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Characterisation of gluten-free dough mixes from traditional food crops with reduced starch digestibility for baking applications

Submitted in fulfilment of the requirements for the degree of

Doctor of Food Science and Technology

Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, Durban, South Africa

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DECLARATION

I declare that the thesis herewith submitted to the Department of Biotechnology and Food Science, Durban University of Technology, for the award of the degree of Doctorate in Food Science and Technology is my work and has not been previously submitted for a degree at any other University or Higher Institution of Education.

As the candidate's supervisor, we agree to the submission of this thesis.

Signature of student **Date**

27/03/2026

Signature of supervisor **Date**

DEDICATION

In loving memory of my Mom and Dad

I dedicate this thesis to my beloved parents (Freddy & Cynthia Govender).
I am because of you.

I am deeply grateful to you both for being my most important supporters, motivators, and inspirations.

I hope I have made you both proud.

My identity and achievements are only possible because of your unwavering support, love and encouragement.

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ABSTRACT

Gluten-free (GF) baking is challenging because gluten, which is absent, gives wheat-based doughs their elasticity, structure, and ability to retain gas. Developing gluten-free dough mixes requires a strategic selection of substitute ingredients that can mimic gluten's functional properties. This calls for the use of various starches, flours, hydrocolloids, and proteins to achieve desirable dough rheology and final product characteristics. The challenge lies in optimizing the interactions between these ingredients to create a cohesive and elastic dough structure that can withstand processing and deliver acceptable sensory attributes. The choice of suitable ingredients and their proportions is crucial for achieving a balance between dough stability, gas retention, and crumb structure. Therefore, food scientists and technologists find it challenging to create baked foods that are gluten-free. For gluten-free products to be more widely accepted by consumers, these ingredients must ensure that they resemble typical bakery goods as much as possible.

Amadumbe, millet, and psyllium offer unique properties that can be harnessed to create gluten-free dough mixes. Amadumbe, a starchy root crop rich in resistant starch, can reduce the glycemic load of baked goods. Millet is a gluten-free grain with high nutritional value, providing vital amino acids, minerals, and antioxidants. Psyllium, a soluble fibre, has been shown to improve dough handling, increase viscosity, and improve the textural properties of gluten-free baked goods. The combination of these ingredients can address the textural and nutritional deficiencies often associated with gluten-free products.

The focus of this research was the characterization of gluten-free dough mixes with reduced starch digestibility for baking applications. This was done by A; investigating the effect of hydrothermal treatments: annealing (ANN) and heat-moisture treatment (HMT) combined with 3% psyllium husk fibre (PHF), on the structural, rheological, pasting, and digestibility properties of maize starch with varying amylose content. B; evaluation of the functional and digestibility profiles of a gluten-free (GF) premix blend made from amadumbe and millet, supplemented with psyllium fibre, and C; the optimized gluten-free flour blend was tested for its dough-forming ability and baking quality.

Hydrothermal treatments: annealing (ANN) and heat-moisture treatment (HMT), combined with 3% psyllium fibre supplementation, significantly ($p < 0.05$) enhanced the rheological, pasting, and starch digestibility profiles. However, hydrothermal treatment alone caused minimal to no change in these functional properties. The results demonstrated that fibre supplementation of both HMT- and ANN-treated starches increased peak viscosity, trough viscosity, final viscosity, and setback viscosity, with

ANN exhibiting significantly higher values. ANN fibre-supplemented normal maize starch showed the largest increase in storage modulus (G'), according to rheological analysis. Profiling of starch digestibility revealed that fibre-supplemented high amylose maize starch produced the greatest levels of resistant starch (RS). These findings highlight the importance of developing healthier starches that promote well-being by reducing digestibility and increasing resistance to enzymatic breakdown. This will support the creation of low-glycemic, fibre-rich products. The results of the investigation proved useful in establishing the criteria for the amadumbe-millet and psyllium flours used to produce gluten-free composite blends.

The evaluation of processed gluten-free (GF) flour blends made from amadumbe, millet, and psyllium flours revealed that the inclusion of psyllium fibre significantly improved the pasting properties (PV, FV, BV) two-fold. The rheological properties showed enhanced viscoelasticity of the dough, as indicated by the storage modulus (G') consistently being greater than the loss modulus (G") across the frequency range. Starch structural analysis using X-ray diffraction (XRD) indicated that all blends exhibited a typical A-type crystalline structure. The analysis of starch digestibility showed a significant increase in resistant starch (RS) and a decrease in rapidly digestible starch (RDS) due to the presence of psyllium fibre. Results obtained from this study suggest that fibre-supplemented gluten-free flours have great potential for baking, owing to their improved functional properties and reduced digestibility profile.

The optimized gluten-free blend of amadumbe, millet, and psyllium (in a ratio of 70-30-3) was further evaluated for its dough-forming properties by examining water absorption capacity (WAC), oil absorption capacity (OAC), rheological properties, and textural properties. All the tests performed showed improvement with the inclusion of fibre. To assess the baking quality of the bread, textural analysis and sensory evaluation were conducted. Sensory evaluation assessed the amadumbe-millet-psyllium bread (AMP1) with a commercially available gluten-free bread. The results for overall acceptance indicated comparable findings (6.62±1.86) for AMP1 bread and (6.91±1.63) for the commercial gluten-free bread. However, starch digestibility results showed a reduction in resistant starch (RS) content, which was attributed to the increased baking temperature and time.

This research contributes to the advancement of gluten-free bread with enhanced nutrition, texture and shelf life, meeting the growing demands of health-conscious consumers and gluten-intolerant individuals. Therefore, this study furnishes valuable insights for food scientists, bakers, and ingredient manufacturers, guiding the preparation of novel gluten-free products with enhanced nutritional and sensory attributes.

PREFACE

This thesis is organized into seven chapters, with Chapter One providing the general introduction to the thesis. Chapter Two presents a critical review of literature highlighting challenges in gluten-free bread and bakery products, nutritional aspects and gaps in gluten-free bread and bakery products and ingredient selection, technological strategies to improve the development of gluten-free bread. Chapter Three focuses on the effect of hydrothermal treatments (annealing: ANN and heat moisture treatment: HMT) with psyllium fibre supplementation on functional properties and digestibility profile of commercial maize starches. Chapter Four presents the optimization of flour blends using amadumbe, millet and psyllium and testing their functionality as well as starch digestibility of the blends. Chapter Five reports on the dough-forming properties and the baking performance of the amadumbe millet psyllium bread. Chapter Six contains a general discussion of the entire findings. Chapter Seven concludes the thesis and suggests recommendations for future studies.

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LIST OF ABBREVIATIONS

ANN:	Annealing
ANOVA:	Analysis of variance
AOAC:	Association of Official Analytical Chemists
CD:	Celiac disease
DF:	Dietary fibre
FAO:	Food and Agriculture Organization of the United Nations
FTIR:	Fourier Transform Infra-Red
G*:	Complex modulus
G″:	Loss modulus
G′:	Storage modulus
GF:	Gluten-free
GFD:	Gluten-free diet
GI:	Glycemic index
GOPOD:	Glucose oxidase-peroxidase
HACS:	High amylose corn starch
HMT:	Heat moisture treatment
IVPD:	In vitro protein digestibility
IVSD:	In vitro starch digestibility
NCS:	Normal corn starch
OAC:	Oil absorption capacity
PHF:	Psyllium husk fibre
RDS:	Rapidly digestible starch
RS:	Resistant starch
SD:	Standard deviation
SDS:	Slowly digestible starch
TPA:	Texture profile analysis
WAC:	Water absorption capacity
WCS:	Waxy corn starch
XRD:	X-Ray Diffraction

LIST OF MATHEMATICAL SYMBOLS

Symbol	Full Term	Measurement
%	Percentage	Mathematics/Statistics
±	Approximately	Mathematics/Statistics
A	Absorbance	Spectrometry
ºC	Degrees Celsius	Temperature
G	Gram	Weight
H	Hour	Time
L	Litre	Volume
M	Molar	Concentration
mg	Milligram	Weight
mg/g	Milligram per gram	Concentration (wt./wt.)
mg/L	Milligram per litre	Concentration (wt./v)
Min	Minutes	Time
mL	Millilitre	Volume
N	Number of samples	Statistics
rpm	Rotations per minute	Speed
s^{-1}	Per second	Time
U/L	Units per litre	Volume
µg/mL	Microgram per millilitre	Concentration (wt./v)
µL	Microliter	Volume

PUBLICATIONS AND CONFERENCES

Publications

- Govender S., and Amonsou E.O. 2025. Modulating Starch Properties Through Hydrothermal Treatment and Psyllium Fibre Supplementation: Influence of Amylose Contents. Starch-Stärke. p.e70073 (Appendix 1)

Submitted manuscript

- Govender S., and Amonsou E. O. 2025. Functional properties and digestibility profiles of gluten-free amadumbe-millet flour blends supplemented with psyllium fibre. Submitted to the Journal of Food Science and Technology. Under review (Appendix 2)

Conference

- Govender Samantha and Amonsou E. O. Influence of hydrothermal processing and psyllium fibre on digestibility and rheological behaviour of maize starch with varying amylose content. 26th SAAFoST Biennial International Congress and Exhibition held at CSIR ICC Pretoria from 25-27 August 2025. Poster presentation.

CHAPTER ONE

1. Introduction

The acceptance of gluten-free diets and lifestyles has increased significantly, primarily because of greater consumer consciousness of gluten-related disorders and gluten intolerance. Recently, the demand for foods specifically designed for nutritional purposes has seen significant growth. A growing number of consumers are opting for foods that meet their expectations and enable them to maintain a healthy lifestyle. Coeliac disorder is a chronic autoimmune condition of the small intestine, caused by exposure to gluten, affecting inherently susceptible individuals. Exposure to gluten causes an inflammatory response, which may cause shortening of the villi lining the small intestine, which, if left untreated, can cause inflammation and severe complications (Lebwohl et al., 2018). The incidence of celiac disease is between 1 - 1.4% globally (Husby et al., 2020). The prevalence of coeliac disease in South Africa remains undetermined, due to diverse symptoms and limited disease consciousness; however, in Europe and America, one out of every 133 persons appears to be impacted by coeliac disorder (Grode et al., 2018). The consumption of gluten-free diets and lifestyles has risen significantly, largely due to improved public consciousness of gluten-related disorders and gluten intolerance (Radoš et al., 2025). The prevalence of celiac disease and the claims for more nutritious foods have increased interest in gluten-free foods.

Globally, gluten-free products continue to expand. However, producing superior gluten-free bread (GFB) continues to be challenging. Gluten, the protein component found in grains like wheat, is crucial for the structural integrity of many baked goods. Its presence in dough provides bread with its characteristic viscoelasticity and baking properties; substituting gluten in dough systems is problematic due to its unique functionality. Doughs made without gluten typically lack elasticity and cohesiveness, often similar to liquid cake batter. (Bender and Schönlechner, 2020). The most popular method for enhancing gluten-free bread-making involves using numerous structural agents, namely starches, proteins, hydrocolloids, and commercial enzymes (Masure et al., 2015). Alternatively, several studies have explored thermal and hydrothermal processing of millet, and sorghum (Robin et al., 2015) to amend the techno-functional characteristics of proteins and starch. These methods, which were first used to boost the performance of inferior wheat flour, have a lot of promise for improving gluten-free flours (Bucsella et al., 2016). Depending on the procedure or parameter selected, both heat and hydrothermal treatments alter the functionality of flour by denaturing proteins and gelatinising starch to varying degrees (Torbica et al., 2019).

One of the most essential ingredients in gluten-free baking is hydrocolloid. Hydrocolloids are the fundamental and most extensively researched additives in gluten-free bread (Anton and Artfield, 2008, Roman et al., 2019). In gluten-free baking, these ingredients enhance batter cohesion and elasticity by forming gels. They stabilize gas cells to improve expansion and bind water, which helps delay retrogradation (Renzetti and Rosell, 2016). A range of hydrocolloids has been explored to improve the quality of gluten-free bread. These hydrocolloids include guar gum, xanthan gum, hydroxypropyl methylcellulose (HPMC), methylcellulose, carboxymethyl cellulose (CMC), locust bean gum, and psyllium gum (Lazaridou et al., 2007, Houben et al., 2012). The inclusion of these hydrocolloids helps in improving the crumb texture of bread. Hydrocolloid addition also improved volume, hardness and porosity, as well as reducing in vitro starch digestibility and estimated glycemic index (Liu et al., 2018).

Furthermore, the dietary fibre supplementation of gluten-free baked products may be required, since it is recommended that the average celiac patient should consume between 25–35 g of dietary fibre per day (Sabanis et al., 2009). Recently diagnosed or poorly controlled celiac patients have been found to show low bone mineral density, imbalanced macronutrients, low fibre intake, and micronutrient deficiencies (Bianchi et al., 2024). The nutritive value of gluten-free products can also be boosted with dietary fibres. The psyllium seed husk-derived bioactive polysaccharide (*Plantago ovata Forsk*) is classified as an arabinoxylan and is commonly referred to as psyllium husk, psyllium mucilage, Ispaghula, or Isabgol. Psyllium husk is a naturally occurring, viscous dietary fibre with demonstrated health benefits, including the regulation of gastrointestinal function and the control of blood glucose and cholesterol levels (Singh, 2007). Sabanis et al. (2009) found that gluten-free breads containing dietary fibres of wheat, maize, oat and barley exhibited higher values for loaf volume and porosity than those without fibre. They determined that dietary fibre and starch work together to help the formation of a structure with greater stability. Apart from the beneficial impacts of dietary fibres on the properties of gluten-free bread, resistant starch has been shown to enhance crumb flexibility (Tsatsaragkou et al., 2014). A greater emphasis has also been placed on substituting more natural ingredients for chemical additives as a result of the recent customer demands for clean-label products (Asioli et al., 2017). In this regard, it has been discovered that psyllium flour satisfies this need while continuing to serve as an additive for baking (Franco et al., 2020). As a result, it is understood that including hydrocolloids in dough formulations containing gluten-free components is a possible strategy for creating gluten-free products.

Traditional food crops continue to be explored in the development of gluten-free products. Amadumbe (*Colocasia esculenta*), also called taro, is an underutilized indigenous crop that is widely grown in

South Africa, can be used as an added gluten-free ingredient. Amadumbe starch or flour has been shown to aid in enhancing the properties of baked products. The outcome of adding amadumbe flour to wheat flour in amounts of 10%, 20%, and 30% (w/w) on the quality of bread-baking was examined by Ammar et al. (2009). The findings indicated that bread made with taro flour up to a 10% substitution level had rheological and organoleptic qualities compared to wheat flour bread. Research conducted using the enzyme laccase to test the rheological properties of amadumbe dough by Manhivi et al. (2018) found that the laccase (1.5%) treated amadumbe dough showed improvement in the rheological behaviour in terms of its elasticity. Amadumbe has been one of the selected sources of flour replacement and as a source of hydrocolloid for this study, because of its low glycemic level, gluten-free status, and its relative non-utilisation as an ingredient in bread processing.

Millet is a drought-tolerant crop extensively grown in the semi-arid tropics of Africa and Asia, serving as a key source of carbohydrates. Due to its role in food security and potential health advantages, millet has gained much focus, and the United Nations General Assembly designated 2023 as the International Year of Millets (IYM 2023) (Faostat, 2016). Nutritionally millet, is gluten free and contains carbohydrates (60%- 75%), essentially in the form of starch, dietary fibre (10% –15%), protein (8% – 15%), lipid (1% –5%) and ash (total minerals) (1% –3%) (Taylor, 2017). The presence of polyphenols and fibre in millets could play an important role in their hypoglycemic properties (Annor et al., 2017). Millets have been linked to a lower risk of cardiovascular disease, some types of cancer, obesity, high blood pressure, and a healthy digestive system (Larzelere and Jones, 2008). According to Wang et al. (2019), using pearl millet flour as a substitute for 50% wheat flour demonstrated potential improvements in the final product's functionality (such as its free soluble phenolic content and antioxidant activity) and nutritional quality (such as its in-vitro starch and protein digestibility). Because of its favourable chemical and nutritional makeup, millet flour was chosen as a substitute for wheat in this study

Ingredient selection is an important consideration in gluten-free baking. When these ingredients are chosen, it is based on their technological properties and functionality, and often with little or no emphasis on nutrition. A critical research gap exists in the development and characterisation of gluten-free dough formulations made from traditional food crops of South Africa that integrate ingredient functionality with targeted nutritional outcomes, specifically reduced starch digestibility and enhanced fibre content, while maintaining suitable technological performance for baking applications. Addressing this gap is essential for advancing gluten-free product development from purely functional substitutes toward nutritionally optimised, health-oriented foods, and most importantly, the inclusion of traditional food crops, amadumbe and millet. This study focused on the development of a model amadumbe flour -based dough incorporating millet and psyllium fibre, and to determine the impact of these ingredients on the starch functionality and digestion kinetics of the dough and resultant bread.

CHAPTER TWO

2. Literature Review

As one of the most enjoyed food commodities, bread, as well as bakery products, dominate the menus of many nations. The unique characteristics of gluten proteins are crucial to the quality of the wheat flour bread dough (Kovács et al., 2021). The amount of gluten, which consists mostly of prolamin and glutelin, fluctuates according to the grain crop and variety. Several protein components, such as wheat prolamins and glutelins, are marked by slight structural and compositional variations, or micro-heterogeneity (Siminiuc and Turcanu, 2020). When prolamin and glutelin form bonds in an aqueous solution, a three-dimensional protein structure is produced that offers resistance and elasticity to dough stretching (Rosell, 2011). As the dough ferments, the gluten network holds the flour's starch granules and gas bubbles in place. These qualities support gluten's central function in the production of baked goods. A well-developed bread with thin pore walls and fine, consistent porosity is guaranteed by gluten that is sufficiently extensible and elastic.

2.1. Gluten-free (GF) bakery products

Increased consumption and high demand for gluten-free (GF) products have increased rapidly in recent years. The growing incidence of gluten-related illnesses and certain consumer demand for GF goods are the primary causes of this growth (Aguiar et al., 2023). A recent study estimates that by 2030, the global market for GF goods will be worth 13.67 billion US dollars (Research, 2025). Of all the GF items on the market, GF bread is the most well-known and has the biggest market share (Zhao et al., 2022). But it is crucial to remember that store-bought gluten-free bread frequently has a high glycaemic index and poor sensory appeal (Aguiar et al., 2023, Santos et al., 2019).

