Yeast and mould count was also higher in B12 and WTB. The observed yeast counts may be because yeasts are widely distributed in nature, inhabit a wide range of habitats, and could easily

contaminate food (Aboaba and Obakpolor, 2010). The microbial population in all the bread samples were far below the acceptable limits recommended by the Ghana Standards Authority (GS 955:2018) and were therefore safe for human consumption.

6.3.2 Sensory Descriptors

Twenty-one (21) unique characteristics were generated to describe the sensory profile of the six bread types. They comprised four (4) appearance attributes, six (6) texture-in-hand attributes, three (3) aroma attributes, four (4) flavour attributes, and two (2) attributes each for mouthfeel and aftertaste. Some attributes applied to more (repeated) than one part of the bread (top crust, bottom crust, crumb); therefore, there were forty-eight (48) attributes altogether. Table 6.2 shows the attributes, definitions and anchors grouped for each modality according to the specific part of the bread (the top crust, bottom crust, and crumb of the bread).

Table 6.2: Sensory descriptors developed for six formulations of gluten-free bread made from Frafra Potato flour

Modality	Part of bread	Attribute	Definition	Anchor	Reference
Appearance	Top Crust	Brown colour	Shade of brown	Light - Dark	Skippy peanut butter (creamy)
		Dry	The product appears to lack moisture	Not - Very	
	Crumb	Dry	The product appears to lack moisture	Not - Very	Bottom crust of butter bread
		Brown colour	Shade of brown	Light - Dark	
	Bottom crust	Smooth	Having a fine surface	Not - Very	
		Homogeneous colour	Even colouration of the product	Not - Very	
		Dry	The product appears to lack	Not - Very	

	Doughy	moisture Having a rubbery, plastic feel when rubbed between the thumb and index finger	Not - Very	Bread dough
	Smooth	Having a fine feel when rubbed between thumb and index finger	Not - Very	Top crust of butter/sugar bread
	Soft	Easily compressed when rubbed between thumb and index finger	Not - Very	The top crust of butter bread/sugar bread
<i>Top crust</i>	Dry	Absence of moisture in the product when rubbed between thumb and index finger	Not - Very	
Texture-in-hand	Smooth	Having a fine feel when rubbed between thumb and index finger	Not - Very	
	Soft	Easily compressed when rubbed between thumb and index finger	Not - Very	
	Flaky	The product breaks into small fragments when rubbed between thumb and index finger	Not - Very	
	Springy	Product bounces back to its original form when compressed	Not - Very	
	Dry	Absence of moisture in product when rubbed between thumb and index finger	Not - Very	
<i>Crumb</i>	Doughy	Having a rubbery, plastic	Not - Very	

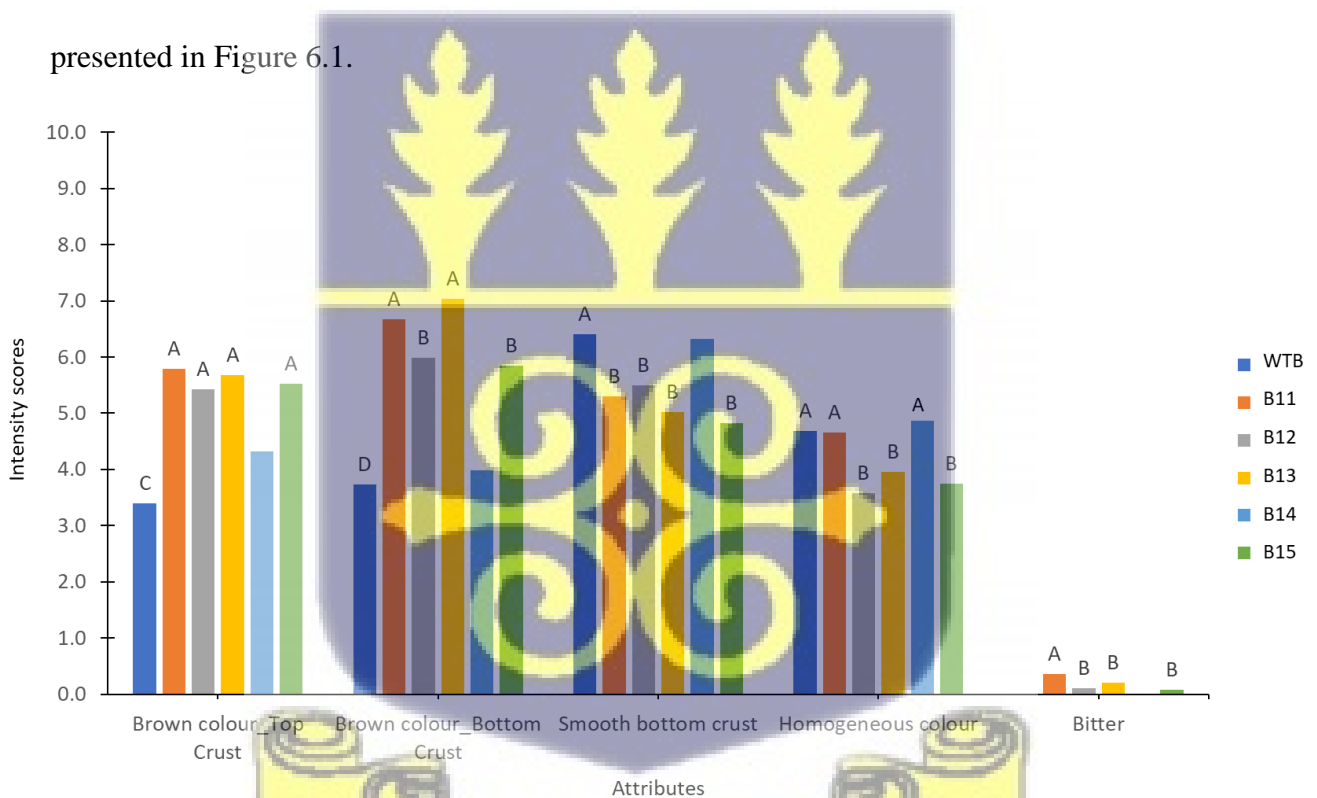
		feel when rubbed between the thumb and index finger	
	Smooth	Having a fine feel when rubbed between thumb and index finger	Not - Very
<i>Bottom crust</i>	Soft	Easily compressed when rubbed between thumb and index finger	Not - Very
	Dry	Absence of moisture in the product when rubbed between thumb and index finger	Not - Very
	Bread	Characteristic aroma like that of bread	Not - Very
<i>Top crust</i>	Nutmeg	Characteristic aroma like that of nutmeg	Not - Very
	Bread	Characteristic aroma like that of bread	Not - Very
	Nutmeg	Characteristic aroma like that of nutmeg	Not - Very
Aroma	Bread	Characteristic aroma like that of bread	Not - Very
	Nutmeg	Characteristic aroma like that of nutmeg	Not - Very
	Bread	Characteristic aroma like that of bread	Not - Very
<i>Bottom crust</i>	Nutmeg	Characteristic aroma like that of nutmeg	Not - Very
	Burnt note	Characteristic aroma like that of burnt bread	Not - Very
	Salty	Basic taste	Not - Very
	Sweet	Basic taste	Not - Very

Flavour	<i>Top crust</i>	Bread	Characteristic flavour like that of bread	Not - Very
	<i>Crumb</i>	Salty	Basic taste	Not - Very
		Sweet	Basic taste	Not - Very
		Bread	Characteristic flavour like that of bread	Not - Very
	<i>Bottom crust</i>	Salty	Basic taste	Not - Very
		Sweet	Basic taste	Not - Very
Bread		Characteristic flavour like that of bread	Not - Very	
Mouthfeel	<i>Top crust</i>	Bitter	Basic taste	Not - Very
		Chewy	Sticky feel in the mouth and teeth as the product is masticated	Not - Very
	<i>Crumb</i>	Soft	Easily compressed in the mouth	Not - Very
		Chewy	Sticky feel in the mouth and teeth as the product is masticated	Not - Very
		Soft	Easily compressed in the mouth	Not - Very
	<i>Bottom crust</i>	Soft	Easily compressed in the mouth	Not - Very
Aftertaste	<i>Top crust</i>	Salty	Basic taste	Not - Very
		Salivation	Production of saliva in the mouth after product is swallowed	Not - Very
		Bitter	Production of saliva in the mouth after product is swallowed	Not - Very
	<i>Crumb</i>	Salty	Basic taste	Not - Very
		Salivation	Production of saliva in the mouth after product is swallowed	Not - Very
		Bitter	Production of saliva in the mouth after product is swallowed	Not - Very
<i>Bottom</i>	Salty	Basic taste	Not - Very	
	Salivation	Production of saliva in the	Not - Very	

crust
mouth after
product is
swallowed

6.3.3 Differences in Bread for Discriminating Attributes

Of the 48 attributes used to describe all the six bread types, only five attributes significantly differentiated them ($p \leq 0.05$). These were four appearance attributes and one flavour attribute: brown top crust, brown bottom crust, smooth bottom crust, homogenous colour, and bitter after taste. There were no significant differences ($p \leq 0.05$) in aroma, flavour (except bitter), texture in hand, mouthfeel and aftertaste. The discriminating attributes are presented in Figure 6.1.



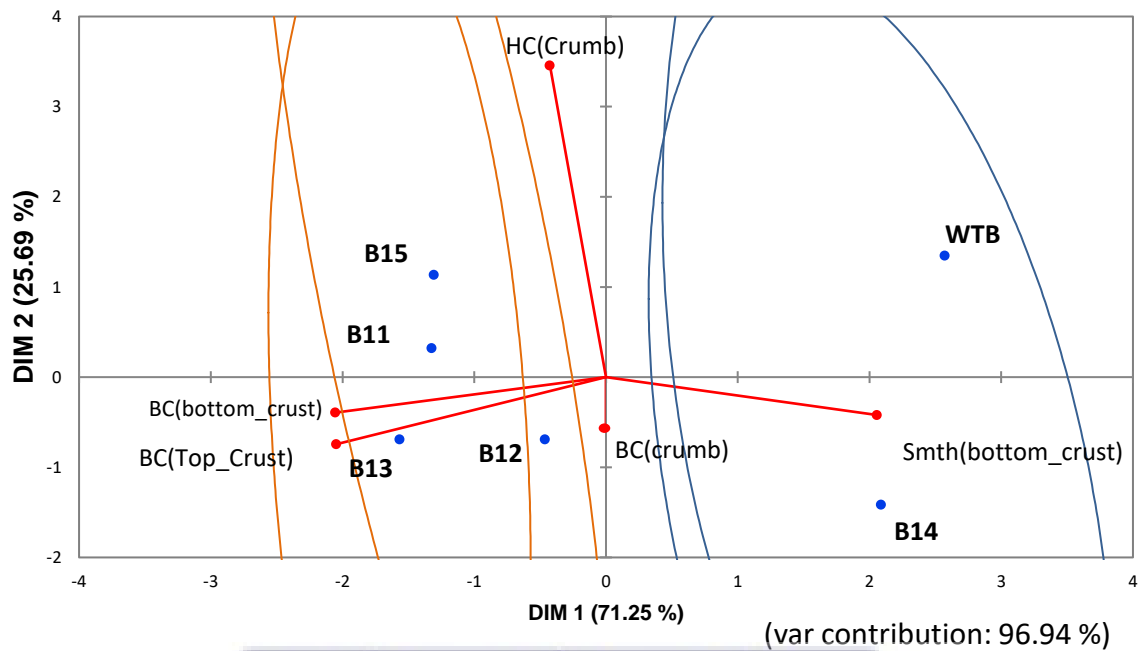
WTB = Wheat bread, B11 = Manga bread, B12 = Nutsugah bread, B13 = Naachem-Tiir, B14 = Maa-Lana bread, B15 = WAAPP bread. Bars, within attributes, “with different letters are significantly different ($p \leq 0.05$)”.

Figure 6.1: Bar chart showing differences between bread formulations for discriminating attributes

The top and bottom crusts of WTB and B14 had the lowest intensity (lightest) of brown colour in the sample set and did not differ statistically ($p \leq 0.05$) from each other (figure 6.1). The top crusts of products B11, B12, B13 and B15 had higher brown colour intensities and were not significantly different ($p \leq 0.05$) from one another. The brown colour of the bottom crust of B11 was not significantly different from that of products B12, B13 and B15. B13 had a significantly higher brown bottom crust colour than all the products except B11 ($p \leq 0.05$). The appearance of the bottom crusts of WTB, B14 was significantly smoother than B13 and B15. The smoothness of the bottom crusts of WTB and B14 were significantly different ($p \leq 0.05$) from the other bread types in the sample set. The homogeneity of the bottom crusts of all the products was generally low (between 3 and 4 out of 10). The homogeneous colour scores of B11, B14 and WTB were significantly higher than those of B12, B13 and B15. A bitter aftertaste was perceived in the FPB products (B11, B12, B13 and B15) except B14. However, this bitter aftertaste was very low (less than one on a 10cm line scale). The bitterness perceived in B11 was significantly more intense than the others ($p \leq 0.05$). The bitter aftertaste could be due to the relative concentrations of the components and compounds, such as hydroximetafurfurals, furfurals and mellanonidens, that are formed during the Maillard reaction that occurred during the baking process, which may cause some bitter aftertaste to be perceived in baked and toasted foods (Alencar *et al.*, 2017).