2.2. Challenges in preparing gluten-free products

For those who suffer from gluten-related diseases, nutritional therapy is essential. From a nutritional and particularly a technological perspective, creating gluten-free food is still a major issue. Bakery goods made without gluten are typically of lower quality and differ greatly from those made with regular wheat flour. The volume of bread is decreased by weaker doughs that are highly permeable to carbon dioxide and struggle to maintain their structure due to the absence of an important protein matrix that may expand and trap air (Ren et al., 2020a). When gluten is not present, the dough's cohesiveness, elasticity, and ability to retain gas are all negatively impacted.

To replicate the viscoelastic qualities of wheat dough, GF bread is produced from ingredients comprising gluten-free flours and starches, incorporating hydrocolloids (Conte et al., 2019). These ingredients must guarantee that gluten-free products are as close to traditional bakery goods as possible to boost customer acceptance (Padalino et al., 2016, Chiş et al., 2020, Šmídová and Rysová, 2022). The formulation of gluten-free bread products poses substantial challenges for food technologists and the scientific community. High-quality GF baked products are created using formulation-focused and technologically advanced methods (Cappelli et al., 2020). (Figure 2.1)

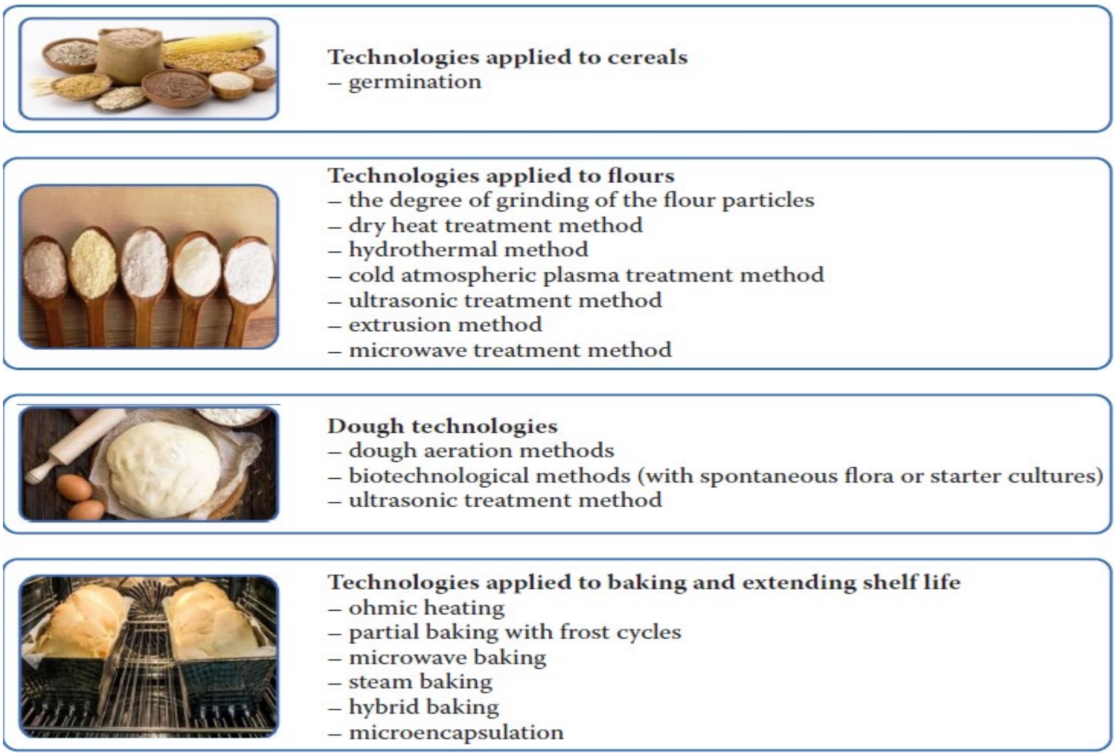

Figure 2. 1. Processing strategies for gluten-free products

Source (Siminiuc and Ţurcanu, 2023, Ramos et al., 2021, Šmídová and Rysová, 2022)

2.3. Product formulation

The formulations or techniques that facilitate the replication of the gluten matrix have been established. Ingredients such as starch, GF flour, proteins, hydrocolloids, emulsifiers and enzymes are the subject of the majority of research. In addition to improving the end product both technologically and sensorially, the ingredients and their interactions often mimic the cohesion and flexibility of traditional dough (El Khoury et al., 2018). However, incorporating raw ingredients, which are often nutritionally advantageous, into gluten-free baked goods may negatively affect the rheological characteristics of the dough and, consequently, the quality standards of the finished goods (Cappelli et al., 2020).

A substantial amount of research has been done to address the technological difficulty of processing non-wheat flours (Siminiuc and Ţurcanu, 2023). Finding substitute ingredients or processing methods that can effectively replace the way gluten proteins work. has been the primary objective of several studies on the creation of new, nutritious bread products.

The market's current selection of bakery goods demonstrates a lack of products made only from rye, oat, sorghum, and millet flours (Wang and Jian, 2022). This phenomenon is a result of these cereals' technological qualities rather than being supported by their nutritional value. Rye dough can be handled using the traditional baking procedure, but the resulting breads still have certain drawbacks, such as a weak flavour or even a sharp taste. However, doughs prepared with millet, oat, and sorghum flour have a more fluid structure and are more viscous than cake batters, making them unsuitable for use in traditional baking methods (Torbica et al., 2019).

2.4. Gluten-free ingredients used in baking

Food scientists face a significant scientific hurdle when it comes to gluten-free products, particularly in the baking industry. The lack of the structure created by the two gluten components, gliadin and glutenin, has been recognised as one of the main issues in baking with gluten-free flour. While gliadin offers the lower weight component that provides extensibility, glutenin contributes to elasticity by giving an increased molecular weight (Ćurić et al., 2007). When gluten is excluded during the bread-making process (both leavened and unleavened processing), a batter rather than a dough is produced, which results in bread with poor colour, texture, and other quality issues.

Gluten-free bread has a limited shelf stability and is inclined to degradation due to its absence of a gas cell structure and its diminished ability to retain moisture (Schober et al., 2007). Most gluten-free bakery goods are less nutrient-dense than their gluten-containing equivalents. Gluten-enriched food items are rich in micronutrients, whereas gluten-free meals lack fibre, B vitamins, magnesium, vitamin D and calcium, due to how they are prepared using processed flours and starches (Moore et al., 2006). Enhancing and adding micronutrients to gluten-free products is therefore crucial. Utilising legumes, tubers, and seed flours has been proposed as a major step in producing gluten-free, nutrient-dense baked items that customers will find acceptable (Culetu et al., 2021b).

Due to the lack of a gas cell structure and inadequate water retention, bread without gluten has a shorter shelf life and a higher risk of staling (Schober et al., 2007). Furthermore, when compared to baked goods that include gluten, gluten-free baked goods had worse nutritional profiles, depending on the type of gluten-free flour used. Gluten-containing foods are generally characterised by a relatively high micronutrient density; however, gluten-free alternatives commonly exhibit nutritional inadequacies, particularly with respect to vitamin D, magnesium, dietary fibre, calcium, and B vitamins. These deficiencies are predominantly attributed to the extensive use of refined flours and purified starches in gluten-free product formulations. (Moore et al., 2006).

Enhancing and improving gluten-free ingredients is therefore essential. The incorporation of legumes, tubers, and seed flours is proposed as a significant step forward towards the processing of gluten-free baked goods. Gluten-free products have been successfully created using millet, sorghum, buckwheat, sweet potatoes, flax, amaranth, cassava, peas, soybeans, and teff grains (Kupper, 2005).

Functional additives were shown to improve gluten-free bread's mouthfeel, volume, structure, capacity, and durability. Protein isolates, emulsifiers, and hydrocolloids are a few examples of functional additives that have been demonstrated to stabilise liquid layers that surround gas molecules in the fermenting slurry by acting as surface-active substances (Lazaridou et al., 2007). Protein-rich components may help create a coherent network in gluten-free systems, improving the structural quality of gluten-free bread, according to earlier studies (Schober et al., 2007, Witczak et al., 2017, Skendi et al., 2021). The proteins' functional qualities (gelation, foaming, and aiding in the increase of elastic modulus) allow for improvement of gluten-free bread's structure. Maillard browning and flavour development are two ways that proteins have been used to enhance the sensory qualities of bread (Starowicz and Zieliński, 2019).

Various challenges are encountered in the formulation of a wholesome, appealing, and high-standard gluten-free leavened bread, which currently remains the focus of much research. Using hydrocolloids in gluten-free mixtures showed their commercial potential as they enhance batter development by retaining gas cells and acting as moisture retainers, thereby delaying retrogradation (Culetu et al., 2021a). However, despite their potential health benefits (particularly their prebiotic impact) and ability to alter food's viscosity and texture, the use and incorporation of fibres (such as oat 8-glucan, psyllium, and arabinoxylans) in the finished product have not attracted much attention. This is significant because a lot of gluten-free products lack nutritional fibre because they are made from processed flours.

2.5. Nutrient Deficiencies in Gluten-Free Products

Although gluten-free grains are capable of supplying essential nutrients, predominantly gluten-free products utilize rice, maize, tapioca, or potato as their primary starch source. These products exhibit a nutritional profile that differs significantly from that of wheat. A diet predominantly composed of wheat is notably abundant in iron, dietary fibre, and B complex vitamins(Shewry and Hey, 2015). Dietary items such as gluten-free bread, pasta, and cereals do not necessitate additional supplementation. Because of this, in regions where wheat is heavily fortified, gluten-free goods have lower amounts of iron, B vitamins, and other vital minerals such as calcium, zinc, magnesium, and protein (Bozorg et al., 2024). Studies examining the nutritional makeup of gluten-free items in comparison to those that contain gluten have found that baked goods and pasta have significantly lower levels of calcium, niacin, and zinc (do Nascimento et al., 2014).

Studies have confirmed that pursuing a gluten-free diet (GFD) might result in nutritional deficits (Caeiro et al., 2022, Babio et al., 2020). One of the main causes of a GFD's low fibre content, according to Caeiro et al. (2022), is the highly processed nature of gluten-free food. In a different study, Babio et al. (2020) discovered that those with coeliac disease tended to consume more added sugars and total fats. Additionally, compared to a regulated diet, they consumed much more animal proteins and processed carbs and significantly less fibre-rich foods. Although these issues have not been thoroughly examined in previous research, they are likely to incur substantial patient and healthcare costs associated with managing nutritional deficiencies related to a GFD.
Considering the improved levels of sugars, fats, and salts, together with the diminished nutrient profiles of numerous gluten-free products, nutritionists have commenced advocating for using naturally gluten-free foods as opposed to packaged processed gluten-free alternatives. Adherence to these recommendations is anticipated to enhance patient health and potentially mitigate future expenditures related to managing comorbidities associated with GFD. Nevertheless, due to the elevated costs linked with naturally gluten-free foods, individuals with constrained financial means may be compelled to continue relying on processed gluten-free items, which probably correlate with both compromised health outcomes and increased societal expenditures in the future (Bozorg et al., 2024).

2.6. Plant polysaccharide: Starch

Starch has been identified as the predominant carbohydrate derived from natural sources in the human diet. It is inherently found in a diverse array of food sources, including legumes, cereals, roots, tubers, fruits, and various processed food products (Rostamabadi et al., 2024). Starch comprises numerous interconnected glucose units that undergo hydrolysis into amylose and amylopectin. It supplies approximately 70%-80% of human calorie consumption (Li et al., 2022).

2.6.1 Molecular structure of starch

The primary components of starch molecules, amylose and amylopectin, are arranged in complex semi-crystalline granules. While amylose and amylopectin (Figure 2.2) are essentially linear and branched molecules, respectively, amylopectin is the primary component that contributes to starch ordering (Yang et al., 2016). Double helices formed by the adjacent amylopectin segments can be classified as A-, B-, or C-type crystalline polymorphs. The next levels of starch organization include semi-crystalline growth rings, which are made up of a periodic sequence of crystalline and amorphous lamellae interspersed with amorphous growth rings; granules; and alternating crystalline and unstructured lamellae, which are created by aggregates of amylopectin double helices and amylopectin branch points, respectively. (Apriyanto et al., 2022; Qiao et al., 2020). Amylose is predominantly amorphous within the granule, primarily situated in the centre and scattered among amylopectin molecules. Furthermore, the quantity of amylose influences the crystalline arrangement inside the starch granule (Yang et al., 2016). The starch structure exhibits differences among botanical sources and differing amylose/amylopectin ratios, leading to starches with a broad range of functions that are appealing for various industrial food uses.

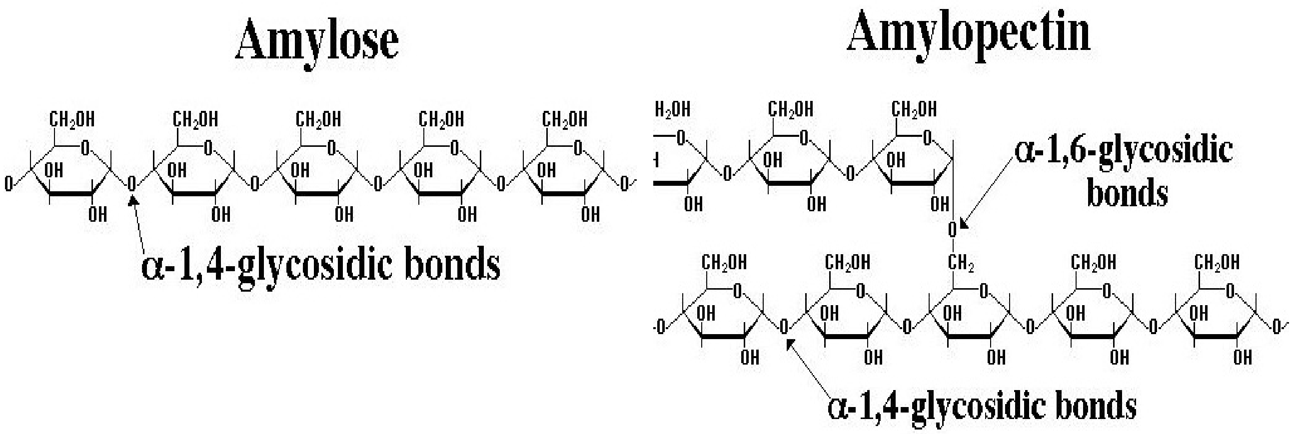

Figure 2. 2. Chemical structure of amylose and amylopectin

Upon treatment, the granules of starch are divided into rings, and the inner crystalline architecture is disrupted (Chen et al., 2020). This adjustment improves the digestibility of starch, leading to a notable rise in blood sugar concentrations following the consumption of starch-rich meals. Substantial fluctuations in blood glucose levels can influence the central nervous system via the gut–brain axis, leading to activation of the brain's reward circuitry. This activation has been associated with behaviours resembling sugar addiction, characterised by heightened cravings and desires that promote increased carbohydrate consumption. (Lennerz and Lennerz, 2018).

Chronic hyperglycaemia is likely to cause type 2 diabetes, obesity, cardiovascular diseases, and other metabolic disorders. Additionally, extreme variations in blood glucose may detrimentally affect cognitive function. Prior investigations have demonstrated that intermittent fasting, which serves to lower serum glucose content, can mitigate memory decline and slow the progression of cognitive impairment as well as energy metabolism dysfunction in individuals afflicted with Alzheimer's disease (Shin et al., 2018). The evolving interest in food health and nutrition has highlighted the control of starch breakdown to sustain glycaemic homeostasis and reduce the digestion of refined carbohydrates (Zhou et al., 2022) as an important subject for investigation.

The expanding applications of starch in the food industry have been constrained by various intrinsic limitations, including inadequate shear and temperature resistance, as well as poor retrogradability or opacity of the resulting paste (Punia Bangar et al., 2024). Currently, the starch modification using chemical, physical, and enzymatic methods represents the most developed strategy to address these challenges. Here, a partial modification of the chemical and physical structures of the molecule of starch can improve paste clarity, reduce the gel's tendency towards syneresis by increasing its water retention capacity, and increase the resistance of starch granules to shear forces during heating (Falsafi et al., 2019, Fan and Picchioni, 2020).

Besides their significant effects on physicochemical and technological functional properties, starch modification may cause significant differences in starch digestibility (Olawoye and Gbadamosi, 2020). From a digestive standpoint, and in light of the significant importance of regulating the glucose absorbed post-consumption of starch-rich meals in relation to public health, numerous initiatives have been undertaken to categorize starches based on their digestive characteristics. Englyst et al. (1992) were among the first researchers to effectively assess starch digestibility. They developed the most widely recognized methodology for classifying starches according to their rates of digestion, wherein starch was subjected to a simulated stomach and colon phase, and the quantity of glucose generated was systematically documented at varying time intervals.

This approach facilitated the separation of starch into 3 separate phases: (1) rapidly digestible starch (RDS), which raises blood glucose levels quickly because it hydrolyses in the first 20 minutes of digestion; (2) slowly digestible starch (SDS), which hydrolyses completely but more slowly (20–120 minutes); and (3) resistant starch (RS), which is not assimilated in the stomach and colonic phases and is transferred intact to the intestines, where probiotic bacteria can ferment it.

2.6.2. Rapidly digestible starch (RDS)

RDS predominantly includes amorphous and scattered starch, found in substantial quantities in starchy meals such as bread and potatoes, which are prepared using moist heat (Sajilata et al., 2006, Zhang et al., 2006b). Cereals (2.5–90 %) possess a greater amount of rapidly digestible starches (RDS) compared to tubers (0.1–21.0 %) and legumes (3.0–21.0 %) (Bajaj et al., 2018). Double helices are unattainable for the shortest A chains (DP 5–10) and may induce crystalline defects by disrupting the ordered crystal structure. Enzymes are more prone to effectively break down starches with an elevated amount of short A chains, leading to an increased level of rapidly digestible starch (Zhang et al., 2006b).

2.6.3. Slowly digestible starch (SDS)

SDS comprises impermeable amorphous starch and pure starch with A- and C-type crystalline forms, while B-type starch shows resistance to enzymatic breakdown. (Lee and Moon, 2015). SDS possesses physical structures that hinder enzyme availability and chemical structures which restrict enzyme activity (Zhang and Hamaker, 2009). The presence of channels in cereal starches facilitates the passage of enzymes into the particle's centre, initiating hydrolysis. This leads to a digestion sequence that occurs sequentially from the inside out, with both amorphous and crystalline regions being assimilated simultaneously. The concurrent breakdown mechanism of native cereal starches results in slow digestion, producing increased slowly digestible starch (SDS), which exceeds 50% (Zhu et al., 2024).

2.6.4. Resistant starch (RS)

Cereal starches with elevated short-chain content (A+B1) exhibited an increased amount of SDS related to potato starch, which contained fewer short chains. This suggests that SDS is linked to the short-chain portion in amylopectin, promoting the development of dual helices and crystalline lamellae within the crystalline section (Zhang et al., 2006a). Consequently, the crystalline area is the primary determinant of the reduced digestion characteristics of pure cereal starches (Zhang et al., 2006a). RS refers to the portion of starch that remains indigestible in the small intestine and will be fermented by bacteria in the large intestine, leading to the formation of short-chain fatty acids and other metabolites (Bello-Perez et al., 2020). RS has a beneficial impact on digestive system function, microbial flora, blood cholesterol levels, glycaemic value, and supports diabetes management (Liu et al., 2019). RS has demonstrated a reduced effect on the sensory attributes of food as a dietary fibre source related to conventional fibre sources, such as whole grains, fruits, or bran (Fuentes-Zaragoza et al., 2010). The resistant starch (RS) content of cereal and pulse starches is influenced by the levels of amylose and amylopectin, as well as their chain lengths (Perera et al., 2010). RS fractions encompass a remarkably extensive and varied spectrum, classified RS1 through RS5.

Currently, these are primarily delineated based on physical and chemical properties. RS1 is found inside either intact or partially crushed grains or seeds, rendering it unavailable (Samarakoon, 2020). RS2 comprises native starch granules, such as those derived from banana and potato, as well as high-amylose starches from high-amylose maize, that are shielded from enzymatic breakdown due to the profile or structure of the starch granule (Fuentes-Zaragoza et al., 2010). This condensed structure limits the access of digestive enzymes and explains the resistant characteristics of RS2 (Fuentes-Zaragoza et al., 2010). RS3 refers to retrograded starches that might develop during the cooling of food that is cooked (Jiang et al., 2020). Foods treated with heat and moisture consequently include some RS3. RS3 exhibits thermal stability, which permits its consistent performance during typical cooking processes, hence positioning it as a viable ingredient in numerous conventional food products (Ma et al., 2020). RS4 is synthesised via chemical modifications such as modification, transformation or cross-linking, which impede the breakdown of starch by restricting enzyme availability and forming bonds that are distinct from α-D-(1→4) and α-D-(1→6) (Coulon et al., 2020, Fuentes-Zaragoza et al., 2010). Because the amylose-lipid combination is resistant to enzymatic hydrolysis, it has been designated as RS5 (DeMartino and Cockburn, 2020). The enzymatic inhibition of the amylose-lipid complex depends on the molecular structure associated with the lipid and the crystalline arrangement of the individual helices (Hasjim et al., 2013).

2.6.5. Advantages of resistant starch for health

Numerous studies indicate that resistant starch (RS) offers several health benefits and, as a component of dietary fibre, possesses the functional attributes of dietary fibre (Jiang et al., 2020, Fuentes-Zaragoza et al., 2010). Resistant starch (RS) serves as an energy source for microbial proliferation in the intestine, while short-chain fatty acids (SCFAs) generated by bacterial fermentation of RS contribute to maintaining reduced pH levels in the gut, thereby inhibiting pathogenic bacterial activity (Jiang et al., 2020). Guo et al. (2020) conducted a systematic review regarding the influence of resistant starch (RS) consumption on obesity and associated health conditions. They concluded that dietary intake of RS does not directly affect body weight or composition; however, it does confer advantageous effects on diabetes management, glucose tolerance, and gut hormone levels. Bacterial fermentation of resistant starch (RS) may significantly contribute to the reduced risk of colorectal cancer, as both in vitro and in vivo studies indicate high butyrate synthesis from starch, which is proposed to exert a defence against colorectal cancer (Amini et al., 2016). The gradual digestion of resistant starch positively influences diabetes management by lowering postprandial glucose and insulin responses (Sajilata et al., 2006). In vivo studies have demonstrated how diets rich in resistant starch lower blood cholesterol and fat levels, showing the beneficial effects of resistant starch on lipid metabolism (Fuentes-Zaragoza et al., 2010). The influence of RS on functional and health challenges may vary according to the structural differences of RS and the specific compositions of microbiota (DeMartino and Cockburn, 2020). The impact of RS on medical disorders is presented in Table 2.1.

Table 2. 1. Health advantages of resistant starch

Health condition	Effect of resistance starch
Colorectal cancer	Lower gut pH, thereby promoting an environment less conducive to the development of malignant tumours.
Diabetes	Reduce postprandial insulin and glucose levels, improve insulin tolerance, and minimise the development of insulin resistance.
Obesity	Decreased caloric content and decreased energy consumption, improved appetite
Blood lipid levels	Suppress the production of cholesterol and fatty acids in the liver
Mineral absorption	Facilitate absorption of calcium and magnesium by short-chain fatty acids
Diarrhoea	Probiotics reduce the duration of rotavirus-induced diarrhoea.

Source (Guo et al., 2020, Jiang et al., 2020, Samarakoon, 2020, Amini et al., 2016)

2.6.6. The effect of amylose in maize starch on digestibility

Maize starch digestibility is largely governed by its molecular composition and granular structure, which determine the accessibility of digestive enzymes during gastrointestinal digestion. Native maize starch is composed of amylose and amylopectin organized in semi-crystalline granules, and increasing amylose content has been consistently associated with reduced enzymatic hydrolysis and lower post-prandial glucose release (Han et al., 2023, Lv et al., 2021). Waxy corn starch, which is rich in amylopectin, is rapidly digested. In contrast, high-amylose corn starch contains a greater proportion of slowly digestible starch (SDS) and resistant starch (RS), attributed to its denser molecular packing, enhanced hydrogen bonding, and more stable crystalline structures (Lv et al., 2021). Structural stability limits α-amylase penetration and delays starch gelatinization and hydrolysis, thereby reducing overall digestibility and glycaemic response (Han et al., 2023, Magallanes-Cruz et al., 2017). In addition, processing and modification strategies such as lipid complexation, ultrasonic treatment, and microwave heating promote the formation of amylose–lipid complexes (RS5), further decreasing corn starch digestibility by increasing enzyme resistance (Photinam et al., 2024). The fraction of corn starch that escapes digestion in the small intestine undergoes microbial fermentation in the colon, producing short-chain fatty acids that support gut health and glucose homeostasis, highlighting the nutritional importance of controlling corn starch digestibility through compositional and structural manipulation (Niu et al., 2025).

2.7. Amadumbe (*Colocasia esculenta*)

As one of the primary categories of plant-based foods worldwide, roots and tubers are vital to human diets and substantially contribute to food security and sustainable development (Scott et al., 2000, Sharma et al., 2016). Potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), yams (*Dioscorea spp*), and cocoyam (*Colocasia spp* and *Xanthosoma spp*) represent a selection of the tubers cultivated internationally. The tubers of *Colocasia esculenta* serve as significant sources of carbohydrates, providing energy and functioning as staple foods in tropical and subtropical climates. Before consumption, these tubers are typically subjected to various processing methods. Such processing enhances digestibility and palatability, prolongs shelf life, and mitigates postharvest losses.

The tropical tuber crop known as amadumbe, or taro (*Colocasia esculenta*), is cultivated primarily for its subterranean corms, made up of 70–80% starch. Anthocyanins with anti-oxidative and anti-inflammatory qualities, such as cyanidin-3-glucoside, pelargonidin-3-glucoside, and cyanidin-3-chemnoside, are abundant in the corms of *Colocasia antiquorum*. Due to the presence of antinutritional components, amadumbe consumption has been negatively impacted, causing noticeable burning and irritation in the oral cavity and throat after consumption (Kaushal et al., 2015). Due to its high gum (mucilage) content and small starch granules, amadumbe is very easily digested and appropriate for many food applications.

South Africa is subdivided into nine provinces, with taro predominantly cultivated along the KwaZulu-Natal coastline. Within the Natal province, taro is colloquially referred to as amadumbe (derived from the Zulu language). Also, the province of Limpopo, parts of Mpumalanga, and the Eastern Cape are home to amadumbe (Mokhele, 2018). In the northern parts of the province and the Midlands, it is seldom grown. There is a lot of agricultural potential in growing amadumbe, especially in terms of giving rural areas work opportunities. The marketing of amadumbe within KwaZulu-Natal has experienced a substantial increase in recent years. This noteworthy increase could be due to small-scale farmers selling amadumbe to well-known stores like Pick 'n Pay and Woolworths Foods (Mapumulo, 2022).

The amadumbe plant is distinguished by its big leaves that protrude above the soil surface and can grow to a height of one to two meters. The corms have fibrous roots and are found underground. As shown in Figure 2.3 (Ghumman et al., 2019), the corms are usually enveloped in a dense layer of brown to slightly brown tissue that protects their fragile and delicate starchy core. Three separate stages make up the amadumbe growth cycle: anagen, vegetative, and initiation, referred to as the mature phase (Ebert and Waqainabete, 2018). Typically, amadumbe is harvested 8 to 10 months after its cultivation, as soon as the plant's foliage starts to noticeably yellow. Although the leaves and corms are both high in nutrients, the corm is the one that is most commonly eaten. The amadumbe plant attains a height of approximately 1 to 2 meters, and it is identified by big leaves that reach higher than the soil surface. Amadumbe may tolerate drought conditions even though it is primarily grown in humid climates (Shelembe, 2020).

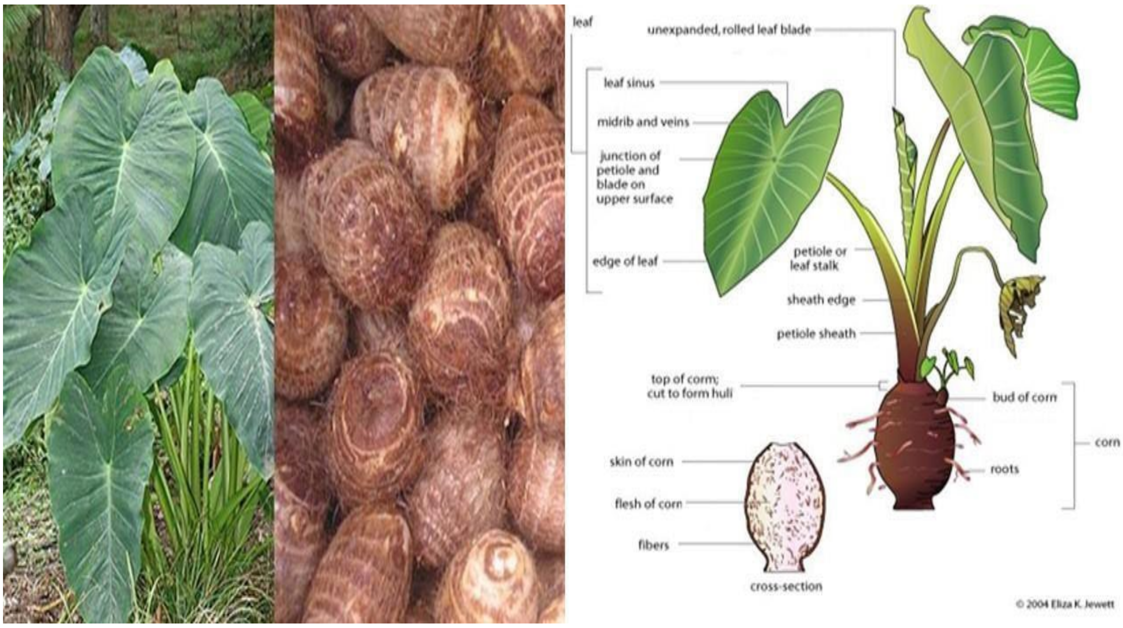

Figure 2. 3. *Colocasia esculenta* plant, corm and a labelled structure of the taro plant and corm. Source (Gajadhar, 2023)

2.7.1. The Nutritional value of amadumbe corms

The intrinsic structural properties of amadumbe encompass both nutritional and anti-nutritional factors, in addition to a diverse array of phytochemicals exhibiting various biological activities (Slavin 2013). Compared to sweet potatoes and potatoes, amadumbe has a higher caloric yield and is a better source of carbohydrates, dietary fibre, sugar starch, ash, and vitamin A (Kapoor et al., 2022). The corms of amadumbe exhibit elevated concentrations of magnesium, calcium, and potassium in comparison to other tuberous plants (Kapoor et al., 2022). On a fresh weight basis, the corms of amadumbe comprise approximately two-thirds water and possess a carbohydrate content ranging from 13% to 29%. Amadumbe has about 7% protein compared to other important root crops such as cassava, sweet potatoes, and yams (Khatemenla et al., 2019). Table 2.2 lists the nutritional composition of amadumbe. Except for reduced amounts of lysine, isoleucine, histidine, and methionine, amadumbe is abundant in all of the necessary amino acids. The comparatively low fat content of amadumbe sets it apart from other roots and tubers (Naidoo et al., 2015). Carbohydrates make up the majority of amadumbe. The presence of mucilage, or water-soluble gums, gives amadumbe its characteristic slimy texture. Moreover, amadumbe produces a significant amount of mucilage. The average mucilage content of amadumbe has been documented to vary between 3% and 19% (Njintang et al., 2014). However, despite evidence that viscous soluble fibres may offer increased health advantages, less is known about the mucilage of amadumbe (Slavin, 2013). The amadumbe corms contain a good amount of starch and a lot of mucilage (Naidoo et al., 2015). Both the particular variety and the growing environment have an impact on the starch content, which varies. Starch is thought to be a contributing factor to its functional properties, including enhanced swelling and solubility. (Falade et al., 2014).

Table 2. 2 Nutritional composition of amadumbe corm

Composition	g/100g
Protein	1.5
Ash	1.2
Carbohydrate	26.46
Dietary fibre	4.1
Sugars	0.4
Total lipids	0.2
Moisture	70.64

Source: Kapoor et al. (2022).

2.7.2. Utilization of amadumbe

Traditionally, the amadumbe corms are milled into flour, added to stews, boiled, or used in porridge. (Plucknett, 2019), which gives economically disadvantaged populations in Africa nutritional security (Mabhaudhi et al., 2014). In West Africa, amadumbe is boiled and subsequently transformed into a paste through the process of pounding. The paste is consumed with different soups and stews. A product similar to crisp or potato chips is made in the United States by thinly slicing corms and frying them in hot oil. According to empirical approaches, products made from amadumbe can include canned, flaked, or frozen corms as well as cereals, flour, bread, cakes, noodles, infant nutrition, and beverage powders (Chukwu, 2020, Saxby et al., 2020). Today, eating amadumbe is associated with several health benefits. like a lower incidence of diabetes, a decreased chance of lung or oral cancers, improved blood circulation, increased cognitive function, and the avoidance of anaemia, excessive gas, bloating, and constipation. It also boosts immune system performance and general health (Dhaliwal and Sharma, 2016, Iwu, 2016).

2.7.3. Nutritional functionality of amadumbe

Mucilage and resistant starch are two nutritionally beneficial components found in tuberous plants. It has been shown that mucilage made from different tubers and roots possesses antioxidative and an enzyme that converts angiotensin-inhibitory properties (Lee et al., 2003). Additionally, tubers and roots are gluten-free, which is a major factor to consider when choosing sources of carbohydrates.

Using tubers instead of gluten-containing carbs as a source of starches could help in lowering the prevalence of coeliac disease (CD) and associated adverse responses (Rekha and Padmaja, 2002).

Roots and tubers are normally transformed into many forms prior to eating. The digestibility of taro, attributed to its diminutive starch granules, coupled with its hypoallergenic characteristics, renders it an exceptional product. When it comes to nutritional content, taro contains a greater range of nutrients and vitamins than other tubers. Protein and starch make up a sizable amount of taro corms (70–80 g/100 g of dry taro). The normal crude starch levels of the six types of taro flour ranged from 41.27% to 61.44%. It also provides a significant amount of calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, and niacin—all essential elements for human nutrition (Nigussie, 2025). According to Amon et al. (2011) taro corms have high potassium and magnesium contents, varying from 2,251 to 4,143 mg/100 g and 118 to 219 mg/100 g of dry matter, respectively. Although the corm has a relatively low caloric value, it is distinguished by its high potassium and carbohydrate content.

2.7.4. Amadumbe utilization and current applications

Amadumbe represents one of the tropical root tubers and remains significantly underutilized within Africa (Ferdaus et al., 2023). This underutilization has been ascribed to the lack of available information regarding its potential, thereby resulting in considerably lower production levels in comparison to root plants (Ugwu, 2009, Kaushal et al., 2015). Amadumbe, the 14th most harvested vegetable/staple in the world, is grown mainly for its corms (Oscarsson and Savage, 2007). According to Mawoyo et al. (2017) is considered an excellent source of dietary starch. Because its starch particles are small (from 0.25 to 0.5 μm), the tuber is readily digested and is applied to create a wide range of products, such as infant food (Kaushal et al., 2015). The tuber possesses mucilage, a hydrocolloid that, following decomposition, releases β-galactose and L-arabinose. Mucilage possesses distinctive texture characteristics and has many possible uses as stabilisers and thickening agents in food (Njintang et al., 2008), making it advantageous in gluten-free formulations.

2.7.5. Mucilage in Amadumbe

Mucilage represents natural plant-derived foods that are occasionally referred to as gums. While they share a close relationship with pectin, they exhibit distinct physical attributes (Haruna et al., 2016, Jangdey et al., 2016). The variance among mucilage and pectin lies in their behaviour when exposed to water; specifically, pectin undergoes gelatinization, whereas gums swell, and mucilage forms slippery aqueous dispersions. Mucilage is present in various plant structures, including canals, sacs, and mucilage-secreting hairs (Ameri et al., 2015), and constitutes components of cells of plants

(Prajapati et al., 2013). Mucilage functions as a water reserve, energy reservoir, and membrane thickener within plant systems.

Mucilages are categorized as exopolysaccharides and glycoproteins (Naqvi et al., 2011). These polysaccharides are enriched with hydroxyl groups, bestowing mucilage with a high capacity for water binding. Such complex polysaccharides fall within the category of dietary fibres and can absorb substantial quantities of water; consequently, their water storage capacity aids certain plants in exhibiting tolerance to arid environments (Njintang et al., 2008, Nguimbou et al., 2014). Mucilage has been employed in food manufacturing to enhance the quality of food products (Haruna et al., 2016).

Tubers demonstrate a restricted shelf life due to their higher moisture content. One of the most effective methodologies for preservation may involve processing for the extraction of flour and/or starches (Pérez et al., 2005). Amadunbe is a great substitute for wheat or maize flour because of its unique high mucilage concentration and remarkably small particle size. Himeda et al. (2014) separated mucilage-free starch and related proteins from several taro (amadumbe) cultivars using a single-step extraction method. According to the monosaccharide profiles, the primary monosaccharides found in the mucilage hydrolysate included galactose, mannose, and arabinose. Aspartic acid/asparagine (14.4–17.2%) together with glutamic acid/glutamine (10.3–13.6%) was found to be the two most prevalent amino acids among the 17 that were analysed in the mucilage and the flour. Along with the protein composition, which is similar to that of flour, the monosaccharide profile shows some stability (Himeda et al., 2014).