6.3.4 Product Map

The Principal Component Analysis (PCA) map generated displays the positioning of the six bread samples in the sensory space. Almost all the variance (96.94%) was explained in the first 2 dimensions (DIM1 = 71.25%), DIM2 = 25.69%) as shown in Figure 6.2.



WTB = Wheat bread, **B11** = Manga bread, **B12** = Nutsugah bread, **B13** = Naachem-Tiir, **B14** = Maa-Lana bread, **B15** = WAAPP bread.

Figure 6.2: PCA biplot display of the breads types in the sensory space

From figure 6.2, B12 and B13 were mainly characterised by brown colours of both the top and bottom crusts and appeared close to B11 and B15 (area of orange ellipses). B14 and WTB were mainly characterised by the smooth appearance of the bottom crusts (area of blue ellipses). The positioning of the six products is distinguished into two groupings, with B14 and WTB in one group and the rest in another group.

6.3.5 Correlation between Sensory profile and the physicochemical characteristics of FPF and Bread samples

Pearson's correlation was employed to ascertain how the physicochemical properties of FPF and bread significantly influenced the sensory profile of FPB. Table 6.4 shows the summary of correlation analysis.

Table 6.3: Correlation between Sensory profile and the physicochemical characteristics of FPF and Bread samples

Correlation Between	Coefficient (R ²)	P-value
Sensory profile and Colour parameters (FPF)	0.98	0.01
Sensory profile and Dough properties	0.95	0.01
Sensory profile and Instrumental texture (TPA)	0.83	0.03

FPF = Frafra potato flour, TPA = Texture profile analysis

Correlation analysis of the data obtained showed that sensory profile (QDA) correlated strongly with optical properties of FPF, dough characteristics and TPA (Table 6.3). This implies that the sensory profile of the bread was influenced strongly by these characteristics. This is supported by findings by Aguilar *et al.* (2015) and Alencar *et al.* (2017; 2015), where the sensory profile of gluten-free bread was strongly influenced by the parameters of texture, colour and dough properties. Hence, in developing gluten-free flours, colour parameters of the flours, dough characteristics, and TPA should be considered.

6.4 Conclusion

The sensory profiles of bread made from Frafra potato flour were determined using nine trained assessors. Only 5 out of the 48 attributes assessed significantly differentiated the bread samples. The formulations were differentiated in appearance by the brown colour of the top and bottom crusts, smooth bottom crust, the homogeneous colour of the bottom crust and a bitter flavour note perceived only in the bottom crust of Frafra potato bread. WTB and B14 (Maa-Lana Bread) had similar colour and texture attributes (for the top and bottom

crusts and smoother bottom crusts). B11 (Manga Moya Bread), B12 (Nutsugah Bread), B13 (Naachem-Tiir Bread) and B15 (WAAPP Bread) had a bitter note, albeit low intensity. The study has shown that the five released accessions of Frafar potato are desirable to continue further studies for a gluten-free bread with a similar sensory profile to typical bread. However, the Maa-Lana accession would be more desirable than the other four.

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CHAPTER SEVEN

7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 General Conclusion

The study showed that Frafra potato cultivars can be processed into fine flour and starch (with high yields and modest extractability respectively), and used as an ingredient for bakery products processing.

The starches from Frafra potato were characterised as A-type allomorphs and were typical of other root crops, with similar amylose/amylopectin ratio, degree of crystallinity, and granule morphology. However, the starches from the different Frafra potato cultivars showed different paste clarity, resistant starch, and thermal properties due to their differences in granule size distribution. PCA clustered them into two main groups based on these differences, with Nutsugah and Maa-Lana in one group and the rest in the other. The characteristics of FPS provide important information for useful applications of the starches and may also influence the advanced selection of accessions of interest for the development of suitable varieties for cooking quality and specific end use such as in baked foods, jellies, powder desserts, thickener.

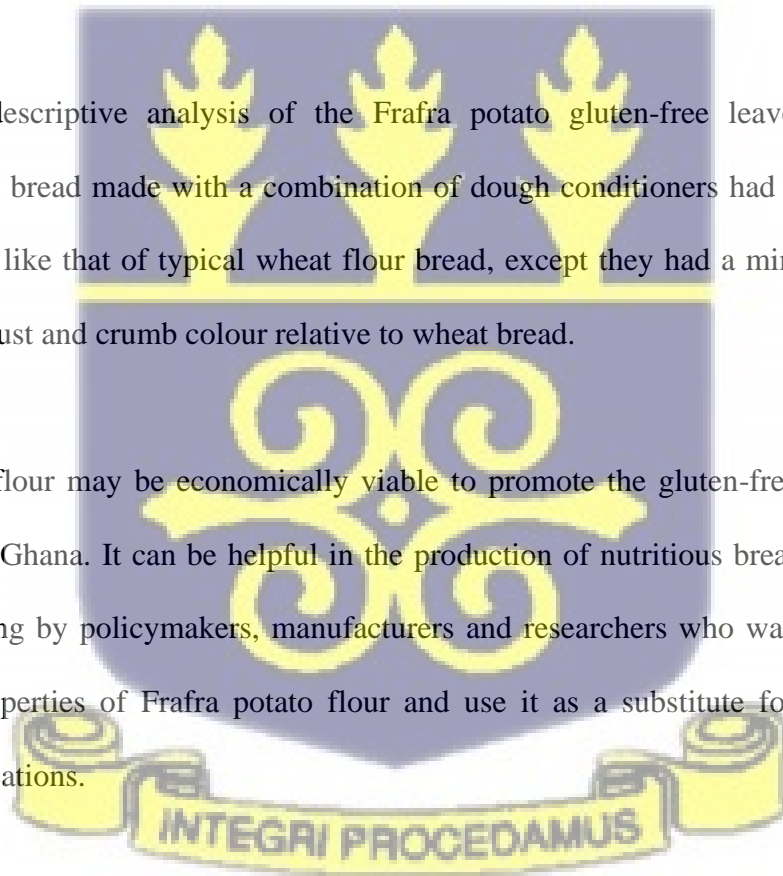
The released cultivars of Frafra potato had higher concentrations of protein and ash and had similar invitro mineral bioavailability. Frafra potato showed relatively higher levels of proteins, consisting mainly of four storage proteins, including a protease inhibitor. Among a profile of 19 amino acids determined for Frafra potato protein, 7 of them were essential amino acids. Moisture sorption behaviour revealed that the flours have a maximum allowable moisture content for safekeeping at 10% at about 0.5 *aw*. Moisture sorption isotherms using the Oswin and Smith models demonstrated that the flours become more

hygroscopic beyond 6.0 *aw* and require good moisture barrier packaging material for longer shelf life. FPF properties make the flours useful in food systems such as baked foods, fruit jellies, powder desserts, thickener, weaning blends, and gluten-free pasta.

The study demonstrated that Frafra potato flour has potential in the gluten-free leavened bread space when used with dough conditioners (gelatin with egg albumin powder combination) followed with transglutaminase, a protein cross-linking enzyme (transglutaminase) to mimic the gluten-like network for better handling, baking capacity and crumb structure.

Quantitative descriptive analysis of the Frafra potato gluten-free leavened bread also confirmed that bread made with a combination of dough conditioners had majority sensory characteristics like that of typical wheat flour bread, except they had a minor bitter flavour note, darker crust and crumb colour relative to wheat bread.

Frafra potato flour may be economically viable to promote the gluten-free leavened tuber flour bread in Ghana. It can be helpful in the production of nutritious bread and encourage decision making by policymakers, manufacturers and researchers who want to harness the nutritional properties of Frafra potato flour and use it as a substitute for wheat flour in culinary applications.



7.2 Recommendations

Based on the findings of the study, the following recommendations are suggested:

1. Breeding studies should investigate the feasibility of developing improved accessions of Frafra potato with higher starch yield, as this could make Frafra potatoes compete, with cassava, as an affordable alternative source of starch for industry players who depend heavily on high starch yielding crops such as the Ayensu Starch Factory in Accra, Ghana.
2. The effect of different moisture barrier packaging on the short- and long-term storage of Frafra potato flours should be investigated to determine the best packaging for the longer keeping of the products under different storage conditions.
3. This study made gluten-free Frafra potato bread close to wheat bread; however, there were significant differences in the dough, baking and texture profile characteristics, which may be addressed through further optimization studies of gluten-free leavened bread formulation and processing conditions. This may promote the use of Frafra potato flours in the production of gluten-free bread, especially in the communities where the tubers are cultivated.
4. To control the high cost of wheat flour importation and promote Frafra potato flours local manufacturing in Ghana, a small-scale tuber flour processing plant could be built at the Savana Agriculture Research Institute (SARI) in the Upper East Region of Ghana. Comparable tuber flour producing plants can be built in all the northern regions, especially at Frafra potato growing communities, as one of the Government's industrialization programs of providing manufacturing factories in each district in Ghana. This will reinforce the efforts of several organisations in Ghana (SARI and West Africa Agricultural Productivity Project, WAAPP) to

promote the utilisation of Frafra potatoes while contributing to food and nutrition security. This can also help fight micronutrients and vitamin A deficiency, especially in areas where the crop is cultivated. Before commencing a full-scale production, FP flour processing standardisation would be conducted in a pilot-scale production unit to establish optimized conditions for upscaling the production.



APPENDICES

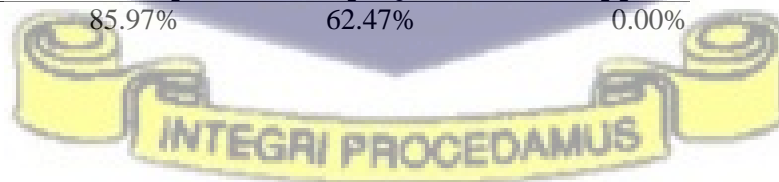
Appendix I: Anova and Comparisons Using Tukey's Hsd

Analysis of Variance – AP-Brown colour (top crust)

Source	DF	Adj SS	Adj MS	F- Value	P- Value
Sample_Name	5	82.36	16.47	14.47	0.000
Panelist_Code	8	111.2	13.91	12.21	0.000
Session_Name	1	0.171	0.171	0.15	0.700
Sample_Name*Panelist_Code	40	72.96	1.824	1.60	0.070
Sample_Name*Session_Name	5	6.376	1.275	1.12	0.366
Panelist_Code*Session_Name	8	5.963	0.745	0.65	0.727
Error	40	45.55	1.138		
Total	107	324.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.06718	85.97%	62.47%	0.00%



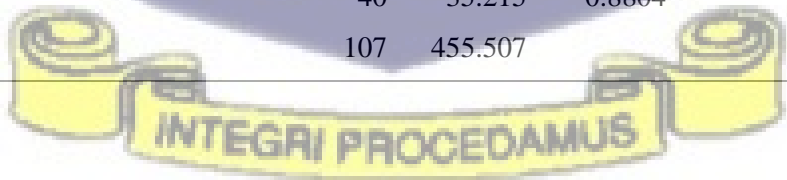
Grouping Information Using the Tukey Method and 95% Confidence

Sample_Na	N	Mean	Grouping
MMYAB	18	5.7833	A
		3	
NKIMB	18	5.6833	A
		3	
WAPPB	18	5.5277	A
		8	
NGAHB	18	5.4277	A
		8	
NLNAB	18	4.3222	B
		2	
CWHTB	18	3.3944	B
		4	

Means that do not share a letter are significantly different.

Analysis of Variance – AP-Brown colour (bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	171.378	34.2756	38.93	0.000
Panelist_Code	8	116.460	14.5575	16.54	0.000
Session_Name	1	0.037	0.0370	0.04	0.839
Sample_Name*Panelist_Code	40	116.709	2.9177	3.31	0.000
Sample_Name*Session_Name	5	1.459	0.2917	0.33	0.891
Panelist_Code*Session_Name	8	14.250	1.7812	2.02	0.068
Error	40	35.215	0.8804		
Total	107	455.507			



Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.938281	92.27%	79.32%	43.64%

Grouping Information Using the Tukey Method and 95% Confidence

Sample_Name	N	Mean	Grouping
NKIMB	18	7.03889	A
MMYAB	18	6.67778	A B
NGAHB	18	5.98333	B
WAPPB	18	5.85000	B
NLNAB	18	3.98333	C
CWHTB	18	3.73333	C

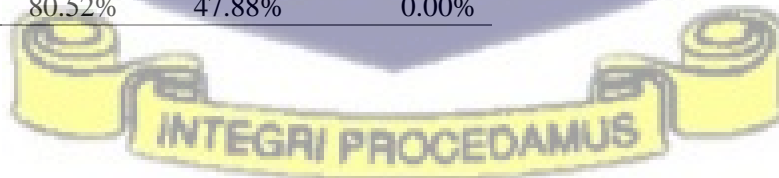
Means that do not share a letter are significantly different.