Due to its distinctive high mucilage content and exceptionally small particle size, amadunbe serves as an excellent alternative to corn or wheat flour, employing a single extraction method to separate the mucilage-free starch and related proteins from a variety amadumbe. The monosaccharide form indicated that galactose, mannose, and arabinose constituted the predominant monosaccharides present in the hydrolysate of the mucilage. The monosaccharide profile, in conjunction with the protein content, is similar to that of flour —exhibiting a degree of stability. Mucilage possesses a greater potential for application owing to its natural origins, cost-effectiveness, and widespread availability.

2.8. Millet

Millet is a popular crop for many farmers in China, Africa, and India because of its hardy nature, inherent biodiversity, and very low input costs (Muskan et al., 2025). As reported by the Food and

Agriculture Organization (FAO) of the United Nations in 2014, millet production reached 12.49, 0.31, 14.83, and 0.79 million tons in Africa, America, Asia, and Europe, respectively (Faostat, 2016). The principal species include pearl millet (*Pennisetum glaucum*), finger millet (*Eleucine coracana*), proso millet (*Panicum miliaceum*) and foxtail millet (*Setaria italica*) (Figure 2.4). These various species of millets are cultivated in distinct regions globally. China predominantly grows foxtail millet, whereas pearl millet is mostly grown in India, Nepal, and Africa (Obilana, 2003).

2.8.1. Proximal chemistry of millet grains

Globally, millets serve as a significant food source in arid and semi-arid regions. Millet is recognized as a substantial source of energy. Millets are distinctive among cereals owing to their elevated amounts of calcium, dietary fibre, proteins, and polyphenols (Devi et al., 2014). They furnish essential protein, fatty acids, dietary fibre, vitamins, minerals, and polyphenols. As illustrated in Table 2.3, millets generally contain 6.2-12.3% protein, 1.3-5% fat, 65.5-83.3% carbohydrates, and 1.98-22% dietary fibre. Typical millet protein is characterized by considerable amounts of essential amino acids, particularly sulphur-containing amino acids (methionine and cysteine), while generally exhibiting low levels of lysine and tryptophan (Devi et al., 2014, Boussard et al., 2009).

Foods made from millet are known to have significant health advantages due to their potential prebiotic qualities as well as the capacity to improve the viability or functionality of probiotics. Additionally, millets have more fat than sorghum, rice, and maize (Obilana and Manyasa, 2002). Pearl millet contains between 8% and 9% dietary fibre (Taylor et al., 2014). The phytates, polyphenols, and tannins found in millets play important roles in preventing ageing and metabolic disorders, and they also contribute to antioxidant activity.

About 65% of millet grains are made up of carbohydrates, with dietary fibre and non-starchy polysaccharides accounting for a sizable amount of this value (Gupta et al., 2025). These substances support a gradual secretion of sugar into the body after eating, assist in reducing blood cholesterol, and help avoid constipation. Elevated consumption of millet is linked with a lower incidence of ulcers in the stomach, heart disease, and hyperglycemia (high blood sugar). Millets are rich in essential vitamins such as thiamine, riboflavin, folate, and niacin. The mineral and fatty acid content of millets is also noteworthy when contrasted with that of rice and wheat (Gupta et al., 2025).

Figure 2.4 Different types of millet: finger millet, pearl millet, prosco millet, foxtail millet.

Source (Annor et al., 2017)

Table 2. 3. Proximate composition of millet grains varieties (g/100g)

Type of Millet	Moisture	Protein	Fat/Lipids	Minerals	Dietary fibre	Carbohydrates
Barnyard millet	11.9	6.2	2.2	4.4	1.98	65.5
Finger millet	7.15-13.1	7.7	1.8	2.7	15.0-22.0	75.0-83.3
Foxtail miilet	11.2	11.5-12.3	2.38-4.3	0.47-3.3	2.5-8.5	60.9-75.2
Kodo millet	12.8	9.8	1.3	2.6	2.47	65.9-66.6
Pearl millet	12.4	11.6-11.8	4.8-5.0	2.2-2.3	11.3	67.0-67.5

Source: (Ramashia et al., 2019)

Saleh et al. (2013) studied the nutritional value and many health advantages of millet. Several research on millets and millet-rich foods have shown that, in addition to being gluten-free, which makes them appropriate for those with coeliac disease, they may also help manage type II diabetes because of their hypoglycemic qualities (Anju and Sarita, 2010, Geetha and Parvathi, 1990, Ren et al., 2016, Shukla and Srivastava, 2014, Ugare et al., 2014).

It's essential to consider that millets' protein digestibility is lower than that of several other cereals (Mertz et al., 1984). This raises concerns, especially since millet is a fundamental grain and the main form of protein for many people in poor nations. Furthermore, certain millet species may experience a reduction in the breakdown of proteins due to manufacturing processes that utilise hydrothermal treatments (Gulati et al., 2017). Knowledge of the processes influencing hypoglycaemic properties and protein digestibility of millets is essential for developing healthier millet-based food products.

2.8.2. Hypoglycaemic property of millet

Societies that eat millet tend to have lower rates of diabetes. Research has shown that phenolics found in millet have the potential to inhibit enzymes, namely pancreatic amylase and alpha-glucosidase (Ofosu et al., 2020). This inhibition helps lower postprandial hyperglycaemia by moderately blocking the enzymatic breakdown of complex carbohydrates. The chance of having diabetic symptoms can be decreased by making healthy food choices and being aware of the severity and possibility of diabetes (Greenwood et al., 2013, Sharma and Niranjan, 2018). Diabetics can control their disease with diet and lifestyle choices in addition to medicine. Diabetes symptoms can be prevented by eating a balanced diet and taking severity and risk factors into account (Bangoura et al., 2013). Dietary fibre is crucial for minimizing spikes in blood glucose levels by improving gastrointestinal function. Research has shown that consistent consumption of millets can significantly decrease cholesterol levels (Anderson et al., 2009, Bangoura et al., 2013, Sharma and Niranjan, 2018).

2.8.3. Impact of processing on the digestibility of millet protein and starch

Numerous studies have examined how processing affects the chemical components of millets (Devi et al., 2014, Taylor and Duodu, 2015). Given the possible health benefits of the finished product, processing has an impact on how easily proteins and carbohydrates can be digested (Singh et al., 2010). Since then, it has been of vital importance to study the influence of various food processing methods on the breakdown of millet and millet-based products. This includes exploring the impact of four major processes: decortication, germination, fermentation, and heat processing.

2.8.3.1. Decortication

Decortication or dehulling is the first step in dry milling. This step entails the extraction of the grain's exterior layers and pericarp. The germ, pericarp, and endosperm are the three primary parts of the seed caryopsis that are separated by this process. Decortication lowers phenolic acids, fibre, fat, and minerals while lowering factors that inhibit nutrition (Annor et al., 2013). In vitro protein digestibility (IVPD) in pearl millet is improved by decortication, most likely as a result of the removal of anti-nutrients that would otherwise delay digestibility (El Hag et al., 2002).

2.8.3.2. Germination

Malting, sprouting, and germination are terms collectively used to explain the method of hydrating the grains with water until saturated and then permitting the grains to germinate under controlled conditions (Annor et al., 2017). Through sprouting, hydrolytic enzymes trigger biochemical changes, resulting in structural modifications and the production of novel bioactive compounds that can enhance the grain's nutritive content and stability. Pearl millet germination has been shown to increase vitamin B levels, increase bioavailability of minerals, improve the essential amino acid composition, in addition to enhancing in vitro protein and starch digestibility (Hejazi and Orsat, 2016).

2.8.3.3. Fermentation

Lactic acid bacteria, either independently or in combination with yeasts, is responsible for fermenting several traditional millet products and drinks, particularly in Africa. Traditionally, certain starter cultures can start fermentation, or it can be natural (i.e., carried out by intrinsic bacteria). Using a segment of the fermented food item or agent, like dough or an inoculum, in the subsequent fermentation is an alternate technique (Hübner and Arendt, 2013). Depending on the cultivar, raw pearl millet's in-vitro protein digestibility (IVPD) varied from 68 to 76%; following 14 hours of dough fermentation, it rose to 82 to 87% (El Hag et al., 2002, Ali et al., 2003).

2.8.3.4. Thermal treatment

Millet is regularly used in traditional cuisine, making use of one of the following processing methods: roasting, boiling and pressure cooking. To increase millet consumption in Western countries, it could be beneficial to introduce millet-rich dishes that are more recognizable to Western customers, such as breads and pastas. In relation to cooking using hot water, roasting or baking millet decreases its starch digestibility, most likely because dry heat processing results in a low degree of starch gelatinisation (Roopa and Premavalli, 2008). Wet heat treatments, including pressure cooking and boiling, did not improve millet's protein digestibility, according to Pushparaj and Urooj (2014). Differences in millet varieties may be the cause of variations in results. Pearl millet's IVPD increased dramatically after roasting, indicating that dry heat treatments work better than wet ones.

2.8.4. Potential health advantages of millets

Millets confer numerous advantageous health effects (Table 2.4) attributable to their substantial nutritional profile and minimal gluten content. Their high fibre percentage plays a crucial role in promoting digestive wellbeing and engendering a sensation of satiety, thus facilitating effective weight control. Due to their low glycaemic index, these grains assist in modulating blood sugar levels and lower the possibility of developing diabetes. Low GI foods are thought to promote a more gradual and long-lasting rise in blood glucose levels, which makes them appropriate for diabetics (Imam et al., 2024). Because millets have a low GI, they help modulate blood sugar levels and decrease the chance of developing diabetes mellitus. Moreover, the soluble fibre in millets helps to improve heart health by reducing cholesterol levels (Imam et al., 2024).

Frequent consumption of millets may contribute to the prevention of two of the greatest health issues: diabetes and cardiovascular disease. Millets are rich in vital nutrients such as iron, magnesium, and phosphorus, and are vital for maintaining bone health and reducing the incidence of anaemia. They promote gastrointestinal health and are appropriate for those with coeliac disease due to them being gluten-free. Furthermore, millets are a great source of antioxidants, which are important in eliminating free radicals and lowering the incidence of chronic illnesses (Imam et al., 2024). Foods rich in millet are conducive to overall health enhancement.

Table 2. 4. Health benefits of Millet grain

Health benefit	Description
Heart Health	Millets may help lower the risk of cardiovascular disorders since they include fibre, antioxidants, and certain phytochemicals that encourage heart health(Ganesan and Xu, 2018). Consuming millet has been associated with better lipid profiles, including decreased levels of LDL cholesterol (Gujral et al., 2013).
Diabetes Management	Millets help regulate blood glucose rates and decrease the incidence of diabetes mellitus since they have a lesser glycaemic index than other grains (Chethan et al., 2018). Additionally, millets' high fibre levels help to improve blood sugar regulation (Bangoura et al., 2013).
Digestive Health	Millets' dietary fibre boosts a healthy gut and may help avoid constipation (Saleh et al., 2013). Millets provide prebiotic properties that enhance gut health by encouraging the development of beneficial gut microbiota (Chethan et al., 2018).
Weight control	Millets' high fibre content contributes the feeling of satiety, which helps you regulate your appetite and weight loss (Gujral et al., 2013). Because of their lower calorie content and high nutrient density, millets are regarded as an excellent choice for diets aimed at promoting weight loss (Shoba et al., 2020).
Gluten-Free Alternative	Due to millets being inherently gluten-free, persons with coeliac disease or gluten intolerance can consume them (Bhullar KS et al., 2018). Their addition to gluten-free diets, millet can provide vital nutrients and menu diversity (Gupta V et al., 2021)
Antioxidant Properties	Antioxidants like flavonoids and phenolic substances, which are abundant in millets, aid in scavenging free radicals and preventing oxidative stress. According to Chethan et al. (2018), antioxidants decrease the probability of chronic diseases and improve overall well-being.
Nutrient-Rich Food Source	According to Bhullar KS et al. (2018), millets are a great source of important nutrients like proteins, vitamins, and minerals. According to Gujral et al. (2013), adding millets to the diet can fulfil nutritional requirements and enhance overall health and well-being.
Sustainable Agriculture	Relative to main cereals, millets possess a lower carbon footprint, need less water, and are more robust to harsh environmental conditions. Growing millets can help ensure food security and promote sustainable farming methods (Shoba et al., 2020).

2.9. Psyllium (*Plantago ovata*) Husk

Psyllium husk, obtained from the kernels of the *Plantago ovata* plant, has found much attention recently because of its remarkable wholesome, functional, and health advantages (Noguerol et al., 2022). As a natural source of soluble fibre, psyllium husk has been historically employed throughout several cultures for its therapeutic qualities (Franco et al., 2020).

Today, it is widely recognized due to its ability to help control cholesterol, promote intestinal health, and aid in weight loss, among other health advantages (Franco et al., 2020). The high fibre content of psyllium fibre is one of its most significant nutritional attributes (Wojciechowicz-Budzisz et al., 2023). Fibre is essential in ensuring a healthy gut, encouraging consistent bowel motions and the prevention of constipation (Snauwaert et al., 2023). Psyllium husk contains both soluble and insoluble fibre, with soluble fibre being particularly beneficial in creating a gel-like substance in the colon, which aids in softening stools and easing their passage (Keller et al., 2024). This unique characteristic makes psyllium husk an effective natural laxative and a valuable dietary addition for individuals suffering from chronic constipation or irritable bowel syndrome (IBS) (Belorio and Gómez, 2021).

The functional properties of psyllium husk extend further than its health benefits, making it a versatile component of the food and pharma industries (Eghbaljoo et al., 2022). It works well as a thickener, stabiliser, and emulsifier in multiple food products, including dairy products, beverages, and gluten-free baked goods, because of its enhanced retention of water and gelling qualities (Jayakody et al., 2023). Additionally, psyllium husk is utilized in the formulation of dietary supplements and medications, highlighting its importance in both preventive and therapeutic health applications.

Generally, psyllium husk is a powerhouse of nutritional and functional benefits, with a profound impact on digestive health, cardiovascular wellness, blood sugar regulation, and weight management. Its diverse applications in the medicinal and food sectors further underscore its significance as a valuable natural ingredient (Medina-López et al., 2022).

2.9.1. Nutritional quality of psyllium

Psyllium husk is well established for its high fibre levels and various health benefits. Its nutritional composition includes a unique combination of macronutrients, vitamins, minerals, and other bioactive compounds that contribute to a vast number of health benefits. A by-product of grinding the seeds, psyllium husk accounts for 10% to 25% of the dried seed's weight (Halász et al., 2020). The husk contains over 60% water-soluble hemicellulose and arabinoxylans (Ren et al., 2020b). The xylan structure of arabinoxylans is linked with galacturonic acid, rhamnose and arabinose units. (Makarova and Shakhmatov, 2021). Psyllium husk is a highly nutritious food (Table 2.5), containing approximately 84.98% carbohydrates, 6.83% fibre, 0.94% proteins, and 4.07% ash (Verma and Mogra, 2013).

Table 2. 5. Nutritional composition of Psyllium husk

Proximate composition	Amount (%)
Crude protein	0.94-6.83
Moisture	4.98-10.53
Crude fibre	20.23
Total carbohydrates	84.98
Crude fat	0.46 - 1.2
Ash	2.34-4.1
Dietary fibres	76.63
Arabinoxylan	46.71
Nitrogen	0.24
Lignin	4.69
Rhamnose	2.39
Arabinose	46.80
Xylose	24.10
Mannose	4.24
Glucose	11.20
Galactose	2.09

(Geremew Kassa et al., 2024)

Of all plant sources, psyllium husk contains the highest concentration of arabinoxylan (Qaisrani et al., 2016). The structure and composition of arabinoxylan differ depending on where it comes from. Arabinoxylan can be added to foods to create functional foods that promote health and treat physiological ailments. Arabinoxylans are non-starch polysaccharides present in grains such as maize, rice, millet, oat, barley, and sorghum (Izydorczyk, 2021). The structure and composition of arabinoxylan change between different sources (Belorio and Gómez, 2021). Arabinoxylan can be used to create functional meals that promote physiological health (Zannini et al., 2022).

2.9.2. Functional benefits of psyllium husk

Psyllium husk offers significant functional benefits when incorporated into food products, especially regarding water solubility index, water absorption capacity, and viscosity (Khan et al., 2021). Psyllium husk is well known for its remarkable capacity to make food items more viscous. When hydrated, psyllium husk forms a gel-like substance due to its high soluble fibre content, primarily composed of arabinoxylans (Noguerol et al., 2022). This gelling property is beneficial in numerous food processes, for example, as thickening agents in soups, sauces, and dressings, and gluten-free baking to enhance texture and structure. The increased viscosity may also contribute to a prolonged feeling of fullness, which is advantageous for weight management and appetite control (Qaisrani et al., 2016). A mucilaginous fibre, psyllium is distinguished by its potent gel-forming properties in water (Belorio and Gómez, 2021).

2.9.3. Health benefits of psyllium husk

Psyllium husk is popular due to its considerable health advantages (Akram et al., 2019, Nie et al., 2018, Wahid et al., 2020) (Table 2.6). Abundant in fibre, both soluble and insoluble, psyllium husk is a versatile and potent natural remedy that can support various aspects of health, ranging from digestive wellness to cardiovascular health (Khan et al., 2021).

Table 2. 6. Health benefits of psyllium husk

Type of disease	Mechanism of psyllium husk inference	Reference
Diarrhoea	– By decreasing the liquid, and helps in the regulation of normal stools – In combination with calcium, it also proved very effective in diarrhoea	(Deng et al., 2022, Lin et al., 2020)
Constipation	– A smooth defecation process is achieved because the water absorbed by the psyllium husk is not absorbed by the gut, making the stools softer and bulkier.	(Yang et al., 2021a)
Diabetes	– By reducing the small intestine's absorption of carbohydrates. – Due to the gel formation in the intestine, this decreases the amount of glucose absorbed.	(Rivers and Kantor, 2020)
Cholesterol	– By binding with bile acids in the ileum, and ultimately reduces the absorption of cholesterol	(Kausar et al., 2024)

2.10. New technologies to enhance gluten-free doughs and baked goods

Gluten-free bread dough is mostly made from processed flour or pure starches, such as rice flour and corn starch, but it is devoid of the three-dimensional protein-starch network seen in gluten-containing bread doughs (Cappelli et al., 2020). To ensure correct gelatinisation of starchy constituents during the baking process and to enhance viscosity and retention of gas capacity, significantly larger amounts of water are required in gluten-free recipes. This substantially alters the dough's viscosity into a batter, impacting the manufacturing process and the overall integrity of the final bread (Bender and Schönlechner, 2020).