Analysis of Variance – AP- Smooth (bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	39.379	7.8759	4.88	0.001
Panelist_Code	8	93.648	11.7060	7.25	0.000
Session_Name	1	0.907	0.9075	0.56	0.458
Sample_Name*Panelist_Code	40	95.076	2.3769	1.47	0.113
Sample_Name*Session_Name	5	3.436	0.6873	0.43	0.828
Panelist_Code*Session_Name	8	34.445	4.3056	2.67	0.019
Error	40	64.586	1.6147		
Total	107	331.479			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.27069	80.52%	47.88%	0.00%



Grouping Information Using the Tukey Method and 95% Confidence

Sample_Name	N	Mean	Grouping
CWHTB	18	6.40000	A
NLNAB	18	6.32222	A
NGAHB	18	5.48889	A B
MMYAB	18	5.30556	A B
NKIMB	18	5.01667	B
WAPPB	18	4.82778	B

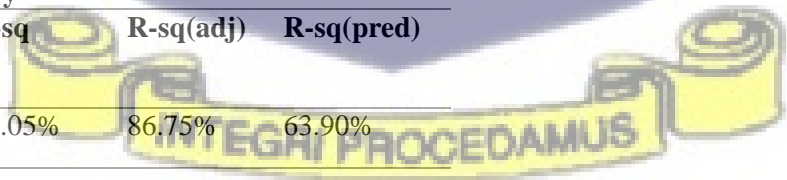
Means that do not share a letter are significantly different.

Analysis of Variance – AP-Homogeneous (bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	27.43	5.487	3.50	0.010
Panelist_Code	8	1093.71	136.713	87.19	0.000
Session_Name	1	0.94	0.945	0.60	0.442
Sample_Name*Panelist_Code	40	66.20	1.655	1.06	0.433
Sample_Name*Session_Name	5	3.96	0.792	0.51	0.771
Panelist_Code*Session_Name	8	11.62	1.453	0.93	0.505
Error	40	62.72	1.568		
Total	107	1266.59			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.25217	95.05%	86.75%	63.90%



Grouping Information Using the Tukey Method and 95% Confidence

Sample_Name	N	Mean	Grouping	
NLNAB	18	4.86111	A	
CWHTB	18	4.68333	A	B
MMYAB	18	4.65000	A	B
NKIMB	18	3.95000	A	B
WAPPB	18	3.73889	A	B
NGAHB	18	3.57778	B	

Means that do not share a letter are significantly different.

Analysis of Variance – Tx-Doughy(top crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.422	0.0844	0.51	0.770
Panelist_Code	8	381.347	47.6683	285.66	0.000
Session_Name	1	0.889	0.8893	5.33	0.026
Sample_Name*Panelist_Code	40	11.618	0.2904	1.74	0.042
Sample_Name*Session_Name	5	2.262	0.4524	2.71	0.034
Panelist_Code*Session_Name	8	3.174	0.3968	2.38	0.034
Error	40	6.675	0.1669		
Total	107	406.387			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.408498	98.36%	95.61%	88.03%



Analysis of Variance - Tx-Doughy(bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	1.590	0.3179	0.95	0.460
Panelist_Code	8	350.909	43.8636	131.07	0.000
Session_Name	1	4.006	4.0059	11.97	0.001
Sample_Name*Panelist_Code	40	20.032	0.5008	1.50	0.103
Sample_Name*Session_Name	5	3.149	0.6297	1.88	0.119
Panelist_Code*Session_Name	8	2.999	0.3749	1.12	0.371
Error	40	13.386	0.3347		
Total	107	396.071			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.578500	96.62%	90.96%	75.36%

Analysis of Variance – TX-Smooth (top crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	1.154	0.2308	0.37	0.864
Panelist_Code	8	197.879	24.7349	40.07	0.000
Session_Name	1	2.139	2.1393	3.47	0.070
Sample_Name*Panelist_Code	40	19.888	0.4972	0.81	0.752
Sample_Name*Session_Name	5	2.029	0.4057	0.66	0.658
Panelist_Code*Session_Name	8	6.549	0.8186	1.33	0.259
Error	40	24.693	0.6173		
Total	107	254.331			

Model Summary

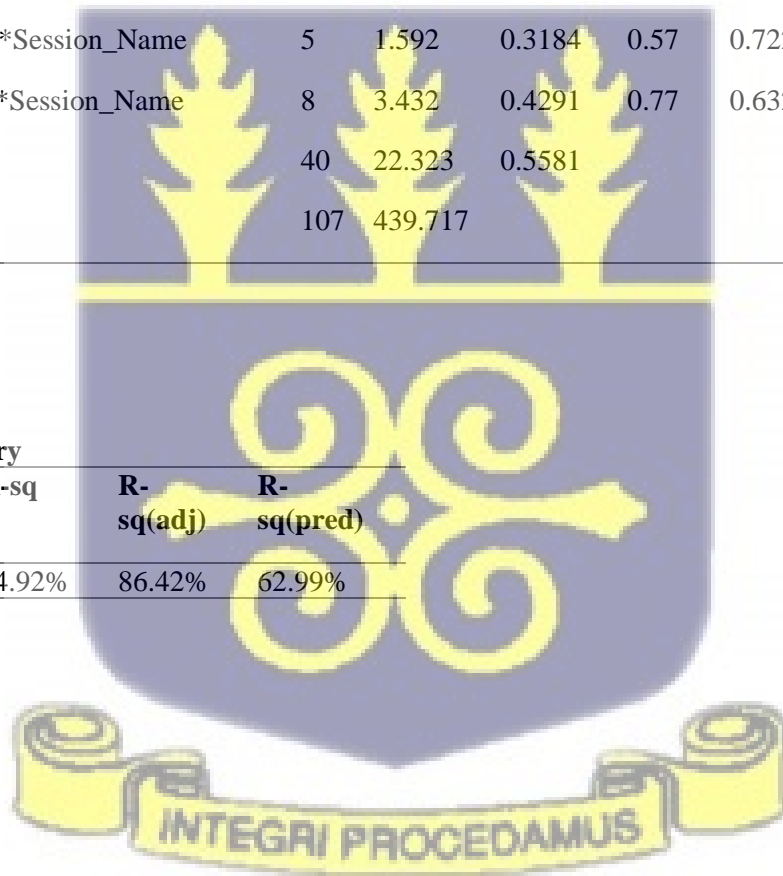
S	R-sq	R-sq(adj)	R-sq(pred)
0.785703	90.29%	74.03%	29.22%

Analysis of Variance – TX-Smooth (crumbs)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	3.629	0.7258	1.30	0.283
Panelist_Code	8	386.408	48.3010	86.55	0.000
Session_Name	1	0.063	0.0626	0.11	0.739
Sample_Name*Panelist_Code	40	22.269	0.5567	1.00	0.503
Sample_Name*Session_Name	5	1.592	0.3184	0.57	0.722
Panelist_Code*Session_Name	8	3.432	0.4291	0.77	0.632
Error	40	22.323	0.5581		
Total	107	439.717			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.747047	94.92%	86.42%	62.99%



Analysis of Variance – TX-Smooth (bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	3.042	0.6083	0.82	0.545
Panelist_Code	8	232.177	29.0222	38.93	0.000
Session_Name	1	0.733	0.7334	0.98	0.327
Sample_Name*Panelist_Code	40	24.649	0.6162	0.83	0.725
Sample_Name*Session_Name	5	1.188	0.2376	0.32	0.899
Panelist_Code*Session_Name	8	16.994	2.1243	2.85	0.013
Error	40	29.819	0.7455		
Total	107	308.603			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.863413	90.34%	74.15%	29.56%

Analysis of Variance – TX- Soft (top crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.480	0.0960	0.53	0.750
Panelist_Code	8	151.200	18.9000	104.98	0.000
Session_Name	1	0.196	0.1959	1.09	0.303
Sample_Name*Panelist_Code	40	5.907	0.1477	0.82	0.733
Sample_Name*Session_Name	5	0.522	0.1044	0.58	0.715
Panelist_Code*Session_Name	8	4.181	0.5226	2.90	0.012
Error	40	7.201	0.1800		
Total	107	169.687			

Model Summary

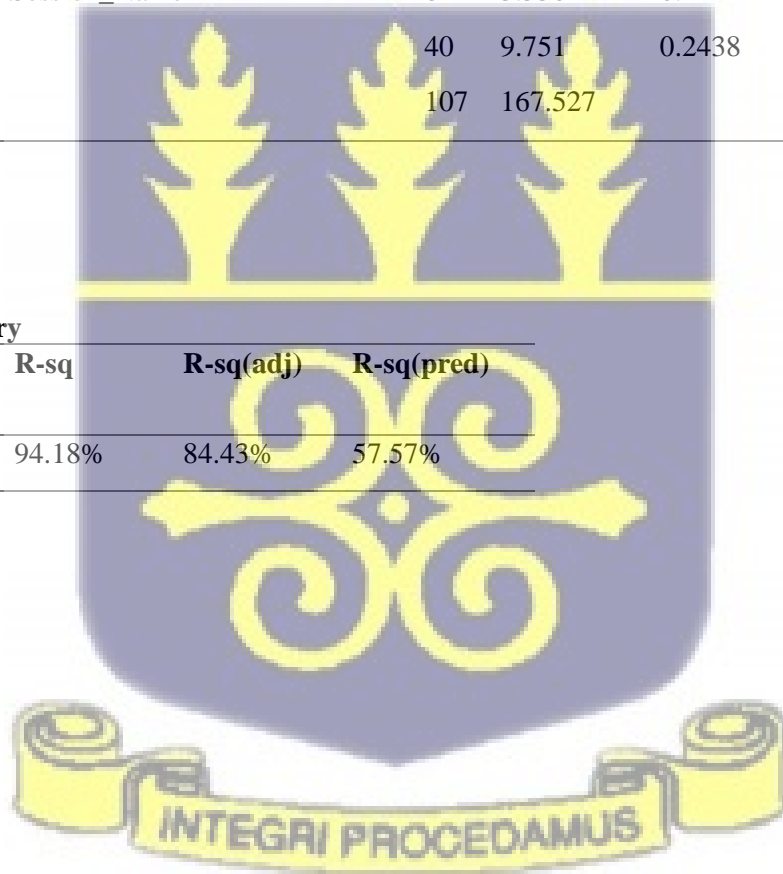
S	R-sq	R-sq(adj)	R-sq(pred)
0.424308	95.76%	88.65%	69.06%

Analysis of Variance – TX- Soft (crumbs)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.660	0.1319	0.54	0.744
Panelist_Code	8	136.004	17.0005	69.74	0.000
Session_Name	1	0.120	0.1200	0.49	0.487
Sample_Name*Panelist_Code	40	15.584	0.3896	1.60	0.071
Sample_Name*Session_Name	5	1.879	0.3758	1.54	0.199
Panelist_Code*Session_Name	8	3.530	0.4412	1.81	0.104
Error	40	9.751	0.2438		
Total	107	167.527			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.493739	94.18%	84.43%	57.57%



Analysis of Variance – TX- Soft (bottom crust)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.848	0.1696	0.38	0.857
Panelist_Code	8	188.443	23.5554	53.34	0.000
Session_Name	1	1.120	1.1204	2.54	0.119
Sample_Name*Panelist_Code	40	16.746	0.4186	0.95	0.567
Sample_Name*Session_Name	5	2.732	0.5464	1.24	0.310
Panelist_Code*Session_Name	8	13.533	1.6916	3.83	0.002
Error	40	17.665	0.4416		
Total	107	241.087			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.664545	92.67%	80.40%	46.58%

Analysis of Variance – TX- Flaky (crumbs)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.175	0.0350	0.22	0.953
Panelist_Code	8	199.076	24.8845	154.99	0.000
Session_Name	1	0.250	0.2504	1.56	0.219
Sample_Name*Panelist_Code	40	4.423	0.1106	0.69	0.879
Sample_Name*Session_Name	5	0.273	0.0546	0.34	0.886
Panelist_Code*Session_Name	8	1.075	0.1343	0.84	0.576
Error	40	6.422	0.1606		
Total	107	211.694			

Model Summary

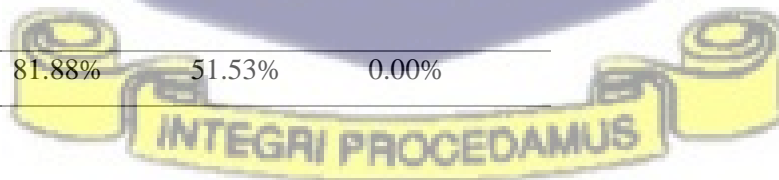
S	R-sq	R-sq(adj)	R-sq(pred)
0.400688	96.97%	91.89%	77.88%