To focus on these challenges and enhance the quality of GF doughs and bakery goods, a variety of strategies have been devised. These strategies encompass the modification of GF flours through several technological methodologies. To improve baking and modify the characteristics of GF dough, various types of technological procedures have been explored (Figure 2.5).

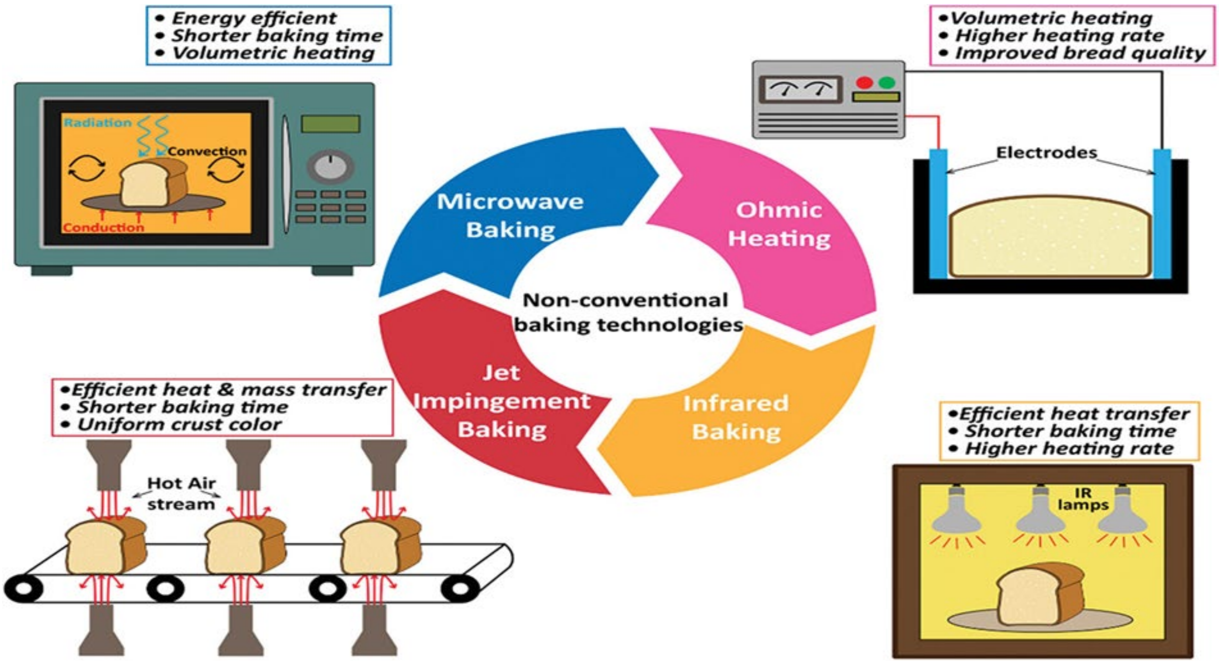

Figure 2. 4. Non-conventional baking technologies and their advantages. Source (Dubey et al., 2025)

2.10.1. Use of Hydrocolloids

Hydrocolloids are water-soluble polysaccharides characterised by diverse chemical structures, elevated molecular weights, and hydrophilic long-chain molecules. Their inclusion in gluten-free products positively affects various attributes, including structure, volume, texture, flavour, overall quality, and shelf life (Padalino et al., 2016, Gao et al., 2018, Zoghi et al., 2021). Hydrocolloids' dispersion qualities, such as their capability to improve water-binding, increase viscosity, speed up hydration rates, and the way temperature affects hydration, play a key role in how effective they are in gluten-free applications (Culetu et al., 2021a). As temperature increases, viscosity tends to decrease for most hydrocolloids (Padalino et al., 2016). Hydrocolloids also facilitate the formation and retention of gases during fermentation.

As seen in Table 2.7, hydrocolloids can be categorised according to their source. Gluten-free products have used a variety of hydrocolloids, such as hydroxypropyl methylcellulose (HPMC), xanthan gum (XG), guar gum (GG), locust bean gum, psyllium, carrageenan, pectin, carboxymethyl cellulose (CMC), konjac gum, gelatine, agarose, agar, β-glucan, gum arabic (GA), and alginate (Salehi, 2019, Xu et al., 2020).

Table 2. 7. Classification of the main hydrocolloids according to their origin.

Type of hydrocolloid	**Origin**
Cellulose-derived molecules	• Methylcellulose • Carboxy methylcellulose • Hydroxypropyl methylcellulose
Plant tissue extracts	• Pectin • ß-glucan
Plant exudates	• Gum Arabic • Tragacanth
Viscous plant substances (mucilages)	• Guar gum • Psyllium • Locust bean gum
Fermentation gums (of microbial origin)	• Xanthan gum • Gellan gum • Dextran
Species of seaweed	• Alginates • Agar • Carrageenan
Animal origin	• Gelatine • Albumin • Caseinate

Source Culetu et al. (2021a)

The characteristics of hydrocolloids significantly influence the quality of gluten-free bread, including their molecular weight, structural configuration, chain length, bonding characteristics, and any chemical modifications they may undergo (Culetu et al., 2021a). The concentration used, the type of flour and other components used, the relationship with other food polymers like starch, and processing conditions like temperature, pH, and shear forces are some other factors that also affect how the efficacy of hydrocolloids (Clapassón et al., 2020, Vidaurre-Ruiz et al., 2019).

Hydrocolloids and water combine to create a gel network during the bread-making process, which greatly increases dough viscosity and strengthens the structural boundaries of gas-expanding cells. The bread's overall volume, structure, texture, and appearance are all enhanced by this interaction, which also increases the bread's ability to hold onto gas during baking and proofing (Miñarro et al., 2012, Mastromatteo et al., 2012, Anton and Artfield, 2008). Additionally, hydrocolloids exhibit a "water-release" effect that is essential for facilitating starch gelatinization during baking (Padalino et al., 2016).

By reacting with proteins, starch, lipids, and water to produce complexes that reduce surface tension inside the dough matrix, emulsifiers further improve the dough's qualities. The interaction between emulsifiers and hydrocolloids can lead to the stabilization of air bubbles dispersed throughout the dough. Consequently, gluten-free breads tend to exhibit smaller and more uniformly sized pores, contributing to improved texture (Mert et al., 2016). Thus, the incorporation of hydrocolloids represents a practical approach to increasing the dietary fibre content in gluten-free baked goods. Because of its inherent bioactive soluble fibre qualities, such as its ability to hold water, form gel, and provide structural reinforcement, psyllium has lately created much attention as a possible hydrocolloid alternative in gluten-free formulations. Psyllium has been shown to effectively regulate crumb texture and can function as an alternative to other commonly used hydrocolloids, such as xanthan gum (XG), guar gum (GG), and hydroxypropyl methylcellulose (HPMC) (Filipčev et al., 2021).

2.10.2. Hydrothermal methods

Hydrothermal treatment is considered easy to use, safe, and readily accessible. This process involves applying heat and moisture under controlled conditions, which changes the physicochemical characteristics of starch. There are two primary methods of hydrothermal treatment: heat moisture treatment (HMT) and annealing (ANN). In order to conduct HMT, starch is heated to a temperature higher than the normal gelatinisation temperature while its moisture content is decreased. In contrast, ANN entails heating the starch below the gelatinisation temperature and adding more moisture (Kim et al., 2023).

2.10.2.1. Heat moisture treatment (HMT)

HMT is a safe and eco-friendly technique of starch alteration that solely uses moisture and temperature (Xia et al., 2016). This important physical modification involves heating starch granules at temperatures between 84 and 120°C with a small amount of water present (less than 35% w/w). For a predetermined amount of time, between 0.25 and 16 hours, this heating takes place above the glass transition point but lower than the gelatinisation temperature (Hoover, 2010).

HMT modifies several of the starches' physicochemical characteristics without compromising the granule's structural integrity, including swelling power, solubility, pasting time, viscosity, crystallinity, gelatinisation, and thermal stability (da Rosa Zavareze and Dias, 2011). Although the granule size and shape remain unchanged, surface modifications, including indentations and partial concavities, develop on the starch granules (Xia et al., 2016). Notably, the starch is more susceptible to novel chemical, physical, or enzymatic modifications when the moisture content employed during modification is reduced, since the degree of these surface changes increases (Gunaratne and Hoover, 2002). Furthermore, the physicochemical and functional properties of root and tuber starches are generally more significantly affected by HMT compared to cereal starches (da Rosa Zavareze and Dias, 2011).

2.10.2.2. Annealing (ANN)

Annealing is a regulated hydrothermal process that keeps starch moist for at least 12 hours while treating it at temperatures over its glass transition temperature and below its gelatinisation threshold (40–55 °C) (Clerici and Schmiele, 2018). This process enables the reorganization of the semi-crystalline form of starch granules, ensuing a reduction in the free energy of the starch molecules and promoting intermolecular interactions within the starch chains (Biliaderis, 2009, da Rosa Zavareze and Dias, 2011).

During the formation of a starch-water slurry, the separation of the liquid phase induces amylose leaching, which modifies the compositional attributes of the starch (Clerici and Schmiele, 2018). According to the literature, annealing is recognized as a treatment that changes the physicochemical properties of starch while preserving its grainy architecture, owing to the treatment temperatures being maintained below the gelatinization point (Jayakody and Hoover, 2008, da Rosa Zavareze and Dias, 2011).

Starch subjected to annealing demonstrates a substantial loss in swelling power and solubility (Biliaderis, 2009). The reorganizational changes within the crystalline architecture of the granules confer enhanced resistance to enzymatic degradation, resulting in a greater percentage of slowly digestible and resistant starch, juxtaposed by a diminished fraction of rapidly digestible starch (Chung et al., 2009). Additionally, annealing encourages greater mobility in the starch's crystalline and amorphous domains, which makes it easier for a variety of helical structures to develop, such as interactions between amylose and amylose, amylose and amylopectin, and amylopectin and amylopectin. This structural change enhances the starch's ability to bind iodine (Adebowale et al., 2009).

According to research findings, the starch-protein interaction during hydrothermal treatment enhances the textural quality of foods (Majzoobi et al., 2016, Malik et al., 2021). Because of its complexity and diversity, the protein-starch interaction—which has a major impact on dough properties—is still an occurrence that is rarely studied (Guo et al., 2020). Protein denaturation and starch gelatinisation happen concurrently and competitively during hydrothermal treatment. Protein coagulation takes place in a continuous network where starch particles stay encapsulated when protein denaturation exceeds starch gelatinisation. The final product's well-preserved forms and structural integrity are characteristics of such a network (Siminiuc and Ţurcanu, 2023).

The quality and shelf life of GF dough and foods are continuously enhanced by innovative technologies. It has been found that some promising techniques do not produce entirely satisfactory outcomes, and therefore, it becomes necessary to incorporate other techniques. This is an area that requires further investigation. The process's economic aspect would be vital to create a product of excellent quality, that also meet the consumer's budget.

2.11. Conclusion

The considerable technological obstacles still facing the creation of gluten-free bread are exemplified by the low quality of GF loaves now on the market. The absence of gluten considerably reduces the rheology of dough and the characteristics of bread in comparison to wheat-based products. This leads to bread with a lesser volume and poor texture, and doughs that are much less cohesive and elastic. It can be challenging to find substitute substances that can replicate the gluten network due to its specific structure and function. Depending on the intended product, it is crucial to choose the best improvement method (or mix of strategies).

The most promising outcomes appear to depend on dry heat treatment of gluten-free flours that improves starch gelatinisation. Several methods could be used to improve the rheology of baked goods and gluten-free dough. Even though the literature is comprehensive, more research is still required, especially regarding the latest and most creative approaches. Additionally, local and alternative sources (such as native legumes or protein sources derived from different production chains), traditional and underutilised indigenous ingredients that are available all through the year, could be utilised to achieve this.

2.12. Aim and Objectives

2.12.1. Aim

To develop a model amadumbe-based gluten-free dough with added millet and psyllium with improved starch functionality and digestion kinetics.

2.12.2. Objectives

1. To study the effect of annealing, heat-moisture-treatment and psyllium fibre addition on the functionality and digestibility profile using maize starch as a model for amylose content variation.
2. To create an optimised flour blend of amadumbe, millet and psyllium flour and test the functionality and starch digestibility profiles of the resulting optimised blends.
3. To evaluate the dough-forming properties and the baking quality of the model amadumbe-millet-psyllium dough and to test its application in bread making.

CHAPTER THREE

3. Effects of hydrothermal treatment and psyllium fibre supplementation on the digestibility, pasting and rheological properties of maize starch as a model for amylose content variation

3.1. Abstract

Although the individual effects of hydrothermal treatment, psyllium fibre, and amylose content on starch properties are well documented, their combined interactions remain largely unexplored. This study explored the impact of heat moisture treatment (HMT) and annealing (ANN), combined with 3% psyllium husk fibre (PHF), on the structural, rheological and digestibility properties of maize starch with varying amylose content. The starch samples were analysed for crystallinity, viscosity, and in vitro digestibility. Frequency sweep and FTIR spectra were also determined. Waxy and normal maize starch showed typical A-type diffraction patterns, while high-amylose starch exhibited B-type diffraction patterns. HMT-treated starches had significantly ($p \leq 0.05$) lower peak and final viscosities, while ANN-treated starches exhibited higher viscosities. HMT and ANN treatments generally increased G', regardless of fibre addition, except for waxy maize starch. Hydrothermal treatments alone had minimal impact on slowly digested starch (SDS) and resistant starch (RS) content, but psyllium significantly increased SDS and RS levels. Findings from the study suggest that combining HMT and ANN with PHF supplementation can significantly modulate starch digestibility by rendering them ideal for functional food uses with prospective health implications, such as diabetes management and improved gut health. Future research should focus on the application of the starch blends in product formulations, as well as the evaluation of their shelf life and sensory properties in the newly developed products.

3.2. Introduction

Starch, a common and sustainable agricultural resource, is the major constituent of staples such as cassava, maize, potato, rice, and wheat. According to Verma et al. (2024), rice, maize, and wheat make up approximately 67% of the global food energy intake. A significant proportion of the starch consumed by humans is in the refined form, primarily through processed food products such as white bread, white pasta, cereals, pizza, samosas, biscuits, and cakes. Unfortunately, the consumption of refined starchy products has been associated with chronic issues such as obesity, cardiovascular diseases and diabetes, which affects more than 500 million people globally, especially between the ages of 20 and 79 years (Magliano et al., 2021). This phenomenon prompted a shift in focus towards healthier starch alternatives, especially resistant starches.

Resistant starch (RS) and slowly digestible starch (SDS) are preferred to attenuate the glycaemic index. RS has now been shown to have prebiotic potential, conferring the needed balance to the gut microbiome as well as modulating the production of postbiotics (Zhang and Bao, 2023). The increasing awareness about these health benefits has raised the demand for healthy starch fractions and the need for starch modification strategies (Wang et al., 2023). Modification via thermal treatment has been a widely adopted starch treatment method due to its lower cost, safety, environmental friendliness and efficiency (Fonseca et al., 2021). Annealing (ANN) and heat moisture treatment (HMT) are thermal modification strategies which are effective in altering the relative crystallinity, water absorption capacity, and pasting properties of starch molecules (Chung et al., 2009, Kim et al., 2023). In HMT, starch is heated above the glass transition temperature (80–140°C) for a predetermined amount of time (1–24 hours) and at low moisture content <35%, whereas in ANN, starch is exposed to temperatures between the glass transition temperature and the gelatinisation onset temperature for a more prolonged amount of time at higher moisture content >40% (Kim et al., 2023).

The interaction between starch and various food constituents, such as dietary fibre (DF) can impact starch functionality (Bello-Perez and Flores-Silva, 2023). Interactions between starch and dietary fibre have been demonstrated to enhance the structural, textural, and functional characteristics of starch in multiple food applications (Mancebo et al., 2015, Nagy et al., 2020, Siminiuc and Ţurcanu, 2023). Structural modifications in both the amorphous and crystalline phases of starch affect granular swelling, as well as thermal properties, and enzyme susceptibility in the presence of dietary fibre (Siminiuc and Ţurcanu, 2023). The inclusion of dietary fibre will result in modulation of starch digestibility, improving the glycaemic response, enhancing Satiety and modifying dough rheology and texture (Kamil et al., 2023). DF encapsulates starch via hydrogen bonding, hydrophobic interactions, as well as physical mechanisms like water competition and steric hindrance, limiting gelatinization and enzyme access, and consequently reducing digestibility (Grundy et al., 2016, Zhou et al., 2021). One such DF is psyllium husk, derived from *Plantago ovata* seeds, which has been associated with improved glycaemic control, decreased cholesterol levels, as well as reduced risks of cardiovascular diseases (Belorio and Gómez, 2021). While other fibres provide various health benefits, psyllium was chosen for this study due to its unique combination of gel-forming ability, water-binding capacity, and proven effectiveness in treating digestive disorders (Ren et al., 2020b). Additionally, psyllium is a non-fermented fibre, which means it does not affect mineral absorption (McRorie Jr et al., 2021). Psyllium husk has immense potential in the production of functional foods as its incorporation into various food preparations enhances the respective nutritional properties and functionality with little or no effect on the original flavour (Franco et al., 2020).

The three most common maize starches include waxy, normal and high amylose. Waxy starches are primarily sourced from waxy corn and consist of nearly 100% amylopectin, resulting in stable, clear pastes with minimal retrogradation. This makes them ideal for frozen foods, canned goods, and gluten-free baking (Le Thanh-Blicharz and Lewandowicz, 2020). Normal starch, derived from corn, wheat, potatoes, or rice, is composed of 70-80% amylopectin and 20-30% amylose. It serves as a versatile thickener and binder in various food products, along with applications in other industries (Goswami and Mahanta, 2021). High-amylose starches, containing over 40% amylose, are used in resistant starch applications for low-calorie foods and improving glycaemic control (Qin et al., 2020, Faisal et al., 2022).

While heat–moisture treatment (HMT) and annealing (ANN) have led to substantial improvements in starch functionality, the incorporation of dietary fibre can further enhance the digestibility and nutritional quality of hydrothermally treated starches. Furthermore, studies have also shown the complex interplay between varying amylose content of starch, moisture, and heat treatment in determining the functional properties of starch (Yang et al., 2019, Yang et al., 2021b). Starch modification strategies face several key limitations. Traditional methods often rely on chemical additives, which do not meet the demand for clean-label products. Additionally, some processes can lower dietary fibre and nutritional value (Bender and Schönlechner, 2020). Many techniques involve complexity and high costs (Siminiuc and Ţurcanu, 2023). Finally, native starch is prone to issues such as retrogradation, syneresis, and instability under varying conditions (Mathobo et al., 2021). While the use of psyllium and heat treatment individually is well-established, the specific synergy between them and the optimization of this combination for particular applications is still relatively unexplored.