Analysis of Variance – Tx-Springy (crumbs)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	1.6341	0.3268	1.79	0.137
Panelist_Code	8	19.7613	2.4702	13.55	0.000
Session_Name	1	0.2315	0.2315	1.27	0.267
	40	7.8943	0.1974	1.08	0.402
Sample_Name*Panelist_Code	5	1.1141	0.2228	1.22	0.317
Sample_Name*Session_Name	8	2.3302	0.2913	1.60	0.156
Panelist_Code*Session_Name					
Error	40	7.2943	0.1824		
Total	107	40.2596			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.427032	81.88%	51.53%	0.00%



Analysis of Variance – TX-Dry

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	5.375	1.075	0.82	0.543
Panelist_Code	8	357.656	44.707	34.09	0.000
Session_Name	1	5.027	5.027	3.83	0.057
Sample_Name*Panelist_Code	40	51.189	1.280	0.98	0.531
Sample_Name*Session_Name	5	6.746	1.349	1.03	0.414
Panelist_Code*Session_Name	8	24.614	3.077	2.35	0.036
Error	40	52.458	1.311		
Total	107	503.065			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.14519	89.57%	72.11%	23.98%

Analysis of Variance – AR-Bread

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.0163	0.00326	0.43	0.823
Panelist_Code	8	69.7580	8.71975	1156.21	0.000
Session_Name	1	0.0033	0.00333	0.44	0.510
Sample_Name*Panelist_Code	40	0.1220	0.00305	0.40	0.997
Sample_Name*Session_Name	5	0.0367	0.00733	0.97	0.446
Panelist_Code*Session_Name	8	0.0383	0.00479	0.64	0.743
Error	40	0.3017	0.00754		
Total	107	70.2763			

Model Summary

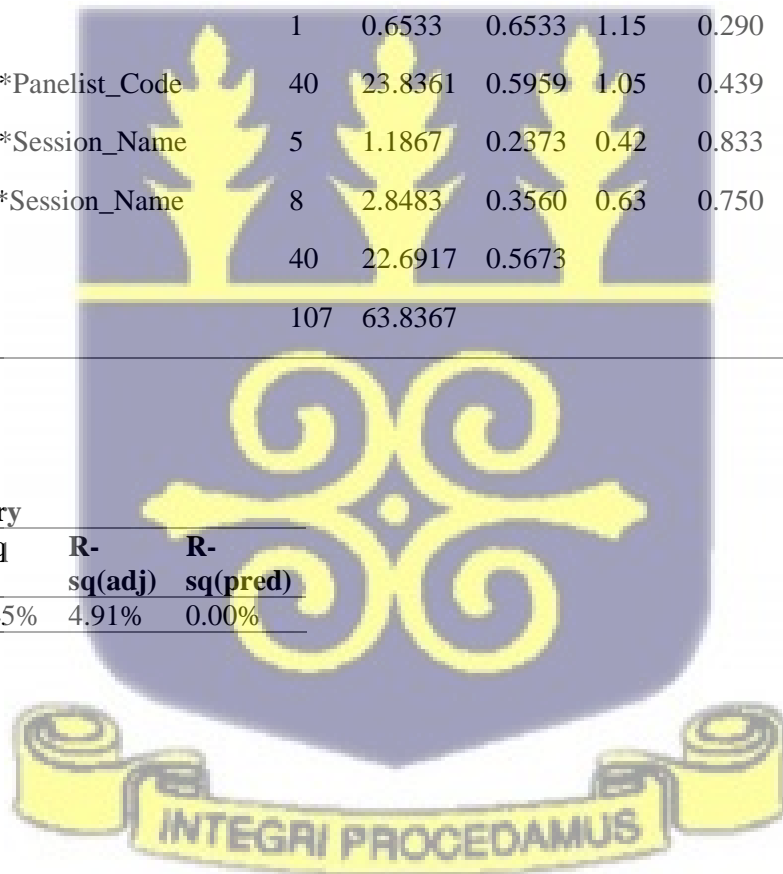
S	R-sq	R-sq(adj)	R-sq(pred)
0.0868428	99.57%	98.85%	96.87%

Analysis of Variance – AR-Nutmeg

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	3.6789	0.7358	1.30	0.285
Panelist_Code	8	8.9417	1.1177	1.97	0.076
Session_Name	1	0.6533	0.6533	1.15	0.290
Sample_Name*Panelist_Code	40	23.8361	0.5959	1.05	0.439
Sample_Name*Session_Name	5	1.1867	0.2373	0.42	0.833
Panelist_Code*Session_Name	8	2.8483	0.3560	0.63	0.750
Error	40	22.6917	0.5673		
Total	107	63.8367			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.753188	64.45%	4.91%	0.00%



Analysis of Variance – AR-Burnt (bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	2.0438	0.4088	2.29	0.063
Panelist_Code	8	2.6074	0.3259	1.83	0.100
Session_Name	1	0.3675	0.3675	2.06	0.159
Sample_Name*Panelist_Code	40	11.0070	0.2752	1.54	0.087
Sample_Name*Session_Name	5	1.6364	0.3273	1.84	0.128
Panelist_Code*Session_Name	8	1.1133	0.1392	0.78	0.622
Error	40	7.1278	0.1782		
Total	107	25.9032			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.422131	72.48%	26.39%	0.00%

Analysis of Variance – FL-Bread

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.0574	0.01148	0.60	0.700
Panelist_Code	8	62.1919	7.77398	405.99	0.080
Session_Name	1	0.0181	0.01815	0.95	0.336
Sample_Name*Panelist_Code	40	0.5059	0.01265	0.66	0.903
Sample_Name*Session_Name	5	0.1007	0.02015	1.05	0.401
Panelist_Code*Session_Name	8	0.1452	0.01815	0.95	0.489
Error	40	0.7659	0.01915		

Total	107	63.7852
-------	-----	---------

Model Summary

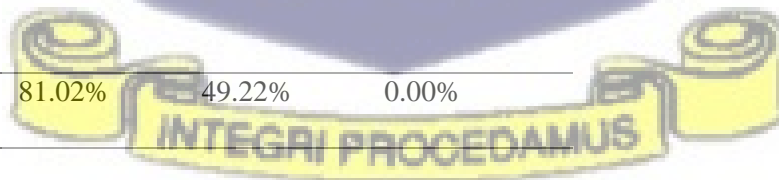
S	R-sq	R-sq(adj)	R-sq(pred)
0.138377	98.80%	96.79%	91.25%

Analysis of Variance – FL-Salty

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	2.276	0.4553	1.25	0.305
Panelist_Code	8	32.879	4.1099	11.28	0.100
Session_Name	1	2.613	2.6133	7.17	0.111
Sample_Name*Panelist_Code	40	11.445	0.2861	0.79	0.776
Sample_Name*Session_Name	5	3.109	0.6218	1.71	0.155
Panelist_Code*Session_Name	8	9.882	1.2352	3.39	0.105
Error	40	14.576	0.3644		
Total	107	76.781			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.603658	81.02%	49.22%	0.00%



Analysis of Variance – FL-Sweet

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.823	0.1647	2.33	0.060
Panelist_Code	8	117.362	14.6702	208.02	0.080
Session_Name	1	0.107	0.1070	1.52	0.225
Sample_Name*Panelist_Code	40	2.885	0.0721	1.02	0.472
Sample_Name*Session_Name	5	0.357	0.0715	1.01	0.422
Panelist_Code*Session_Name	8	1.735	0.2168	3.07	0.009
Error	40	2.821	0.0705		
Total	107	126.090			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.265562	97.76%	94.02%	83.69%

Analysis of Variance – FL-Bitter(bottom crust)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	1.6707	0.33415	2.76	0.031
Panelist_Code	8	1.4441	0.18051	1.49	0.191
Session_Name	1	0.1481	0.14815	1.22	0.275
Sample_Name*Panelist_Code	40	4.4026	0.11006	0.91	0.618
Sample_Name*Session_Name	5	2.2619	0.45237	3.73	0.007
Panelist_Code*Session_Name	8	0.7752	0.09690	0.80	0.606

Error	40	4.8448	0.12112
Total	107	15.5474	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.348024	68.84%	16.64%	0.00%

Grouping Information Using the Tukey Method and 95% Confidence

Sample_Name	N	Mean	Grouping
MMYAB	18	0.355556	A
NKIMB	18	0.205556	A B
NGAHB	18	0.111111	A B
WAPPB	18	0.083333	A B
NLNAB	18	-	B
		0.000000	
CWHTB	18	-	B
		0.000000	

Means that do not share a letter are significantly different.

Analysis of Variance – MF-Chewy

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	3.200	0.6399	1.06	0.396
Panelist_Code	8	317.293	39.6617	65.74	0.000
Session_Name	1	65.178	65.1779	108.03	0.000
Sample_Name*Panelist_Code	40	18.621	0.4655	0.77	0.792
Sample_Name*Session_Name	5	2.234	0.4468	0.74	0.598
Panelist_Code*Session_Name	8	152.910	19.1137	31.68	0.000
Error	40	24.134	0.6033		
Total	107	583.569			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
---	------	-----------	------------

0.776751 95.86% 88.94% 69.85%

Analysis of Variance – MF-Soft

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	3.876	0.7752	0.68	0.642
Panelist_Code	8	137.289	17.1611	15.03	0.000
Session_Name	1	5.558	5.5579	4.87	0.033
	40	51.658	1.2915	1.13	0.349
Sample_Name*Panelist_Code	5	5.254	1.0508	0.92	0.478
Sample_Name*Session_Name	8	20.270	2.5337	2.22	0.046
Panelist_Code*Session_Name					
Error	40	45.664	1.1416		
Total	107	269.568			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.06845	83.06%	54.69%	0.00%

Analysis of Variance – AF-Salty

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.8930	0.17859	1.29	0.286
Panelist_Code	8	70.2207	8.77759	63.55	0.000
Session_Name	1	0.2700	0.27000	1.95	0.170
Sample_Name*Panelist_Code	40	2.9004	0.07251	0.53	0.978
Sample_Name*Session_Name	5	0.3256	0.06511	0.47	0.795
Panelist_Code*Session_Name	8	0.8300	0.10375	0.75	0.647
Error	40	5.5244	0.13811		
Total	107	80.9641			

Model Summary

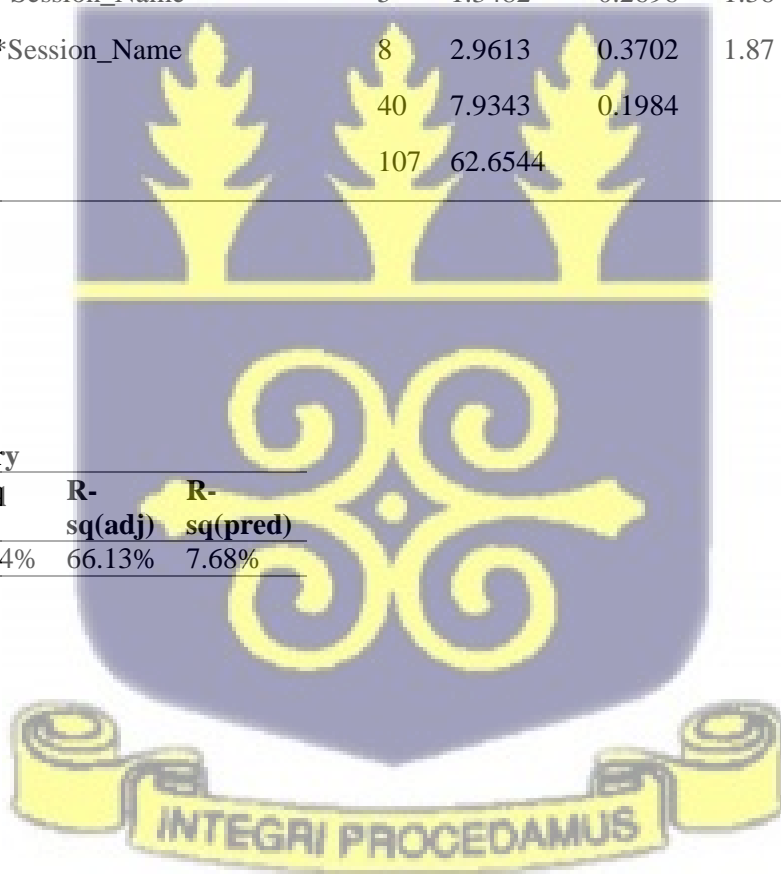
S	R-sq	R-sq(adj)	R-sq(pred)
0.371633	93.18%	81.75%	50.26%