Therefore, the combination of hydrothermal treatment and psyllium fibre supplementation will increase resistant starch content and slow digestibility than either treatment alone.

HMT modifies the internal structure of starch granules and reduces enzyme accessibility (Li et al., 2024). ANN enhances crystal perfection and improves starch's ability to resist retrogradation (Yamaguchi et al., 2019). HMT and ANN together with psyllium fibre further boost the formation of resistant starch, benefiting glycaemic control and gut health (Baptista et al., 2024). Additionally, HMT enhances starch stability under high temperatures and varying pH levels (Mathobo et al., 2021), leading to a heat-stable and retrogradation-resistant starch (Wang et al., 2015) suitable for low-glycaemic index food products. Thus, the current study examined the synergistic influence of psyllium husk and hydrothermal treatment of maize starch with varying amylose contents on starch structure modification and digestibility. The study hypothesized that combining hydrothermal treatment (HMT/ANN) of maize starch with psyllium husk will synergistically increase the resistant starch content compared to either treatment alone. HMT/ANN modifies starch structure to be less digestible, while psyllium may further hinder enzymatic access to starch. Varying amylose content could influence the extent of this synergism (Lv et al., 2021). This study investigated the effect of heat moisture treatment (HMT) and annealing (ANN), combined with 3% psyllium husk fibre (PHF), on the structural, rheological and digestibility profiles of maize starch containing varying amylose content.

3.3. Materials and methods

3.3.1. Materials

100% Maize starches (waxy and normal) were purchased from Sigma-Aldrich, and maize starch (high amylose) (100%) was obtained from Ingredion. The average amylose content of waxy, normal and high amylose starches where >1%, 27% and 55%, respectively, as per the manufacturer's information. Psyllium husk fibre (PHF) was purchased from a health shop in Durban, South Africa and did not include any nutritional information. Amyloglucosidase (260 U/mL) from *Aspergillus niger* and porcine pancreas α-amylase type VI-B (10 U/mg) were procured from Merck-Sigma-Aldrich. Glucose oxidase-peroxidase (GOPOD) assay kits were procured from Megazyme International Ireland Ltd. (Wicklow, Ireland). All reagents, solvents, and chemicals utilised in this investigation were of analytical grade and provided by local vendors. HMT and ANN treated starch samples with added fibre were obtained by adding 3% psyllium husk fibre (w/w) to the heat-modified maize starch samples (waxy, normal and high amylose).

3.3.2. Proximate analysis

Moisture, fat and ash contents of the psyllium husk powder were determined using AOAC methods 990.03, 945,16 and 942.05 respectively (Horwitz, 2000). Using a commercial assay kit (K-TDFR, Megazyme, Ireland), an enzymatic–gravimetric analysis was conducted to determine the total dietary fibre content. Protein content was determined by the Kjeldahl method (6.25xN), and total carbohydrates were determined using the phenol sulphuric acid method.

3.3.3. Heat moisture treatment (HMT) of starch

HMT was executed using the method described by Franco et al. (1995) with slight adjustments. The moisture content of the maize starch samples (waxy, normal and high amylose) was adjusted to 20% and left to equilibrate at 4 °C for 24 h in stoppered flasks. Following equilibration, these samples were then placed at 110°C for 16 h in a Prolab Incubator (Model ODC 400, SA) and subsequently brought down to room temperature. Samples were then air dried at 40°C for 12 h, milled to a final particle size of 180 µm. The HMT-treated starch was stored in airtight containers until further analysis.

3.3.4. Annealing (ANN) of starch

During ANN treatment, starch was held at a moisture level exceeding 40% and subjected to controlled heating within the range defined by its glass transition and gelatinization onset temperatures for an extended period. ANN was executed using a method earlier outlined by Liu et al. (2015b). Briefly, a starch slurry, obtained by mixing maize starch (waxy, normal, high amylose) in deionised water (1:4 w/v), was left to equilibrate overnight at 4°C in stoppered flasks. Thereafter, it was incubated at 55 °C in a constant temperature convection oven (Lasec, Model DHG9240A, SA) for 24 h. Following cooling to room temperature, the samples were air-dried at 40 °C for 12 h. Thereafter, the samples were ground until a final particle size of 180 µm was obtained. They were kept in airtight containers until further analysis.

3.3.5. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectra (ATR FTIR) of dry native (waxy, normal and high amylose) and modified (HMT and ANN) maize starches were obtained using a Fourier transform infrared spectrometer (Agilent Cary 630 FTIR, USA), ranging in wavelength from 4000 to 600 cm^{-1}. The resolution was 4 $cm^{-1,}$ and a total of 32 scans were done.

3.3.6. X-ray diffraction

X-ray diffraction measurements of dry native maize starches (waxy, normal, high amylose), heat-treated ANN and HMT maize starches (waxy, normal, high amylose) and heat-treated ANN and HMT maize starches with 3% added psyllium were conducted using a X-ray diffractometer (PANalytical X'pert PRO -Netherlands) fitted with a Cu Kα (λ = 0.154 nm) radiation source and operated at 40 kV and 40 mA. The diffractograms were scanned between 5 to 55° (2θ), with a scanning speed of 1°/min. The relative crystallinity for all samples was measured (Lopez-Rubio et al., 2008).

3.3.7. Determination of pasting properties

The pasting parameters of native and modified maize starches were investigated using a Rapid Visco-Analyzer (RVA-4500, Perten Instruments, Sweden), according to Liu et al. (2016), except that 3 g of starch was used. Pasting parameters, including peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperatures, were directly measured from the RVA.

3.3.8. Rheological properties of native, HMT and ANN starch paste

The rheological parameters of the starch pastes were assessed utilising a rheometer (MCR102 Rheometer, Anton Paar, Austria), as outlined by Zhong et al. (2023). The measurement equipment utilised consisted of a parallel plate with a diameter of 50 mm and a spacing of 1 mm. The paste was applied between the plates with a spatula, and a thin layer of mineral oil was applied around the rim to inhibit drying before the commencement of the test. The investigation was conducted at a constant temperature of 25 °C and a strain of 0.5% within the linear viscoelastic area. Frequency sweep experiments were conducted within the range of 0.1 to 10 rad/s. The dynamic rheological metrics employed to assess the paste were the storage modulus (G′), loss modulus (G″), and complex modulus (G*).

3.3.9. In vitro starch digestibility

The analysis of starch digestibility was conducted using, with minor adjustments, the methodology of Chen et al. (2017), Englyst et al. (1992). Two hundred milligrams of starch sample (dry basis) was precisely measured into a 50 ml centrifuge tube with a screw cover. Subsequently, ten glass beads (5 mm in diameter) were introduced, followed by the addition of 15 ml of sodium acetate buffer (0.2 mol/L, pH 5.2). After extensive mixing, the tubes were then stabilised in a water bath at 37°C for 10 min. Thereafter, 5 ml of sodium acetate buffer containing amyloglucosidase (15 U/ml) and porcine pancreatic α-amylase (290 U/ml) was introduced into each tube, which were then subjected to oscillation at 300 rpm in a constant temperature shaking incubator at 37°C. Aliquots of the hydrolysed solution (0.5 ml) were collected at 20 and 120-min intervals and promptly transferred into a centrifuge tube containing 4 ml of 100% ethanol to inactivate the enzymes. The tubes were subsequently centrifuged at 4000 × g for 10 min. The glucose concentration of the supernatant was quantified using the glucose oxidase-peroxidase (GOPOD method) assay kits (Wicklow, Ireland). The percentages of RDS, SDS and RS in the starch samples were analysed according to their definition by Englyst et al. (1992).

The equations were as follows:

$$RDS\ (\%) = (G20 - G0) \times 0.9 \times 100$$

$$SDS\ (\%) = (G120 - G20) \times 0.9 \times 100$$

$$RS\ (\%) = [(TS - RDS - SDS)/TS] \times 100$$

where G20 and G120 refer to the level of glucose released after 20 and 120 minutes, respectively; G0 denotes the free glucose content, while TS represents the total starch weight. The proportion of hydrolysed starch was determined by multiplying the glucose content by a factor of 0.9.

3.3.10. Statistical analysis

All experiments were conducted in triplicate unless specified differently. Data analysis was conducted using Analysis of Variance (ANOVA), and means were compared utilising Fisher's Least Significant Differences Test ($p < 0.05$ and $p < 0.01$).

3.4. Results and discussion

3.4.1. Proximate composition of psyllium husk fibre (PHF)

The dietary fibre content was high (approximately 76%) and compared favourably with literature (Alim et al., 2024, Aldughpassi et al., 2021, Waleed et al., 2022). Typical of all fibres, the ash and total fat observed in this study were low; however, while the ash content (2.98 ±0.05%) was similar to those documented recently (Waleed et al., 2022, Alim et al., 2024), the fat content (1.66 ± 0.11%) showed a significant increase compared to that reported by Waleed et al. (2022) and Aldughpassi et al. (2021). The protein content of 3.72 ± 0.35 recorded in this experiment was comparable to values reported by (Waleed et al., 2022, Franco et al., 2020); however, it was significantly higher than that of Aldughpassi et al. (2021). These observed discrepancies could be due to origins and source, purity and composition and processing methods of psyllium fibre,

Table 3. 1 Nutritional composition of psyllium husk fibre

NUTRIENTS	%
Protein	3,72 ± 0,35
Fat	1,66 ± 0,11
Moisture	10,32 ±0,04
Ash	2,98 ±0,05
Total carbohydrates	79.32 ±0.06
Total dietary fibre	76.09 ±0,00

Results are represented as mean ±SD (n = 3)

3.4.2. Structural characteristics of modified starches

The FTIR spectra of the waxy, normal and high amylose starches before and after the physical treatments showed similar peak intensities (Figure 3.1). The characteristic peaks of the three starch samples did not show any significant shift. The spectra recorded in this study displayed strong signals in the carbohydrate zone and less intense signals in the protein zone which were designated to be 900-1200 cm^{-1} and 1500 cm^{-1} -1750 cm^{-1} respectively as shown in previous studies (Huang et al., 2016, de Carvalho Batista Muniz et al., 2023). The intensity of bands slightly decreased after heat modification by ANN and HMT. However, the native maize starches, except WCS, showed higher peak intensities compared to those of the heat-modified starches. This may be due to heat-facilitated starch gelatinization, which leads to the absorption of water by starch particles, resulting in their expansion resulting in hydration reactions inside the starch particles and finally fractures in the intermolecular hydrogen bonds (Wang et al., 2016)

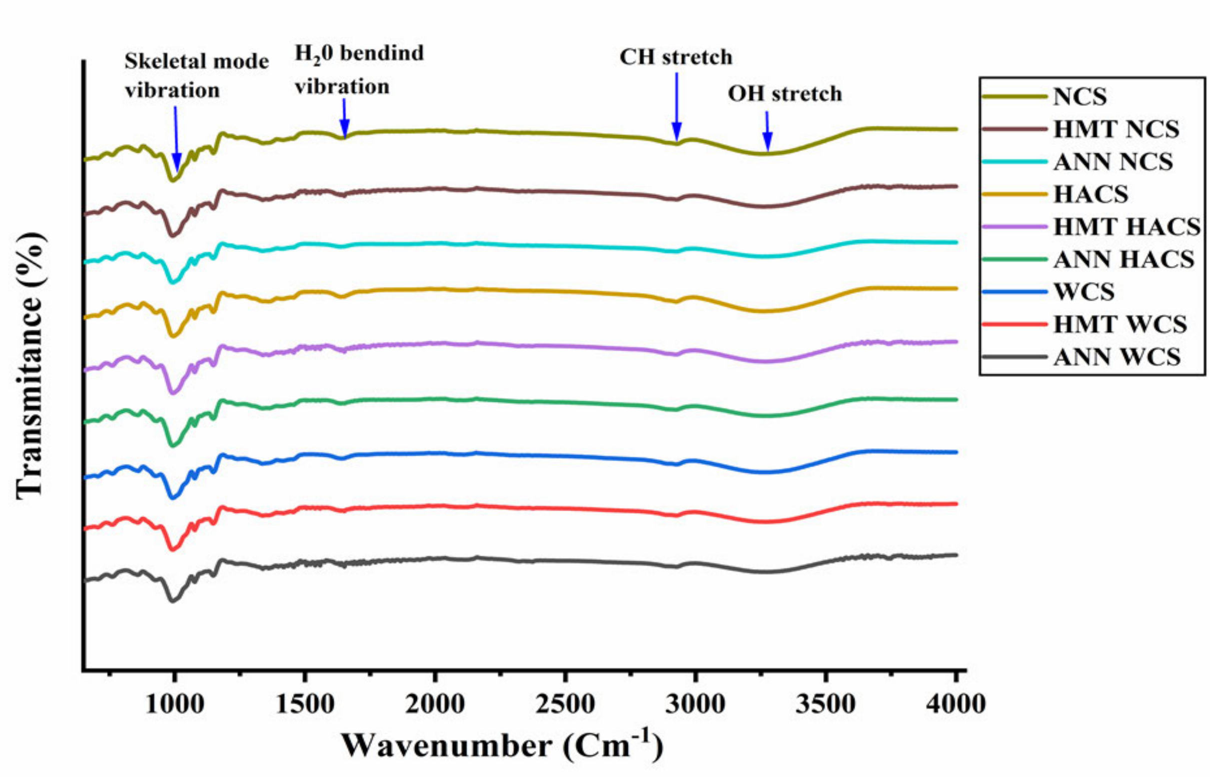

Figure 3. 1. FTIR spectra patterns of native waxy (WCS), normal (NCS), high amylose (HACS) maize starches, and hydro thermally treated: annealed (ANN) and heat moisture treatment (HMT) maize starches

The XRD patterns of corn starch in the native forms and the physically modified forms are illustrated in Figure 3.2. The native waxy and normal maize starches showed diffraction peaks that indicated typical A-type crystallinity (ranging from 15 to 23°) with a major peak at 15.12°, a double peak at

17.17° and 18.01° and another single peak at 23.10°. However, high-amylose maize starch showed typical B-type crystallinity, with a single major peak at 15.09° and 17.99° and a minor peak at 23.03°. Furthermore, the peak positions were not affected by HMT or ANN, as all starch samples maintained their A-type crystallinity. Previous research has demonstrated that heat treatment methods, especially ANN, do not lead to major modifications in the diffraction peaks of native starch (de Carvalho Batista Muniz et al., 2023). However, what was noticed was the weakening and strengthening of diffraction intensities at 17° and 18°.

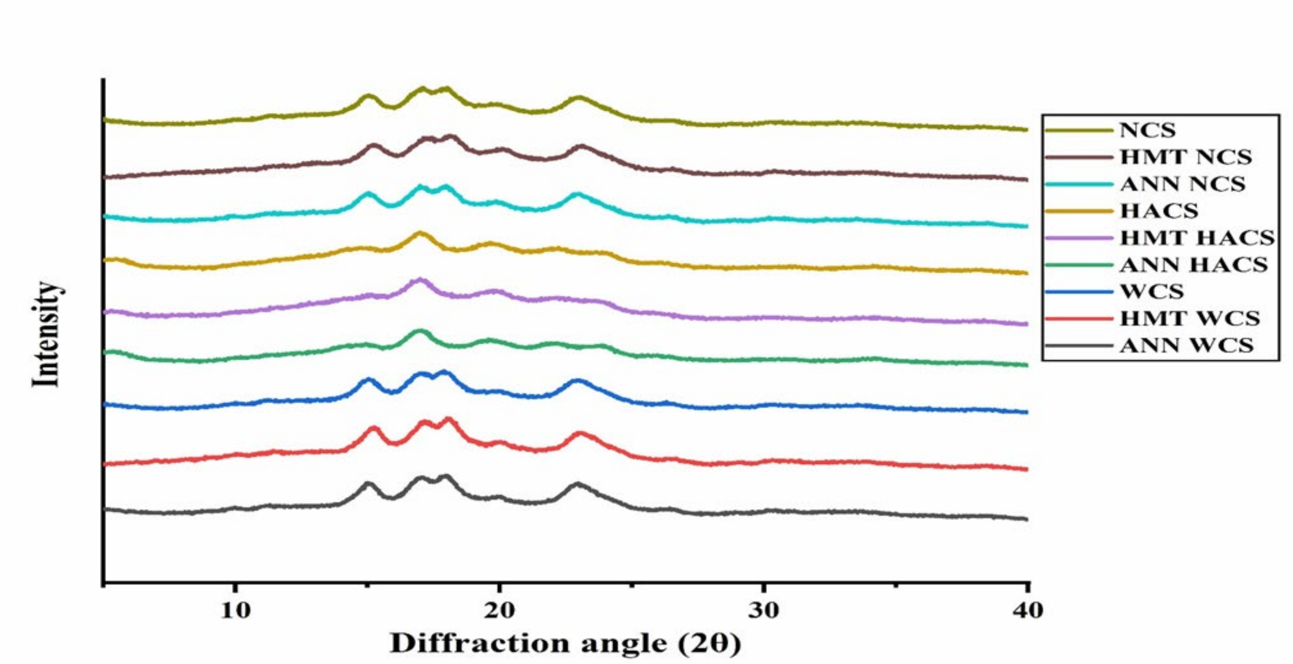

Figure 3. 2. X-ray diffraction patterns of native waxy (WCS), normal (NCS) and high amylose (HACS) maize starch, hydrothermally treated: Heat moisture treatment (HMT) and annealed (ANN) maize starch samples

HMT resulted in a similar structure for WCS and NCS; however, HACS showed significant changes to the internal structure of the starch. It is suggested that HMT elicited significant changes in the crystallinity of HACS due to the disruption of its predominant amylose regions, and this has been established to improve resistant starch levels in high amylose rice starch (Lee and Chung, 2022). Starches with an A-type crystalline configuration are generally more prone to enzymatic hydrolysis and, therefore, are more readily digestible (Suma P and Urooj, 2015). This is because the A-type structure has a less dense crystalline packing, allowing enzymes easier access to the glycosidic bonds. B-type starches tend to have increased resistance to enzymatic degradation as compared to A-type (Pan et al., 2018). This is often attributed to their higher degree of crystallinity, the presence of double helices, and a more compact structure that limits enzyme accessibility (Pan et al., 2018).

3.4.3. Pasting characteristics

The pasting curves of the maize starches (Figure 3.3) and pasting parameters (Table 3.1) showed notable differences in the pasting properties of the different maize starches after both thermal modification as well as the addition of the fibre. Results showed that with WCS and NCS (with or without fibre supplementation), there was no significant variance in the pasting temperature (PT) after ANN treatment; however, an increase was observed after HMT. HMT has been demonstrated in previous studies to cause an increase in PT, which signifies an increase in the strength of the native starch due to higher resistance of the starch granules to swelling and gelatinization (Pinto et al., 2012). HMT maize starch had significantly lower peak viscosity (PV) than that of ANN and native starch, while the HMT-modified starch had a significantly lower PV compared to the unmodified. ANN-modified starches were observed to have higher PV values compared to the unmodified starch. Heat modification was observed to alter the setback viscosity (SB) of all maize starches. The SB was significantly different for both HMT and ANN. HACS results showed that a higher temperature was required to form a paste, which impacted all other parameters during the analysis. Lv et al. (2021) showed that HACS forms a paste at high temperatures, has a lower peak viscosity during heating, but creates a stronger gel with higher setback viscosity upon cooling, possibly reducing digestibility. Pasting properties significantly affect digestibility because retrograded starches are less digestible than gelatinized starches. Amylopectin content influences setback viscosity, indicative of retrogradation (Mancebo et al., 2015), thus impacting digestibility. Higher amylopectin content usually reduces setback viscosity (Chen et al., 2021, Della Valle et al., 1996), potentially enhancing starch digestion.

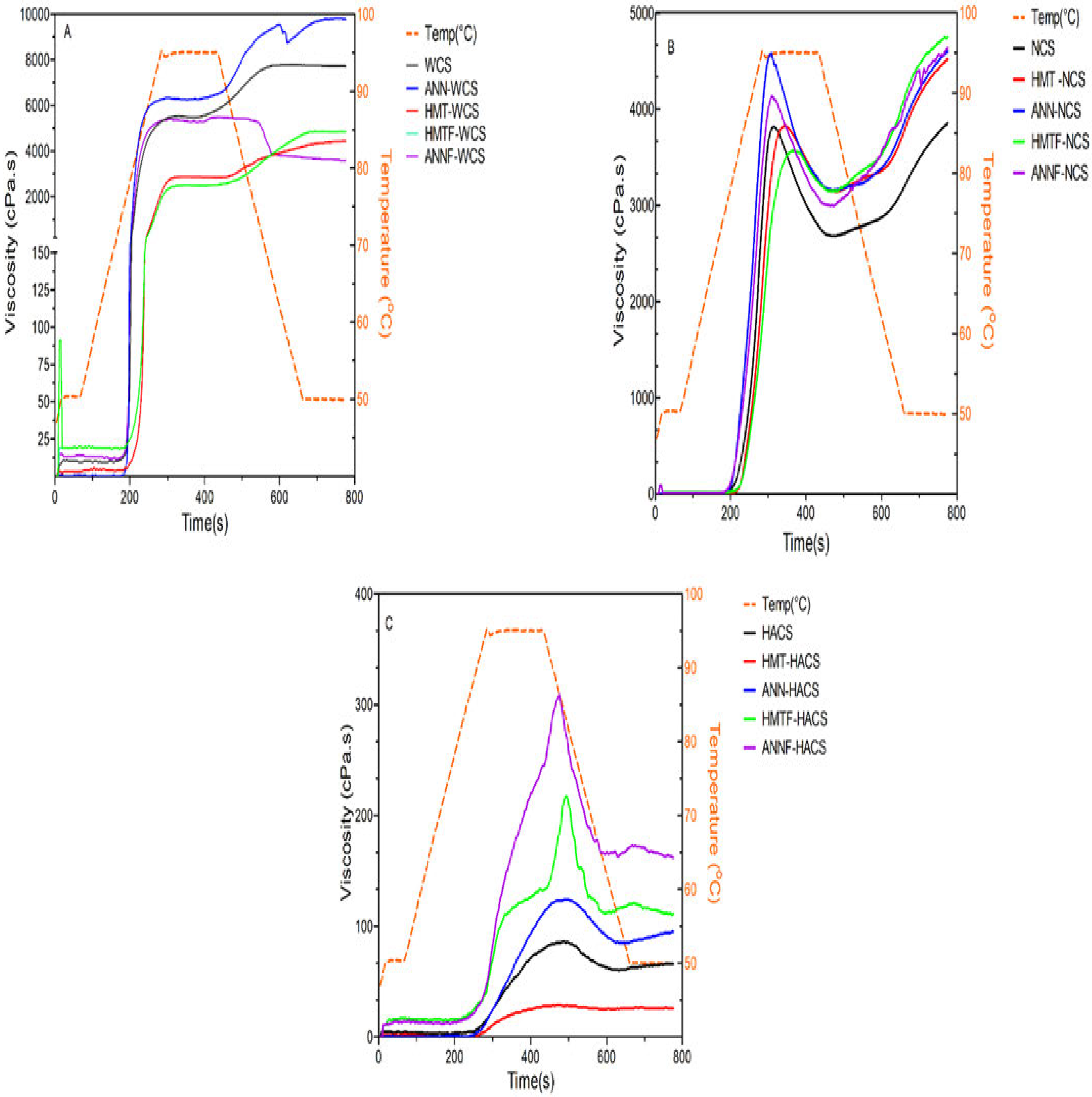

Figure 3. 3. Pasting profiles of native: waxy (WCS), normal (NCS) and high amylose (HACS), hydro thermally treated: annealed (ANN) and heat moisture treatment (HMT), and heat-treated maize starches with 3% added psyllium fibre (HMTF) (ANNF). (A) waxy corn starch, (B) normal corn starch and (C) high amylose corn starch

Table 3. 2 Pasting parameters of native and modified corn starch

Sample	Pasting temperature	Peak Viscosity	Trough Viscosity	Final Viscosity	Breakdown Viscosity	Setback Viscosity
	°C			cPa.s[1]		
Waxy corn starch						
Native	77.88±0.60^{a}	5788±111.72^{b}	5510±43.00^{a}	7267±347.19^{b}	246±12.02^{b}	2335±109.60^{a}
HMT	84.78±0.04^{a}	2091±0.71^{c}	2073±13.44^{c}	2933±34.65^{d}	26±0.71^{b}	885±41.72^{c}
ANN	78.28±0.04^{a}	6568±198.70^{a}	6136±114.55^{a}	8742±210.72^{a}	218±10.61^{b}	2463±122.33^{a}
HMTF	84.38±0.53^{a}	2209±34.65^{c}	2189±32.35^{c}	3775±82.73^{c}	19±0.71^{b}	1586±50.20^{b}
ANNF	78.30±0.00^{a}	6210±50.20^{ab}	3876±189.50^{b}	4075±202.94^{c}	2239±106.07^{a}	593±21.21^{c}
Normal corn starch						
Native	79.88±0.04^{a}	3821±67.88^{a}	2962±89.10^{ab}	3849±38.89^{c}	859±21.21^{ab}	881±41.72^{bc}
HMT	85.60±0.00^{a}	3178±0.71^{b}	2757±45.25^{b}	2509±124.45^{d}	437±21.21^{cd}	687±27.58^{c}
ANN	79.03±0.11^{a}	4070±8.49^{a}	3108±14.14^{a}	4259±2.83^{b}	962±5.66^{a}	1151±16.97^{b}
HMTF	84.35±0.57^{a}	3268±34.65^{b}	3089±7.07^{a}	4150±17.68^{bc}	201±9.90^{d}	1061±10.618^{b}
ANNF	77.85±0.57^{a}	3917±43.84^{a}	3264±27.58^{a}	4828±184.55^{a}	654±16.26^{bc}	1618±80.61^{a}
High amylose corn starch						
Native	0.00±0.00^{a}	57±0.00^{b}	44±0.71^{c}	55±0.71^{b}	14±0.00^{b}	11±0.71^{bc}
HMT	0.00±0.00^{a}	12±0.71^{c}	11±0.71^{d}	13±0.00^{c}	1±0.00^{c}	3±0.71^{d}
ANN	0.00±0.00^{a}	59±1.41^{b}	44±1.41^{c}	59±2.12^{b}	15±0.00^{b}	15±0.71^{b}
HMTF	0.00±0.00^{a}	61±0.71^{b}	54±0.71^{b}	61±1.41^{b}	7±0.71^{c}	8±0.00^{cd}
ANNF	0.00±0.00^{a}	137±0.71^{a}	100±2.83^{a}	161±2.83^{a}	39±1.41^{a}	59±2.83^{a}

Results are represented as mean ±SD (n=2)
Values in the same column with ^{a,b,c} are significantly different ($p < 0.05$)
HMT- Heat moisture treated starch; HMTF- Heat moisture treated starch with fibre; ANN- Annealed starch; ANNF- Annealed starch with fibre

3.4.4. Rheological properties

The frequency sweep of the cooled starch pastes was analysed to characterize the gel viscoelasticity (Figure 3.4). Results revealed that all starchy pastes presented a typically “solid-like” gel behaviour with G' > G" (Qiu et al., 2015). Results for WCS showed that both HMT and ANN treatment increased the G’ moduli of the starch samples. Whilst the inclusion of fibre resulted in reduced G’. Furthermore, the resultant effect of the fibre on the final moduli was significantly more pronounced than that of the hydrothermal modifications, judging from the close similarity between the G” of both ANNF WCS and HMTF WCS. Being low in amylose, the addition of fibre to WCS leads to a more pronounced decrease in both moduli as WCS depends on amylopectin (which is more easily disrupted), rather than amylose, for gel formation. This indicates that in WCS systems, fibre is the primary determinant of final viscous behaviour, outweighing the contribution of hydrothermal modifications. From a formulation perspective, this highlights the need to align product texture expectations with the intrinsic limitations of amylopectin-dominated starch networks. Typically, increases in both storage and loss moduli are recorded on the addition of fibre to starch samples, as most of the previous studies have been on native starches (Qiu et al., 2015, Liu et al., 2019). The complex modulus (G*) reflects the overall resistance of a substance to deformation under both elastic (storage modulus, G') and viscous (loss modulus, G") forces (Qiao et al., 2023). The general trend for G* observed in this study was ANNF > HMTF > HMT > ANN > unmodified starch. As expected, the effect of HMT alone on the starch samples was more pronounced than that of ANN concerning the increase in the G*, as HMT tends towards a more molecular rearrangement and granule rigidity when compared to ANN. Characterization of hydrothermally treated maize starches revealed structural changes linked to amylose content, thereby affecting enzyme accessibility and digestibility (Siminiuc and Ţurcanu, 2023). The combination of hydrothermal treatment and psyllium supplementation synergistically reduces starch digestibility by rendering the starch less susceptible to enzymatic breakdown, due to hydrothermal treatment and further obstructing enzyme accessibility because of psyllium addition (Belorio and Gomez, 2020), especially in fibre-supplemented ANN-treated NCS, where the most noticeable increase in G’ was observed.

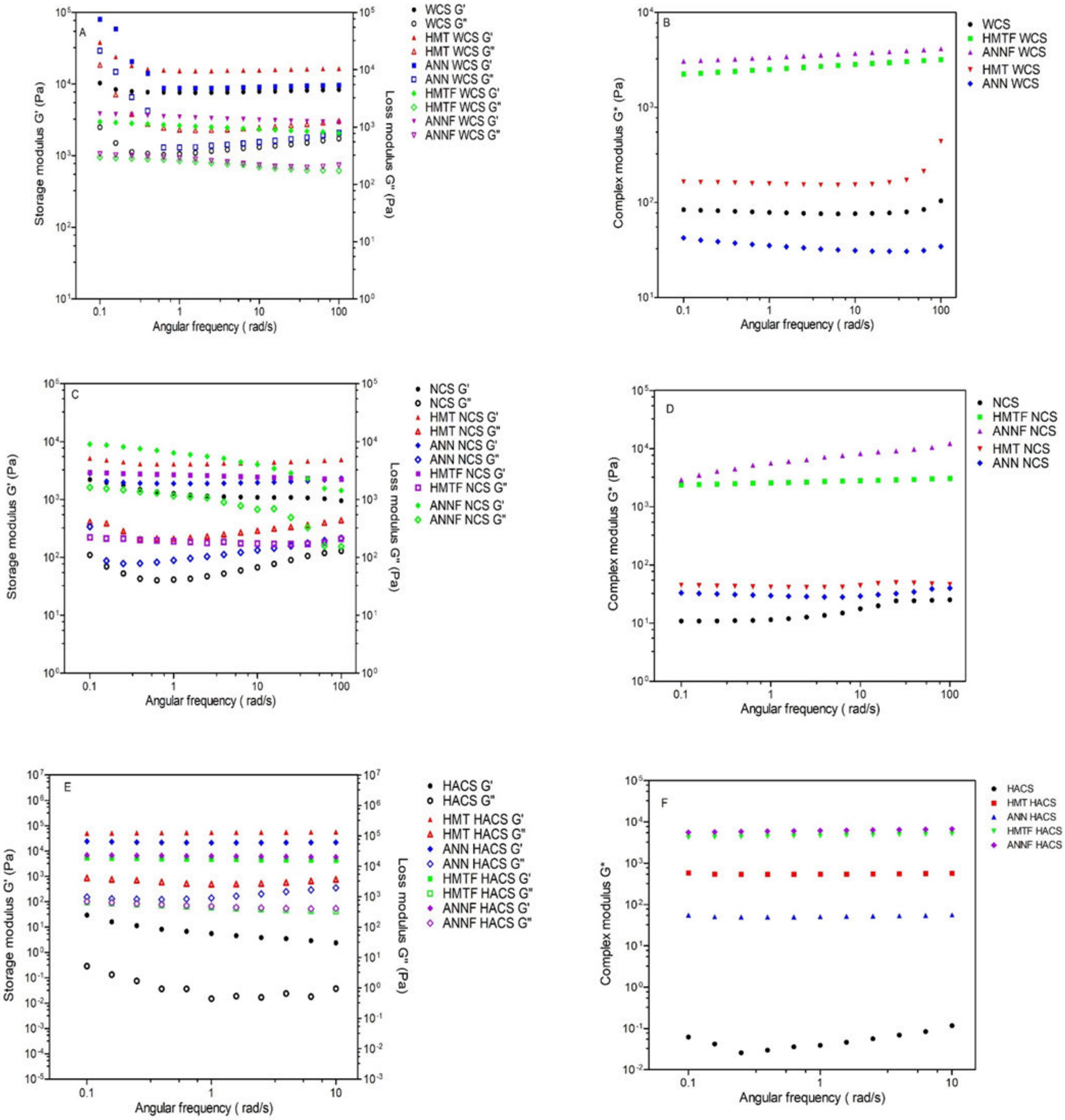

Figure 3. 4. Frequency sweep data of native (WCS, NCS, HACS), heat moisture treatment (HMT) and annealed (ANN), and heat moisture treatment and annealed starches with 3% added psyllium (HMTF, ANNF) of maize starch samples. A, C, E show the storage modulus (G') and loss modulus (G") graphs, and B, D, F show the complex modulus (G*) data graphs. A&B, C&D, E&F represent waxy corn starch, normal corn starch and high amylose corn starch, respectively.

3.4.5. Starch digestibility of native, annealed, and heat-moisture-treated samples with or without 3% psyllium

ANN and HMT significantly impacted the digestibility profile of the different starches (Table 3.2). HMT and ANN modified starch showed a general increase in resistant starch (RS) and slowly digestible starch (SDS) content and a slight decrease in RDS (rapidly digestible starch), with a few exceptions. The enhancement of starch resistance was largely dependent on the levels of amylose present in each starch. According to da Rosa Zavareze and Dias (2011), the starch amylose content is the most important variable accountable for the rise in starch resistance, hence, the modification of the starch digestibility profile. Reduced starch digestibility is generally demonstrated by lower RDS content and a concurrent increase in SDS and RS content (Liu et al., 2015a). This arises as a result of structural modifications in starch granules, which become more robust, hindering enzyme susceptibility during breakdown (Chung et al., 2012). Thus, for WCS, the most significant modification was observed with ANN + 3% psyllium, with an increase of 17.09% amongst the SDS and RS fractions in comparison to native WCS. In the case of normal corn starch, SDS and RS fractions significantly increased ($p \leq 0.05$) by 24.87% and 14.88% for HMT +3% psyllium and ANN +3% psyllium, respectively. With high amylose corn starch, there was a significant increase ($p \leq 0.05$) in SDS and RS fractions of 17.97 and 21.21% for HMT + 3% psyllium and ANN + 3% psyllium in that order. In a recent study, HMT increased the SDS and RS of yam starch by 1.71–3.52% and yam flour by 1.52–4.43%, respectively (Yu et al., 2021). RDS, SDS and RS contents attained in this study exceeded those reported by (Wang et al., 2016, Sharma et al., 2015). This may result from the synergistic action of hydrothermal treatment and psyllium supplementation. Digestibility data demonstrated that the addition of PHF played a major role in reducing the digestibility of the starches, as observed in the rise in the RS and SDS levels of the fibre-supplemented starches. The increase in resistant starch observed when dietary fibre was incorporated into hydrothermally treated high-amylose starch may be primarily due to the inhibition of starch digestion (Li et al., 2024). Soluble dietary fibres, such as psyllium, enhance the viscosity of the mixture (Ren et al., 2020a) and can establish a physical block that impedes the accessibility of digestive enzymes. Furthermore, dietary fibres can interact with starch molecules to form complexes that exhibit resistance to enzymatic breakdown (Ren et al., 2020a). For instance, specific fibres may bind to amylose, resulting in a complex structure that is less amenable to enzymatic hydrolysis.