Analysis of Variance – AF-Salivation

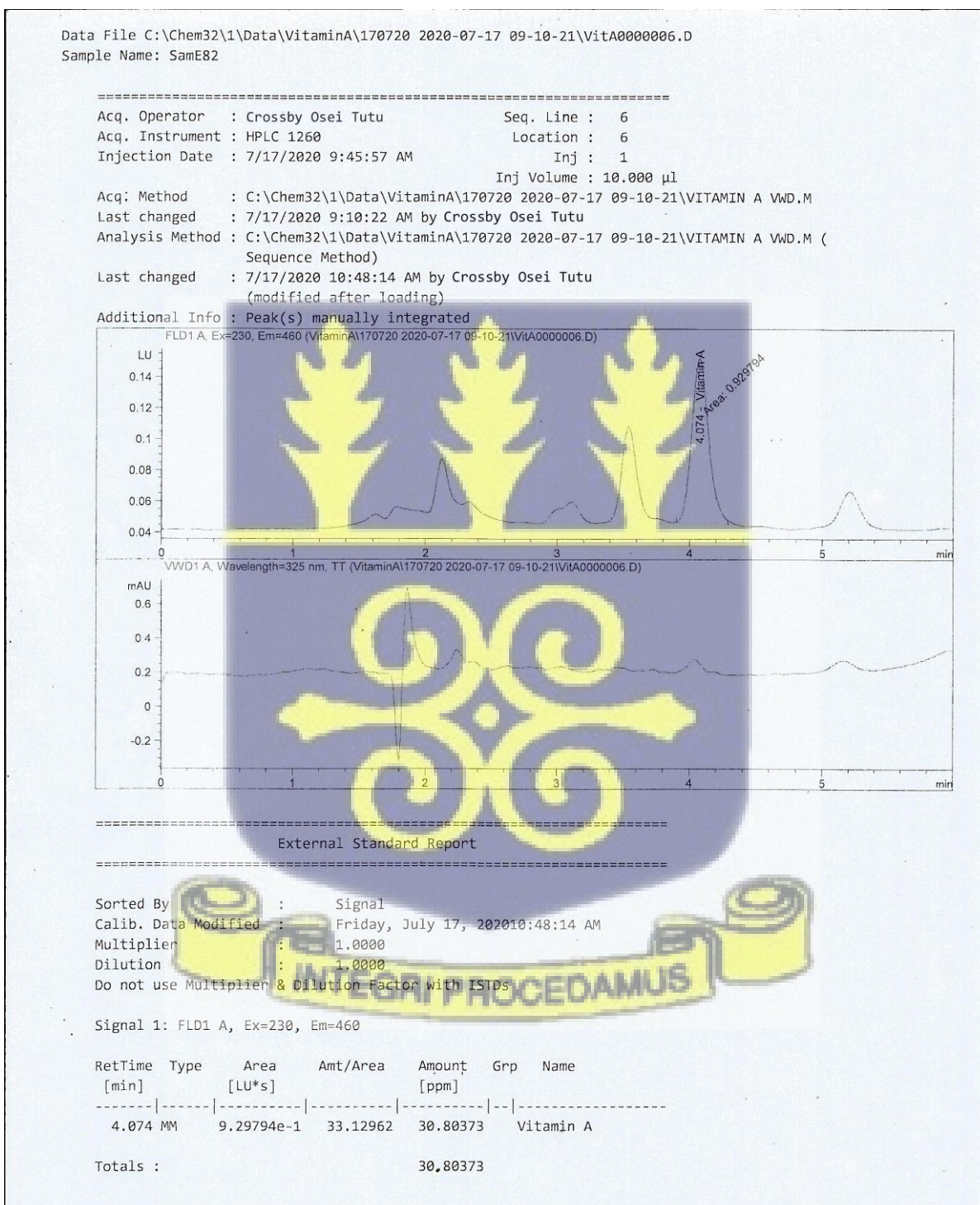
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample_Name	5	0.9460	0.1892	0.95	0.457
Panelist_Code	8	40.3635	5.0454	25.44	0.000
Session_Name	1	0.1712	0.1712	0.86	0.358
Sample_Name*Panelist_Code	40	8.9298	0.2232	1.13	0.355
Sample_Name*Session_Name	5	1.3482	0.2696	1.36	0.260
Panelist_Code*Session_Name	8	2.9613	0.3702	1.87	0.093
Error	40	7.9343	0.1984		
Total	107	62.6544			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.445372	87.34%	66.13%	7.68%



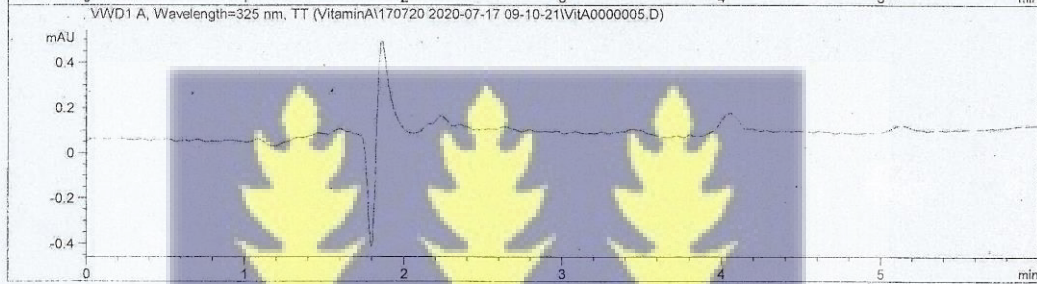
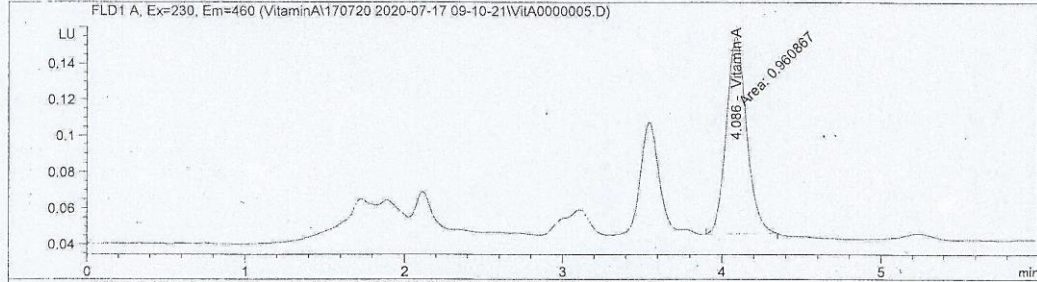
Appendix II: Vitamin A Analysis using HPLC



Data File C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VitA0000005.D
 Sample Name: SamE11

```

=====
Acq. Operator   : Crosby Osei Tutu           Seq. Line :    5
Acq. Instrument : HPLC 1260                  Location  :    5
Injection Date  : 7/17/2020 9:39:02 AM      Inj       :    1
                                           Inj Volume: 10.000 µl
Acq. Method    : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M
Last changed   : 7/17/2020 9:10:22 AM by Crosby Osei Tutu
Analysis Method: C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M (
                Sequence Method)
Last changed   : 7/17/2020 10:48:14 AM by Crosby Osei Tutu
                (modified after loading)
Additional Info : Peak(s) manually integrated
    
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External Standard Report

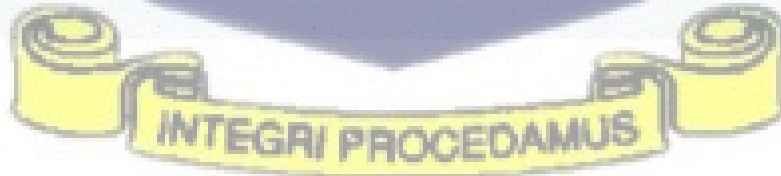
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Calib. Data Modified : Friday, July 17, 2020 10:48:14 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
4.086	MM	9.60867e-1	33.11265	31.81683		Vitamin A

Totals : 31.81683

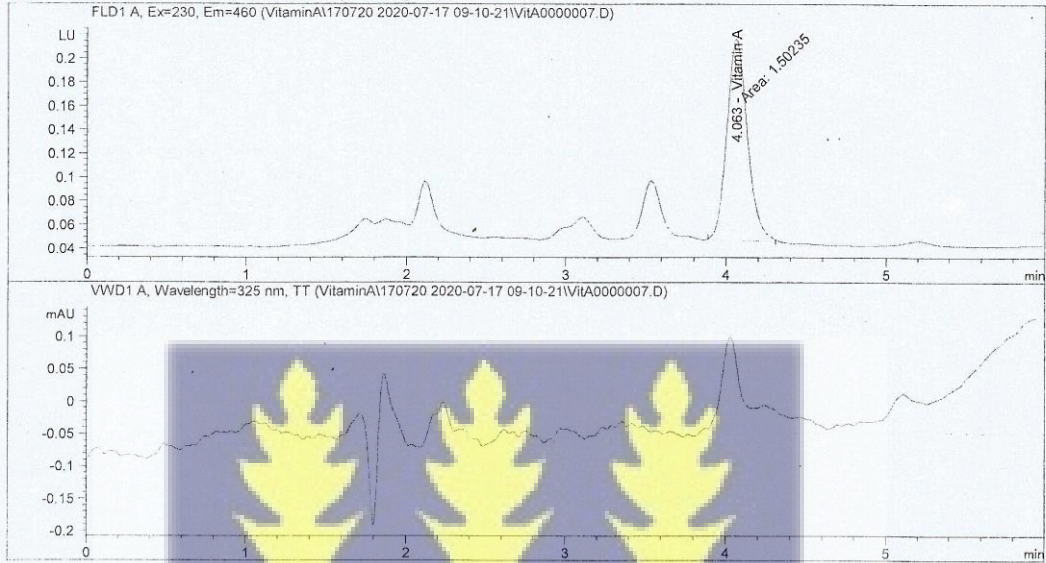


Data File C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VitA0000007.D
 Sample Name: SamE132

```

=====
Acq. Operator   : Crossby Osei Tutu           Seq. Line :    7
Acq. Instrument : HPLC 1260                 Location  :    7
Injection Date  : 7/17/2020 9:52:55 AM      Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M
Last changed    : 7/17/2020 9:10:22 AM by Crossby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M (
                  Sequence Method)
Last changed    : 7/17/2020 10:48:14 AM by Crossby Osei Tutu
                  (modified after loading)
Additional Info  : Peak(s) manually integrated
    
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External Standard Report

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Sorted By      : Signal
Calib. Data Modified : Friday, July 17, 2020 10:48:14 AM
Multiplier    : 1.0000
Dilution      : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
4.063	MM	1.50235	32.92957	49.47182		Vitamin A

Totals : 49.47182



Data File C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VitA0000008.D
 Sample Name: SamE134

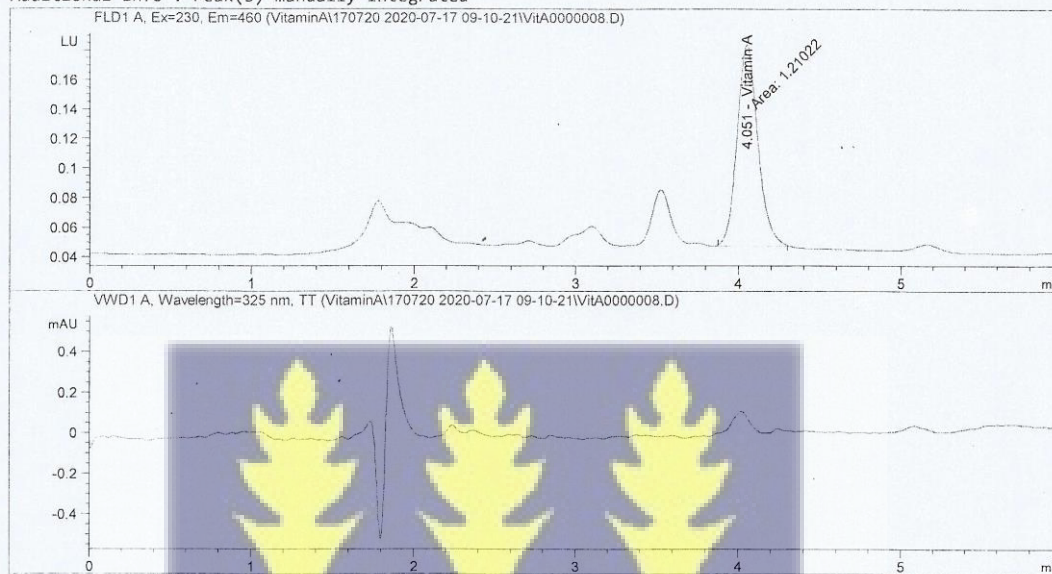
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Acq. Operator   : Crosby Osei Tutu           Seq. Line :    8
Acq. Instrument : HPLC 1260                  Location  :    8
Injection Date  : 7/17/2020 9:59:54 AM      Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M
Last changed    : 7/17/2020 9:10:22 AM by Crosby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M (
                  Sequence Method)

Last changed    : 7/17/2020 10:48:14 AM by Crosby Osei Tutu
                  (modified after loading)

Additional Info : Peak(s) manually integrated
    
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External Standard Report

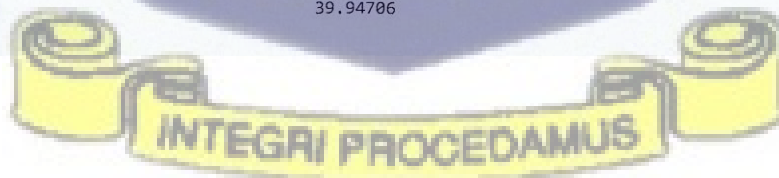
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Calib. Data Modified : Friday, July 17, 2020 10:48:14 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
4.051	MM	1.21022	33.00799	39.94706		Vitamin A

Totals : 39.94706

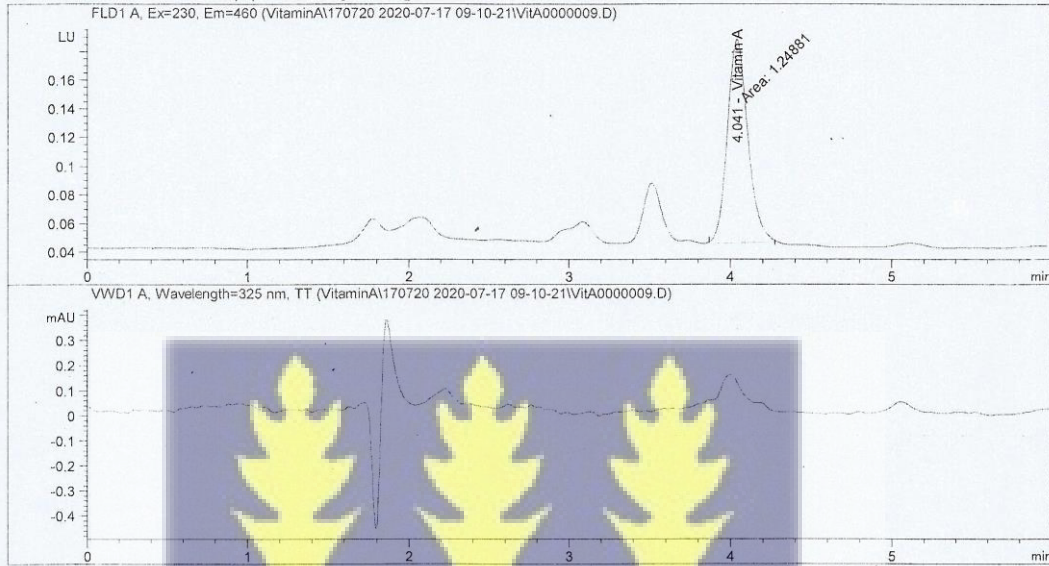


Data File C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VitA0000009.D
 Sample Name: SamE145

```

=====
Acq. Operator   : Crossby Osei Tutu           Seq. Line :    9
Acq. Instrument : HPLC 1260                  Location  :    9
Injection Date  : 7/17/2020 10:06:51 AM      Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M
Last changed    : 7/17/2020 9:10:22 AM by Crossby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M (
                    Sequence Method)
Last changed    : 7/17/2020 10:48:14 AM by Crossby Osei Tutu
                    (modified after loading)
Additional Info  : Peak(s) manually integrated
    
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External Standard Report

```

=====
Sorted By       : Signal
Calib. Data Modified : Friday, July 17, 2020 10:48:14 AM
Multiplier      : 1.0000
Dilution        : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
4.041	MM	1.24881	32.99553	41.20516		Vitamin A

Totals : 41.20516

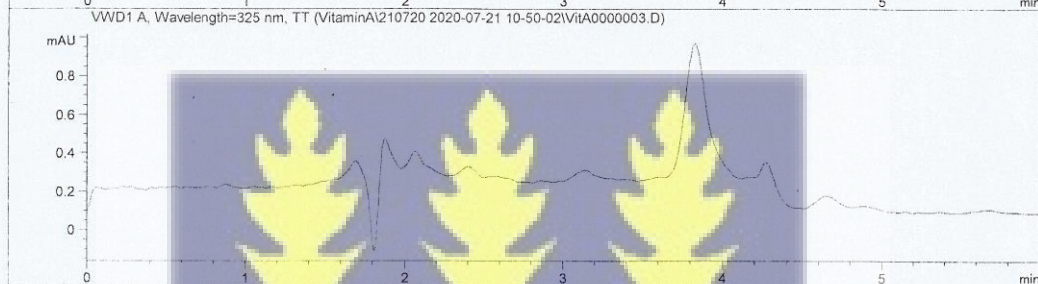
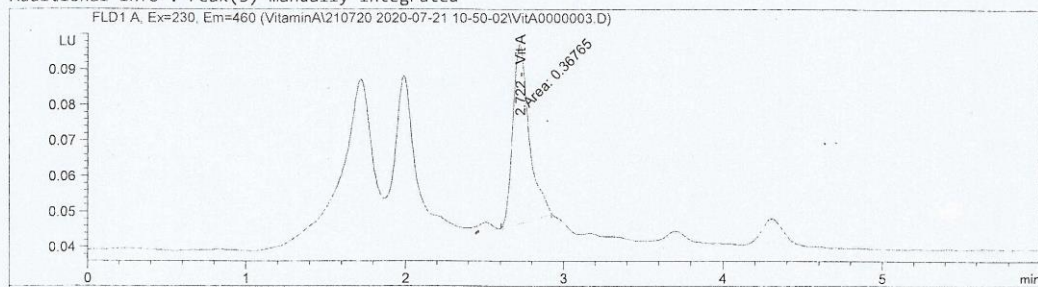


Data File C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VitA0000003.D
 Sample Name: Sam MM

```

=====
Acq. Operator   : Crosby Osei Tutu           Seq. Line :    3
Acq. Instrument : HPLC 1260                 Location  :    3
Injection Date  : 7/21/2020 11:04:50 AM     Inj       :    1
                                           Inj Volume: 10.000 µl
Acq. Method    : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M
Last changed   : 7/21/2020 10:50:03 AM by Crosby Osei Tutu
Analysis Method: C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M (
                Sequence Method)
Last changed   : 7/21/2020 11:58:51 AM by Crosby Osei Tutu
                (modified after loading)
    
```

Additional Info : Peak(s) manually integrated



External Standard Report

```

=====
Sorted By      : Signal
Calib. Data Modified : 7/21/2020 11:58:51 AM
Multiplier    : 1.0000
Dilution      : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
2.722	MM	3.67650e-1	21.99229	8.08546	Vit A	

Totals : 8.08546



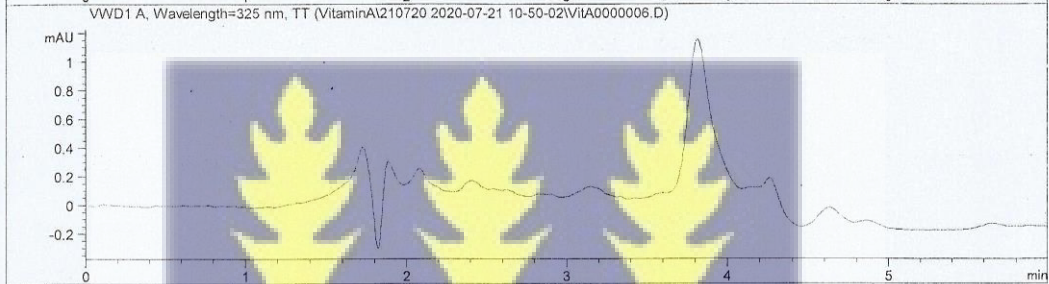
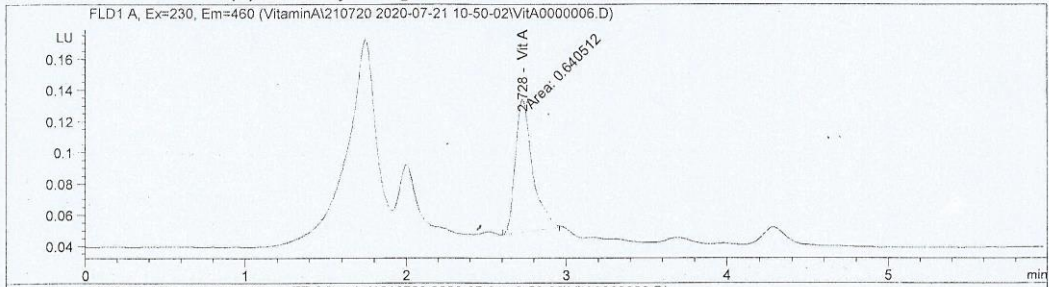
Data File C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VitA0000006.D
 Sample Name: Sam NAL

```

=====
Acq. Operator   : Crossby Osei Tutu           Seq. Line :    6
Acq. Instrument : HPLC 1260                  Location  :    6
Injection Date  : 7/21/2020 11:25:41 AM      Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M
Last changed    : 7/21/2020 10:50:03 AM by Crossby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M (
                  Sequence Method)
Last changed    : 7/21/2020 11:58:51 AM by Crossby Osei Tutu
                  (modified after loading)
    
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Additional Info : Peak(s) manually integrated



External Standard Report

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Sorted By      : Signal
Calib. Data Modified : 7/21/2020 11:58:51 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
2.728	MM	6.40512e-1	21.91280	14.03541		Vit A

Totals : 14.03541



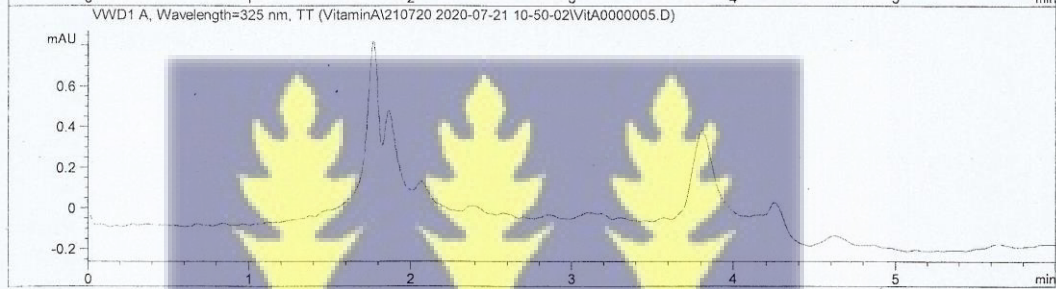
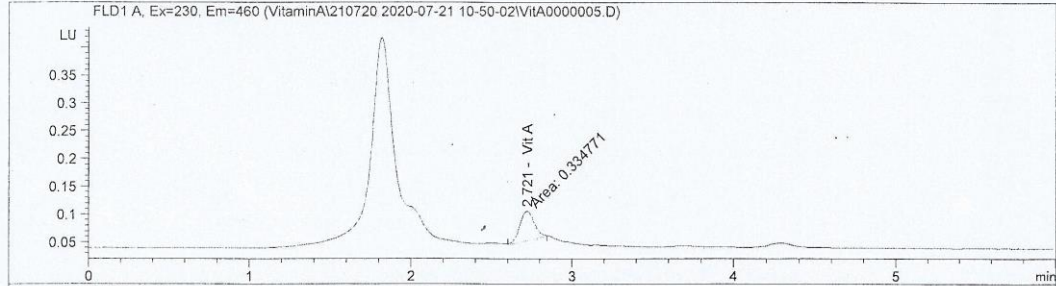
Data File C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VitA0000005.D
 Sample Name: Sam NAK

```

=====
Acq. Operator   : Crossby Osei Tutu           Seq. Line :    5
Acq. Instrument : HPLC 1260                  Location  :    5
Injection Date  : 7/21/2020 11:18:44 AM      Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M
Last changed    : 7/21/2020 10:50:03 AM by Crossby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M (
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                    (modified after loading)
    
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Additional Info : Peak(s) manually integrated



External Standard Report

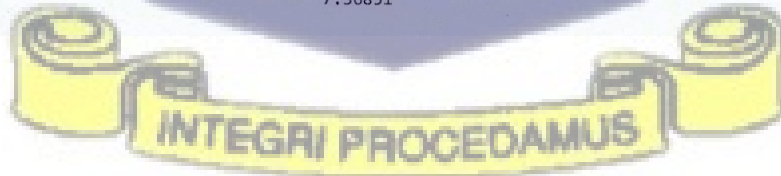
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Dilution        : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
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Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
2.721	MM	3.34771e-1	22.01062	7.36851		Vit A

Totals : 7.36851

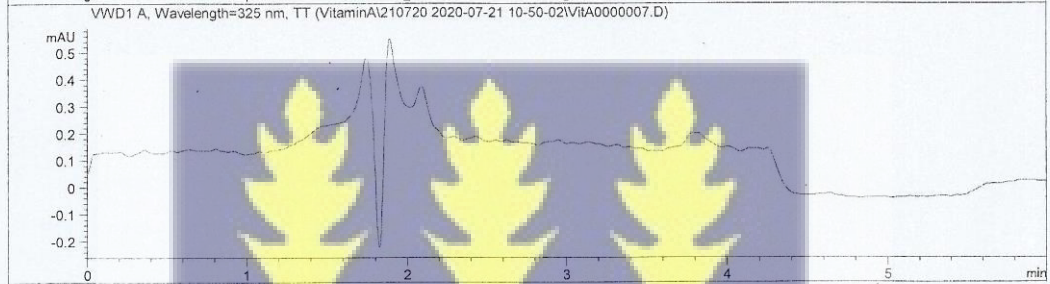
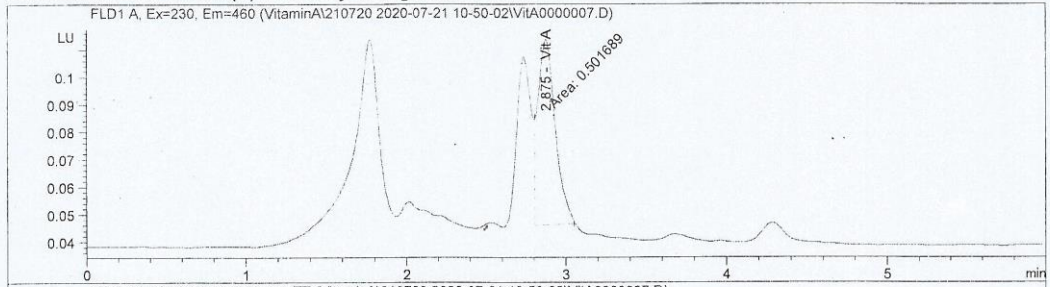


Data File C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VitA0000007.D
 Sample Name: Sam NUT

```

=====
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Acq. Instrument : HPLC 1260                 Location  :    7
Injection Date  : 7/21/2020 11:32:38 AM     Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method    : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M
Last changed   : 7/21/2020 10:50:03 AM by Crosby Osei Tutu
Analysis Method: C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M (
Sequence Method)
Last changed   : 7/21/2020 12:09:18 PM by Crosby Osei Tutu
                (modified after loading)
Additional Info : Peak(s) manually integrated
    
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External Standard Report

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Calib. Data Modified : 7/21/2020 12:09:18 PM
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Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
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Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
2.875	MM	5.01689e-1	21.94244	11.00828		Vit A

Totals : 11.00828



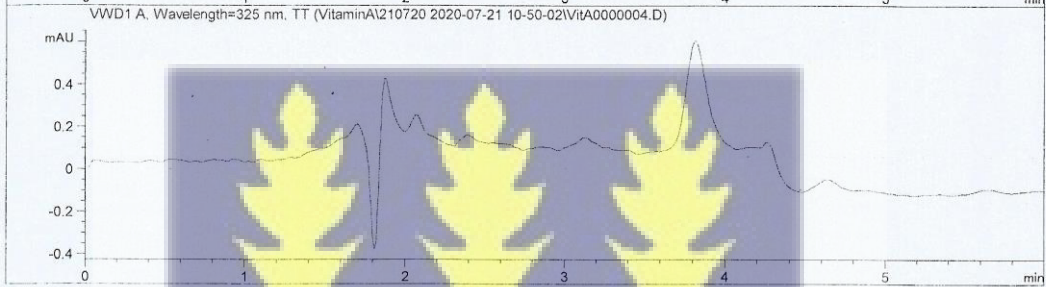
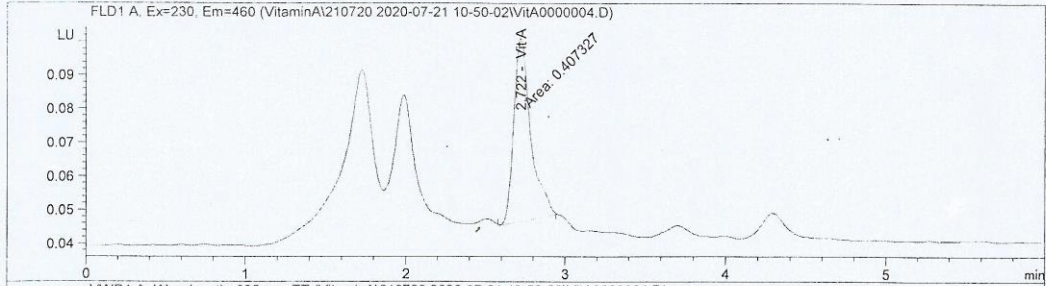
Data File C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VitA0000004.D
 Sample Name: Sam WP

```

=====
Acq. Operator   : Crosby Osei Tutu           Seq. Line :    4
Acq. Instrument : HPLC 1260                 Location  :    4
Injection Date  : 7/21/2020 11:11:48 AM     Inj       :    1
                                           Inj Volume: 10.000 µl

Acq. Method    : C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M
Last changed   : 7/21/2020 10:50:03 AM by Crosby Osei Tutu
Analysis Method: C:\Chem32\1\Data\VitaminA\210720 2020-07-21 10-50-02\VITAMIN A VWD.M (
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Last changed   : 7/21/2020 11:58:51 AM by Crosby Osei Tutu
                (modified after loading)

Additional Info : Peak(s) manually integrated
    
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External Standard Report

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Sorted By      : Signal
Calib. Data Modified : 7/21/2020 11:58:51 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
2.722	MM	4.07327e-1	21.97412	8.95065		Vit A

Totals : 8.95065



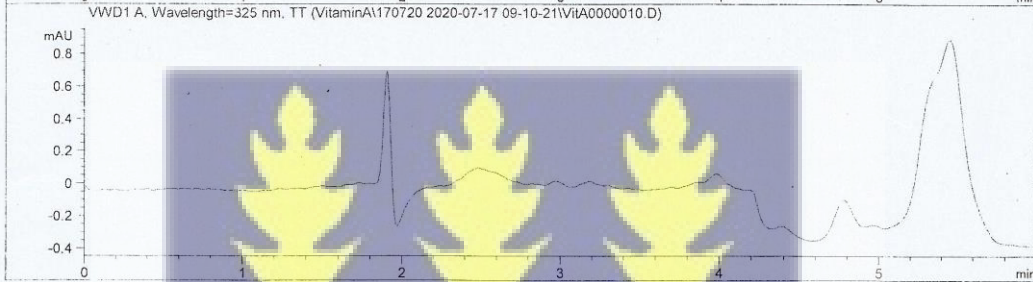
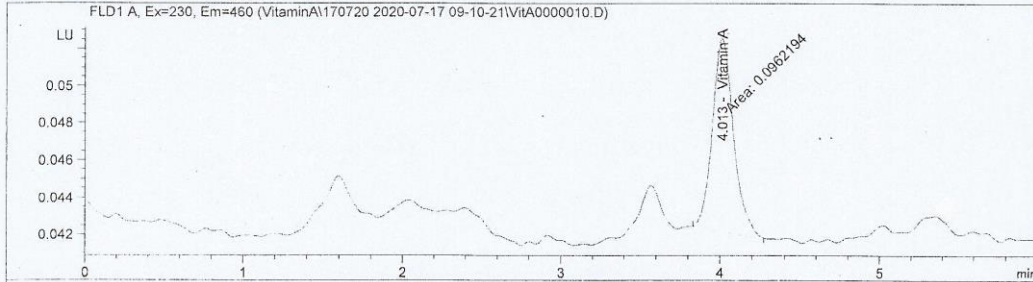
Data File C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VitA0000010.D
 Sample Name: SamIRS

```

=====
Acq. Operator   : Crosby Osei Tutu           Seq. Line : 10
Acq. Instrument : HPLC 1260                 Location  : 2
Injection Date  : 7/17/2020 10:13:50 AM     Inj       : 1
                                           Inj Volume: 10.000 µl

Acq. Method     : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M
Last changed    : 7/17/2020 9:10:22 AM by Crosby Osei Tutu
Analysis Method : C:\Chem32\1\Data\VitaminA\170720 2020-07-17 09-10-21\VITAMIN A VWD.M (
                  Sequence Method)
Last changed    : 7/17/2020 10:48:14 AM by Crosby Osei Tutu
                  (modified after loading)

Additional Info : Peak(s) manually integrated
    
```



External Standard Report

```

=====
Sorted By      : Signal
Calib. Data Modified : Friday, July 17, 2020 10:48:14 AM
Multiplier     : 1.0000
Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: FLD1 A, Ex=230, Em=460

RetTime [min]	Type	Area [LU*s]	Amt/Area	Amount [ppm]	Grp	Name
4.013	MM	9.62194e-2	37.67714	3.62527		Vitamin A

Totals : 3,62527

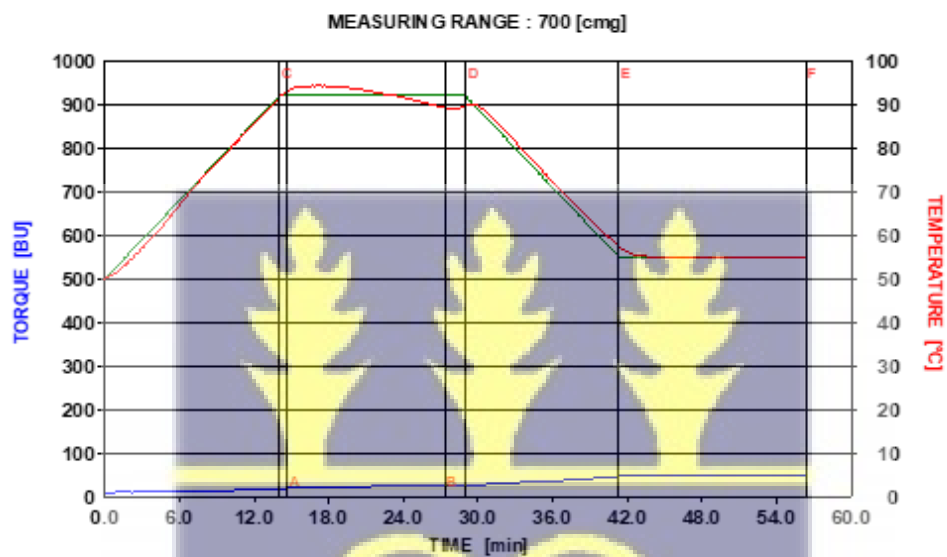


Appendix III: Visco-Amylograph of Flours

BRABENDER VISCOGRAPH E (USB)

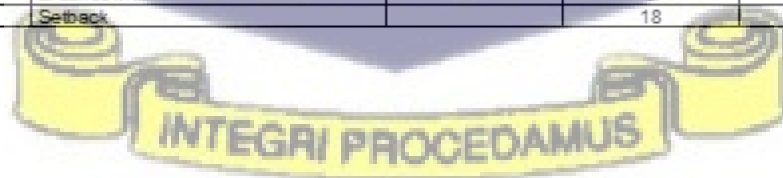
Version 2.4.11

<u>Parameter</u>			
Operator	: CROSSBY	Date	: 24/10/20
Sample	: GNX 1	Method	: Corrected
Moisture	: 7.99 [%]	Correction	: 0 [%]
Sample weight	: 40 [g]	Corr. to 0%	: 43.4 [g]
Water	: 420 [ml]	Corr. to 0%	: 416.6 [ml]
Note	:		
Note	:		
Speed	: 75 [1/min]	Meas. range	: 700 [cmg]
Start temperature	: 50 [°C]	Heat/Cool. rate	: 3.0 [°C/min]
Max. temperature	: 92 [°C]	Upp. hold. time	: 15 [min]
End temperature	: 55 [°C]	Fin. hold. time	: 15 [min]



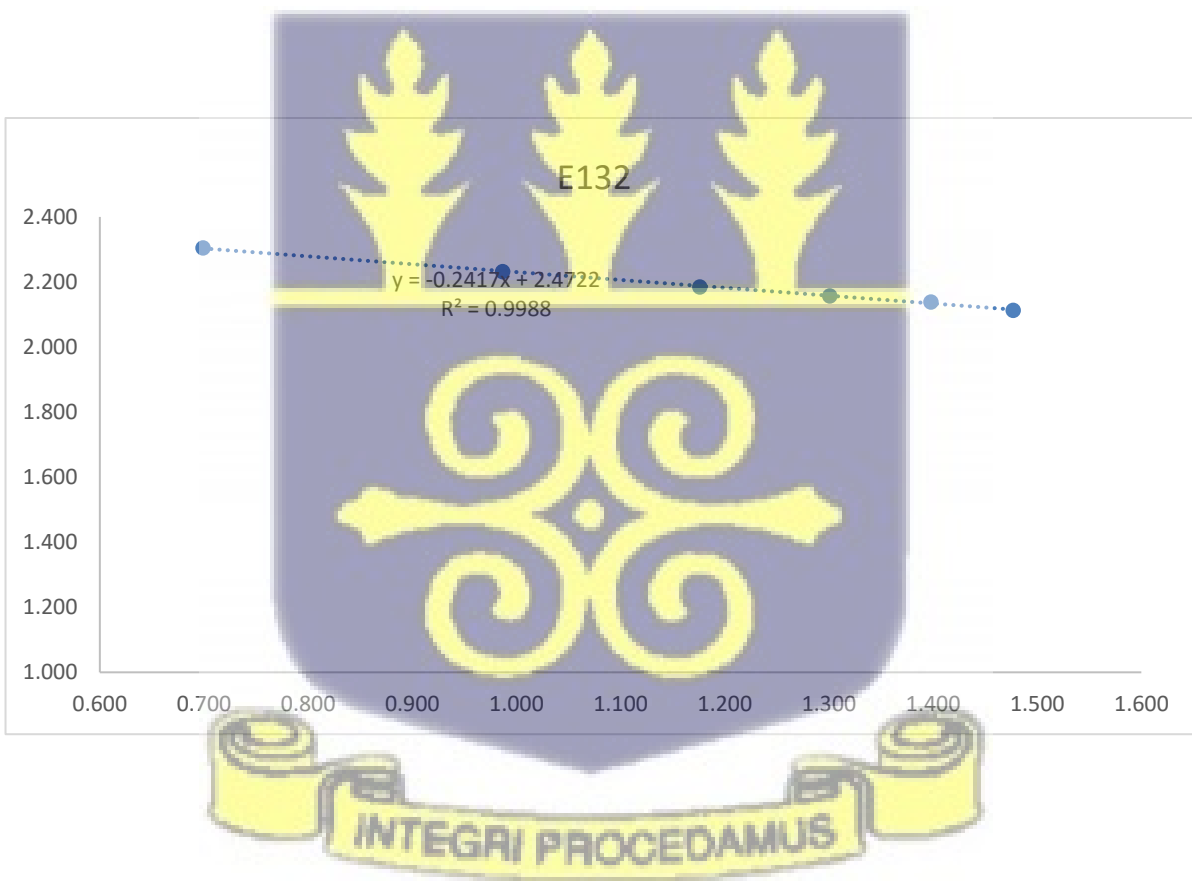
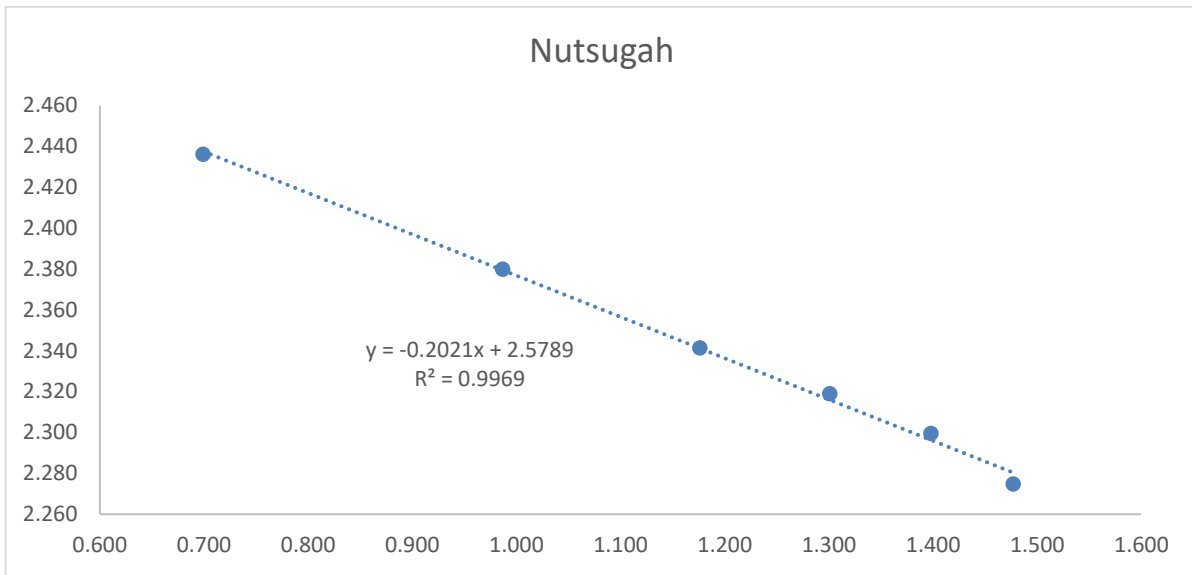
Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:14:40	20	93.2
B	Maximum viscosity	00:27:20	28	89.3
C	Start of holding period	00:14:00	18	91.5
D	Start of cooling period	00:29:00	28	89.7
E	End of cooling period	00:41:20	46	57.4
F	End of final holding period	00:56:20	49	54.9
B-D	Break down		0	
E-D	Setback		18	



File : Measurement

Appendix IV: Power Law Model Plots

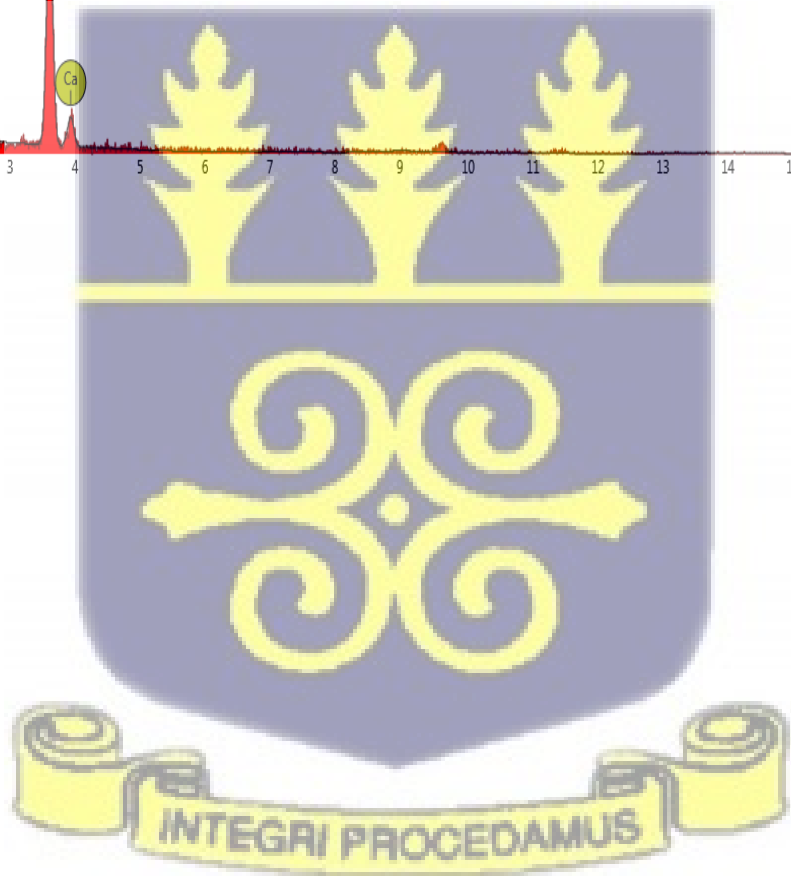
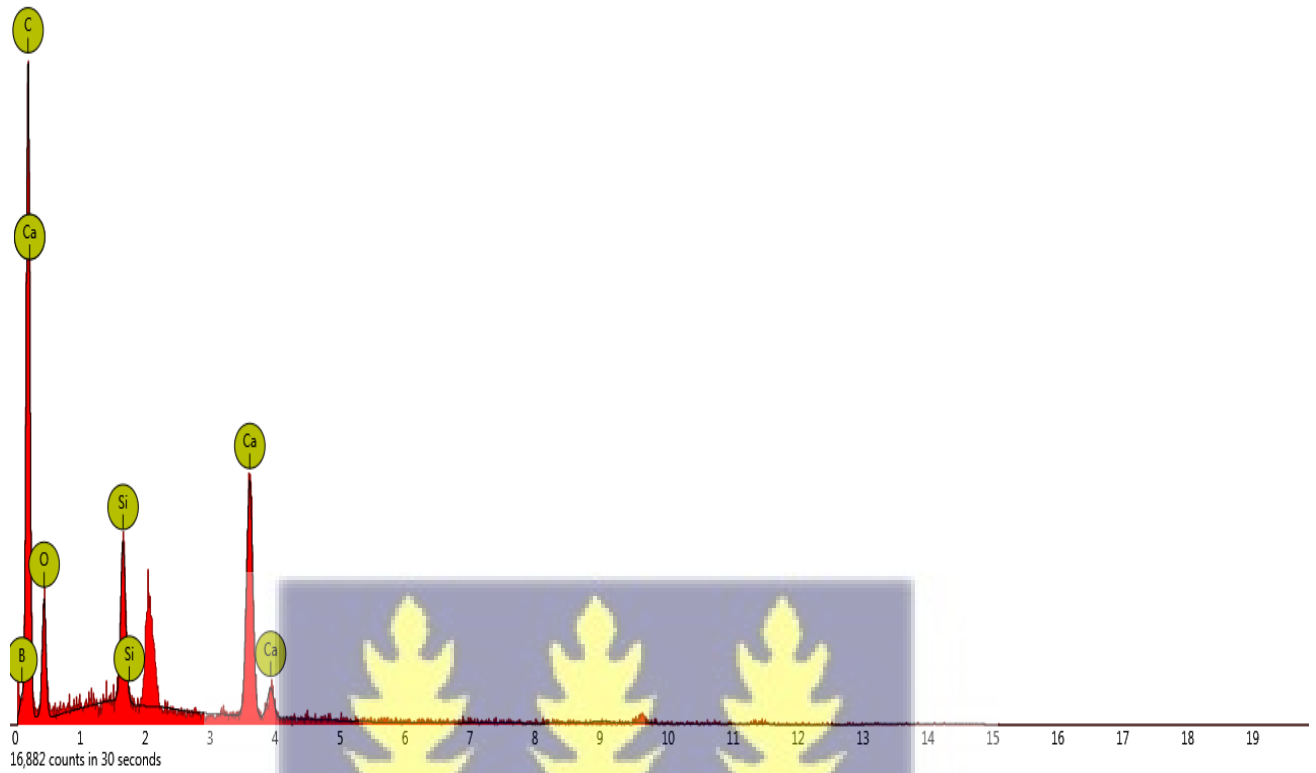


Appendix V: LC/MS Summary Report for Amino Acids Profile of FPF

Quantitative Analysis Results Summary Report							Agilent Technologies
Batch Path	D:\ForAgilentServiceProfessionalsOnly\office15\office\data\15.0.4420.1017\std\20200058\QuantResults\1.batch.bin						
Analysis Time	7/7/2020 4:54:32 PM	Analyst Name	Crossby Osei Tutu				
Report Time	7/7/2020 4:54:46 PM	Reporter Name	Crossby Osei Tutu				
Last Calib Update	7/7/2020 4:54:32 PM	Batch State	Processed				
Quant Batch Version	B.07.00	Quant Report Version	B.07.00				
Sequence Table							
Data File	sample Name	Sample Type	Position	Inj Vol	Level	Acq Method File	
10PPB AMINO ACID STANDARD.d	10PPB AMINO ACID STANDARD	Calibration	P1-F9	0.05	L1	MV3 trial.m	
20PPB AMINO ACID STANDARD.d	20PPB AMINO ACID STANDARD	Calibration	P1-F9	0.1	L2	MV3 trial.m	
50PPB AMINO ACID STANDARD.d	50PPB AMINO ACID STANDARD	Calibration	P1-F9	0.25	L3	MV3 trial.m	
100PPB AMINO ACID STANDARD.d	100PPB AMINO ACID STANDARD	Calibration	P1-F9	0.5	L4	MV3 trial.m	
200PPB AMINO ACID STANDARD.d	200PPB AMINO ACID STANDARD	Calibration	P1-F9	1	L5	MV3 trial.m	
Blank1.d	Blank	Blank	Vial 1	-1		MV3 trial.m	
1.d	MANGA MEYA	Sample	P1-E1	-1		MV3 trial.m	
2.d	WAAPP PIESA	Sample	P1-E2	-1		MV3 trial.m	
3.d	E134	Sample	P1-E3	-1		MV3 trial.m	
4.d	NA KIM TIER	Sample	P1-E4	-1		MV3 trial.m	
5.d	E82	Sample	Vial 1	-1		MV3 trial.m	
6.d	E111	Sample	P1-E1	-1		MV3 trial.m	
7.d	E132	Sample	P1-E2	-1		MV3 trial.m	
8.d	E145	Sample	P1-E3	-1		MV3 trial.m	
9.d	NA LAANA	Sample	P1-E4	-1		MV3 trial.m	
10.d	NUTSUGA PIASAA	Sample	P1-E1	-1		MV3 trial.m	
Quantitation Results							
Compound: L-Lysine							
Data File	Sample Type	RT	Resp	Final Conc	Exp. Conc	Accuracy	
10PPB AMINO ACID STANDARD.d	Calibration	1.380	2749	12.3699	10.0000	123.70	
20PPB AMINO ACID STANDARD.d	Calibration	1.397	4375	17.8306	10.0000	89.15	
50PPB AMINO ACID STANDARD.d	Calibration	1.397	13677	49.0667	10.0000	98.13	
100PPB AMINO ACID STANDARD.d	Calibration	1.397	29085	100.8021	10.0000	100.80	
200PPB AMINO ACID STANDARD.d	Calibration	1.406	58606	199.9307	10.0000	99.97	
Blank1.d	Blank	1.406	574	5.0680	10.0000		
1.d	Sample	1.440	13251	47.6339	10.0000		
2.d	Sample	1.440	17476	61.8228	10.0000		
3.d	Sample	1.440	11730	42.5293	10.0000		
4.d	Sample	1.449	13574	48.7179	10.0000		
5.d	Sample	1.474	2140	10.3247	10.0000		
6.d	Sample	1.431	11318	41.1445	10.0000		
7.d	Sample	1.440	18774	66.1798	10.0000		
8.d	Sample	1.440	10872	39.6465	10.0000		



Appendix VI: SEM Export Report for FPB



Appendix VII: Texture Profile Analysis of FPB