Table 3. 3 In vitro starch digestibility of waxy, normal and high amylose maize starches

STARCH	RDS (%)	SDS (%)	RS (%)
WAXY CORN STARCH			
Native	34,38±0,00^{a}	3,78±0,15^{d}	61,84±0,15^{e}
HMT	31,68±0,05^{b}	2,07±0,05^{e}	66,25±0,00^{c}
ANN	31,61±0,15^{b}	5,97±0,13^{b}	62,42±0,02^{d}
HMTF	28,00±0,13^{c}	4,75±0,05^{c}	67,25±0,08^{b}
ANNF	17,29±0,07^{d}	8,73±0,00^{a}	73,98±0,07^{a}
NORMAL CORN STARCH			
Native	55,98±0, 09^{a}	3,96±0,07^{c}	40,06±0,02^{e}
HMT	52,83±0,04^{b}	2,88±0,07^{d}	44,29±0,06^{c}
ANN	52,92±0,06^{b}	3,69±0,03^{c}	43,39±0,02^{d}
HMTF	31,11±0,00^{d}	22,70±0,26^{a}	46,19±0,26^{b}
ANNF	41,10±0,14^{c}	9,20±0,03^{b}	49,70±0,11^{a}
HIGH AMYLOSE CORN STARCH			
Native	31,32±0,05^{b}	26,37±0,06^{a}	42,31±0,01^{e}
HMT	30,06±0,02^{c}	3,87±0,01^{c}	66,07±0,03^{c}
ANN	32,04±0,10^{a}	3,51±0,38^{c}	64,45±0,08^{d}
HMTF	13,35±0,03^{d}	6,30±0,02^{b}	80,35±0,01^{b}
ANNF	10,11±0,02^{e}	1,35±0,11^{d}	88,54±0,08^{a}

Results are represented as mean ±SD (n=2)
Values in the same column with ^{a,b,c} are significantly different ($p < 0.05$)
RDS – Rapidly digestible starch, SDS – Slowly digestible starch, RS – Resistant starch, HMT- Heat moisture treated starch; HMT- Heat moisture treated starch with fibre; ANN- Annealed starch; ANN- Annealed starch with fibre

3.5. Conclusion

The study findings highlighted that hydrothermal treatment (HMT and ANN) on maize starch, along with the supplementation of psyllium fibre, presents a practical and promising method for enhancing the formation of resistant starch. HACS is particularly effective in increasing resistant starch fractions because of its natural tendency to retrograde. The addition of dietary fibre can further promote this retrogradation, resulting in even higher levels of resistant starch. Increasing the resistant starch content in foods can improve their nutritional profile. Resistant starch serves as a type of dietary fibre, providing advantages that include greater glycaemic regulation, heightened satiety, and improved gastrointestinal health. Applications in the food industry include bakery products, snack foods, pasta and noodles formulation, as well as breakfast cereals. These applications align with the increasing consumer interest in foods with improved nutritional value and specific health benefits, and the ability to tailor starch properties to aid in creating specialized products for specific dietary needs. The application of the starch blends in product formulations, as well as the evaluation of their shelf life and sensory properties in the newly developed products, should be the focus of further research. Further research is needed to validate scalability for industrial food processing.

CHAPTER FOUR

4. Techno-functional properties and digestibility profiles of gluten-free amadumbe-millet flour blends supplemented with psyllium fibre

4.1. Abstract

The development of gluten-free products with improved nutritional quality and texture remains a significant challenge, particularly for individuals with celiac disease and gluten intolerance. In response to current emerging trends, additive-free, clean-label gluten-free (GF) products, a GF flour blend of millet, amadumbe and psyllium fibre was developed. These ingredients are locally sourced, economical, nutritionally dense, and provide potential health-promoting properties. This study investigates the techno-functional and digestibility profiles of a GF flour blend made from amadumbe and millet, supplemented with psyllium fibre. Viscosity, structural stability, and pasting properties of the blend. were analysed. *In vitro* starch digestibility tests were performed to evaluate the nutritional starch fractions. Results revealed that the supplementation of psyllium fibre with the GF flour blend improved the viscoelasticity and pasting properties. contributing to improved functional properties. For structural analysis, XRD showed a typical A-type configuration with no major changes, and FTIR displayed no changes in the peaks identified. Pasting properties of the GF blends supplemented with fibre showed a substantial increase in PV, FV and BV parameters. The GF flour blend with added fibre resulted in reduced starch digestibility, with a 7.7% increase in RS fraction compared to non-supplemented formulations. Findings from the study highlight the potential of psyllium fibre- supplementation of GF flour blends to attenuate starch digestibility by promoting potential health benefits in diabetes, cholesterol and weight management.

4.2. Introduction

A chronic autoimmune disorder of the colon, coeliac disease is brought on by eating gluten-containing foods like wheat, barley, and rye; as well as food products derived from these plants, and has been identified as one of the most prevalent nutritional disorders. Recent estimates show that the global prevalence of celiac disease is approximately 1.4% women and 0.7% men (Bianchi et al., 2024). In South Africa and most other African countries, the incidence of celiac disease is not yet clearly defined; however, in Europe and America, one out of every 133 people seems to be affected by this disease (Grode et al., 2018). In genetically predisposed individuals, gluten exposure triggers an inflammatory immune response that damages the small intestinal mucosa, resulting in villous atrophy and impaired nutrient absorption. If left untreated, this malabsorption manifests as gastrointestinal symptoms such as abdominal pain, diarrhoea, and bloating, and may progress to long-term complications, including iron-deficiency anaemia and osteoporosis. (Gujral et al., 2012). Consequently, a gluten-free diet has been identified as the most effective treatment for the illness; however, this presents many difficulties for people, nutritionists, and food scientists.

The endosperm of wheat, rye, and barley contains a mixture of storage proteins called gluten. Gluten has been identified as a key element in both traditional and modern food production, especially for its role in facilitating the viscoelasticity and texture of baked foods, like bread. It also has applications as a binding agent, thickener, or stabilizer in sauces, soups, and meat products (Zhang et al., 2023). However, as earlier stated, gluten has been highly implicated in the pathology of celiac disease. Furthermore, it has also been linked to other nutritional disorders such as non-celiac gluten intolerance and wheat hypersensitivity (Singla et al., 2024). One of the easiest ways to treat these nutritional problems is to follow a gluten-free diet. However, this option is not without other attendant problems such as low fibre intake, deficiency in micronutrient and higher risk of overnutrition (Świercz et al., 2023). It could result in limited food choices, and economic burden (Bozorg et al., 2024), especially in low-income countries. In this regard, the formulation of sustainable and locally available healthy ingredients from pseudocereals and gluten-free grains such as rice, sorghum, maize and millet has been the focus in recent times (Siddiqui et al., 2022).

Millet (*Pennisetum glaucum*), a vital drought-resistant crop, is extensively grown in the semi-arid tropics of Africa and Asia, and is are gaining attention as a nutritious, GF cereals with potential in food processing. They are considered underutilized plant crops by many, even though they are rich in essential nutrients, dietary fibre, and bioactive compounds (Saleem et al., 2023). Similarly, taro (*Colocasia esculenta*), is another underutilized indigenous crop that is widely grown in South Africa, where it is locally referred to as amadumbe. It is rich in carbohydrates, dietary fibre, vitamins, and minerals, with higher protein content than many root crops; according to Kapoor et al. (2022), the small size and relatively resistant starch granules, as well as its hypoallergenic properties, make it a viable alternative in flour, bread, cookies, and noodles production. Furthermore, the tuber contains mucilage, a hydrocolloid with specific rheological characteristics that may be used as both food stabilizers and thickeners (Njintang et al., 2008). In contrast, the husk of Psyllium (Plantago species) has a naturally occurring, viscous dietary fibre that provides health benefits. Because of its distinctive qualities, including its great capacity to bind water, gel, and construct structure, as well as its compatibility with other food ingredients, psyllium has emerged as a promising gluten substitute, particularly in baked goods (Bernardes et al., 2024).

Further to their fore-mentioned health advantages and functional properties, millet, amadumbe, and psyllium are quite affordable, indigenous to South Africa, and are available all year-round. It is well accepted that including traditional and indigenous foods in the diet can improve public health by offering a range of nutrients in ways that are acceptable to the culture (van Zonneveld et al., 2021). Additionally, using locally sourced ingredients in food processing is in complete harmony with the growth of local content in the African industrial sector and the recent consumer desire for clean label ingredients (Bender and Schönlechner, 2020). Hence, this work study aimed to formulate GF composite flour blends utilizing locally available functional materials and assess some key techno-functional properties and digestibility profiles as the basis for future product innovations.

4.3. Materials and methods

4.3.1. Materials

Millet was sourced from Agricol in the Midlands, of the KwaZulu-Natal Province (KZN), South Africa. This was washed, dried and milled into a flour with a final particle size of 250µm. Amadume corms were sourced from local farmers in Jozini in the North Coast, KZN and Umbumbulu District in Durban South, KZN, South Africa. The corms were washed, peeled, cleaned and dried at 40 °C for 24 hrs, and then milled into flour with a final particle size of 250µm. Psyllium husk fibre was procured from a local health shop in Durban, South Africa.

4.3.2. Gluten-free flour blends preparation

The GF blends were prepared using amadumbe, millet and psyllium fibre in varying proportions as shown in Table 4.1. The proportion of flour blends was optimized, derived from numerous preceding experimental trials. The flours were blended using a mixer to achieve uniform mixtures that were subsequently subjected to testing. 3% psyllium fibre was the percentage fibre addition selected based on prior experimentation and testing.

Table 4. 1. Composition of gluten-free flour blends

Sample id	Amadumbe flour (g)	Millet flour (g)	Psyllium husk fibre (g)
A	100	-	-
AM1	70	30	-
AM2	50	50	-
AMP1	70	30	3
AMP2	50	50	3

4.3.3. Proximate Composition analysis

AOAC procedures were used to determine the moisture, crude fat, and total ash content of the composite flours (Horwitz, 2000). The sample was heated to 105°C in an oven to determine its moisture content. For ash content, samples were then set in a muffle furnace at 550°C. Kjeldahl's total nitrogen content, using the conversion factor %N× 6.25, was used to determine the protein content, and Soxhlet extraction was used to determine its total fat content. Using a commercial assay kit (K-TDFR, Megazyme, Ireland), an enzymatic–gravimetric analysis was conducted to determine the total dietary fibre content. By means of the difference method, the following equation was used to determine the amount of available carbohydrates:

Available Carbohydrates = [100 – (protein + fat + moisture + ash + dietary fibre)]

4.3.4. Amylose content determination

Amylose content of GF flour blends was analysed using methods described by Williams (1970) Twenty milligrams of each sample were weighed into a 50 mL centrifuge tube. The samples were combined with 10 ml of 0.5 N KOH for 5 minutes or until thoroughly mixed. The sample was subsequently transferred to a 100 mL volumetric flask and diluted to the mark with distilled water, ensuring thorough cleaning of the beaker. A 10 mL aliquot of the test starch solution was subsequently introduced into a 50 mL volumetric flask, followed by the addition of 5 mL of 0.1 N HCl. Following that, 0.5 mL of iodine reagent was incorporated, and the total volume was adjusted to 50 mL. After five minutes, the absorbance of the resulting blue colour was measured at 625 nm. The amylose content was calculated using the equation from the calibration curve for standard amylose.

4.3.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of GF flour blends were recorded on a Fourier transform infrared spectrometer (Agilent Cary 630 FTIR, USA) in a range of wave numbers from 4000 to 600 cm^{-1}. Dry flour samples were used for spectral analysis.

4.3.6. X-ray Diffraction

X-ray diffraction measurements of dry GF flour blends were done by means of a X-ray diffractometer (PANalytical X'pert PRO -Netherlands) fitted with a Cu Kα (λ = 0.154 nm) radiation source and operated at 40 kV and 40 mA. The diffractograms were scanned at a range of 5 to 55° (2θ), with a scanning speed of 1°/min. The relative crystallinity for all samples (0.1g) was measured (Lopez-Rubio et al. 2008).

4.3.7. Assessment of pasting characteristics of gluten-free flour blends

The pasting characteristics of GF flour blends were assessed using a Rapid Visco-Analyzer (RVA-4500, Perten Instruments, Sweden), according to a previous method by Liu et al. (2016) with slight modifications. Water addition was adjusted according to the moisture levels of each sample and added to an RVA canister. This was then followed by the addition of 3g of flour for each flour blend. After being heated to 50 °C and maintained at 50 °C for 1 min. Then they were heated to 95 °C and maintained at 95 °C for 5 min. The gel was then cooled to 50 °C for 4 min. Pasting parameters: peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperature were recorded using the Thermo cline for windows software (Perten RVA 4500, Australia). All experiments were done in duplicate.

4.3.8. Rheological properties of gluten-free flour blends

Rheological properties of the GF flour blend gels were investigated using a rheometer (MCR102 Rheometer, Anton Paar, Austria), as outlined by Wani et al. (2021) with modifications. The measuring system consisted of a parallel plate (50 mm diameter, 1mm gap). The gel was positioned between the plates using a spatula. A thin layer of mineral oil was placed around the rim to avoid drying before the test began. Constant temperature of 25 °C and 0.5% strain within the linear viscoelastic region were used for the analysis. Dynamic rheological parameters used to assess the paste were the storage modulus (G′), loss modulus (G″), tan δ and complex modulus (G*). Frequency sweep tests were carried out between 0.1 and 10 rad/s, and the storage modulus (G') and loss modulus (G") values were recorded. All experiments were done in duplicate.

4.3.9. Measurement of in vitro starch digestibility

Starch digestibility was analysed using the methods of Chen et al. (2017) and Englyst et al. (1992) with minor modifications. (200 mg, dry basis) of flour sample was precisely weighed into a 50 ml centrifuge tube, together with ten glass beads (5 mm diameter). Subsequently, 15 ml of sodium acetate buffer (0.2 mol/L, pH 5.2) was added. After thorough vortexing and mixing, the tubes were incubated in a water bath at 37 °C for 10 min. Thereafter, 5 ml sodium acetate buffer containing amyloglucosidase (15 U/ml) and porcine pancreatic α-amylase (290 U/ml) was introduced to each tube, followed by shaking at 300 rpm in a constant temperature shaking incubator at 37 °C. Then, 0.5 ml aliquots of hydrolysed solution were removed at 20- and 120-min intervals and immediately placed into a centrifuge tube containing 4 ml of absolute ethanol to deactivate the enzymes. Tubes were then centrifuged at 4000 × g for 10 min. The glucose content of supernatant was analysed using glucose oxidase-peroxidase (GOPOD method) assay kits (Wicklow, Ireland). The percentages of RDS, SDS and RS in the starch samples were analysed according to their definition by Englyst et al. (1992). The equations were as follows:

$$RDS\ (\%) = (G20 - G0) \times 0.9 \times 100$$

$$SDS\ (\%) = (G120 - G20) \times 0.9 \times 100$$

$$RS\ (\%) = [(TS - RDS - SDS)/TS] \times 100$$

G20 and G120 represent the glucose content released after 20 and 120 minutes, respectively; G0 represents the free glucose content, while TS indicates the total starch weight. The percentage of hydrolysed starch was determined by multiplying the glucose content by a factor of 0.9. Each sample was analysed in duplicate.

4.3.10. Statistical analysis

Unless otherwise stated, tests were carried out in triplicate. Analysis of variance (ANOVA) was used to analyse the data, and Fischer's Least Significant Differences Test was used to compare means ($p < 0.05$).

4.4. Results and discussion

4.4.1. Compositional analysis of gluten-free flour blends

The proximate composition of amadumbe flour and the gluten-free flour blends are presented in Table 4.2. The protein, moisture and amylose contents of the different flour samples showed minor differences for all the GF blends. However, the percentage of crude fat in the samples was recorded to be between 0.70 and 3.11%. All flour blends had significantly higher fat content (>2%) compared to amadumbe flour. This might be attributed to the higher fat content (±7%) of millet, which forms a major component of the blend (Obadina et al., 2016), as opposed to amadumbe (0.28-0.84%) (Mawoyo Bruce et al., 2022). The ash content was recorded to be from 2.28 to 3.24%, while the carbohydrate content ranged from 66.45 to 70.54%. The effect of the inclusion of psyllium fibre in the flour blends can be observed as AMP1 and AMP2 had higher total dietary fibre contents of 3.4% and 2.88% which were both higher than the non-supplemented flour blends. It must be noted that the data obtained for amadumbe flour agrees with previous studies (Mawoyo et al., 2017, Naidoo et al., 2015). Furthermore, the carbohydrate, dietary fibre, protein and fat contents recorded for the gluten-free flour blend in this study also compare favourably with those of Anna-Sophie and Elke (2013). The amylose content of the gluten-free flour blends was within the range as reported by Kumar et al. (2024) for millet and (Oyeyinka and Amonsou, 2020) for amadumbe flour.

Table 4. 2. Proximate composition and amylose content of gluten-free flour blends

Parameter	**A**	**AM 1**	**AM 2** **%**	**AMP 1**	**AMP 2**
Protein	8.43 ± 0.11^{a}	8.71 ± 0.05^{a}	8.31 ± 0.05^{a}	8.64 ± 0.37^{a}	8.31 ± 0.04^{a}
Fat	0.70 ± 0.00^{a}	2.60 ± 0.04^{b}	3.11 ± 0.10^{c}	2.03 ± 0.05^{d}	2.51 ± 0.06^{b}
Moisture	11.04 ± 0.20^{a}	11.25 ± 0.08^{a}	11.38 ± 0.02^{a}	$11.24 \pm 0{,}04^{a}$	$11{,}43 \pm 0{,}24^{a}$
Ash	3.24 ± 0.13^{a}	2.52 ± 0.13^{b}	2.30 ± 0.03^{b}	2.72 ± 0.01^{c}	2.28 ± 0.10^{d}
Total CHO	70.15 ± 0.45^{a}	69.39 ± 0.31^{a}	70.54 ± 0.10^{a}	66.45 ± 0.29^{b}	68.27 ± 0.31^{c}

Total dietary fibre	6.44±0.00[a]	5.52±0.03[b]	4.36±0.00[c]	8.92±0.01[d]	7.20±0.01[e]
Amylose	12.15±0.00[a]	11.83±0.02[b]	12.05±0.00[c]	11.95±0.00[d]	11.92±0.01[e]

Results are represented as mean ±SD (n=3)
Values in the same row with [a,b,c] are significantly different ($p < 0.05$)

4.4.2. FTIR spectroscopy for starch structure of the composite flour blend

The FTIR spectra of GF blends are shown in Figure 4.1. FTIR characterization was performed to determine the influence of combining different flours with or without fibre on starch characteristics using the functional group approach. All of the flour blends had comparable FTIR spectra, with a noticeable difference in peak intensity but no noticeable shift in peak number. It is believed that the variations in spectral intensity may be mainly due to varying content of dietary fibre as recorded in the proximate analysis. The skeletal vibration mode of the glucose pyranose ring was responsible for the complex vibrations observed in GF blends at low wavenumbers (< 800 cm^{-1}) (Mawoyo Bruce et al., 2022). C-O stretching may be responsible for the peaks seen at 861 and 995 cm-^{1}, whereas C-O and C-C stretching may be the reason for peaks seen at 1148 cm^{-1} in starches (Zeng et al., 2011). The sharp peak seen at wavenumber 1636 cm^{-1} could be explained by the bending vibrations of water molecules in the starch's non-crystalline area (Kizil et al., 2002). C-H bond stretching may be the reason of a distinctive band in the 2800–3000 cm^{-1} area, with a peak at 2922 cm^{-1}. The absorption of starch in this area may be dependent on the proportion of amylose to amylopectin. Strong and wide absorption peaks were seen at 3272 cm^{-1} in all flour samples, possibly as a result of the glucose unit's O-H stretching vibration (Jain et al., 2019, Zeng et al., 2011). Furthermore, none of the flour blends exhibited the characteristic peak of gluten's α-helical secondary structure, which is typically centred at approximately 1650 cm^{-1} (Jia et al., 2020), thus confirming that all the flour samples are gluten-free.

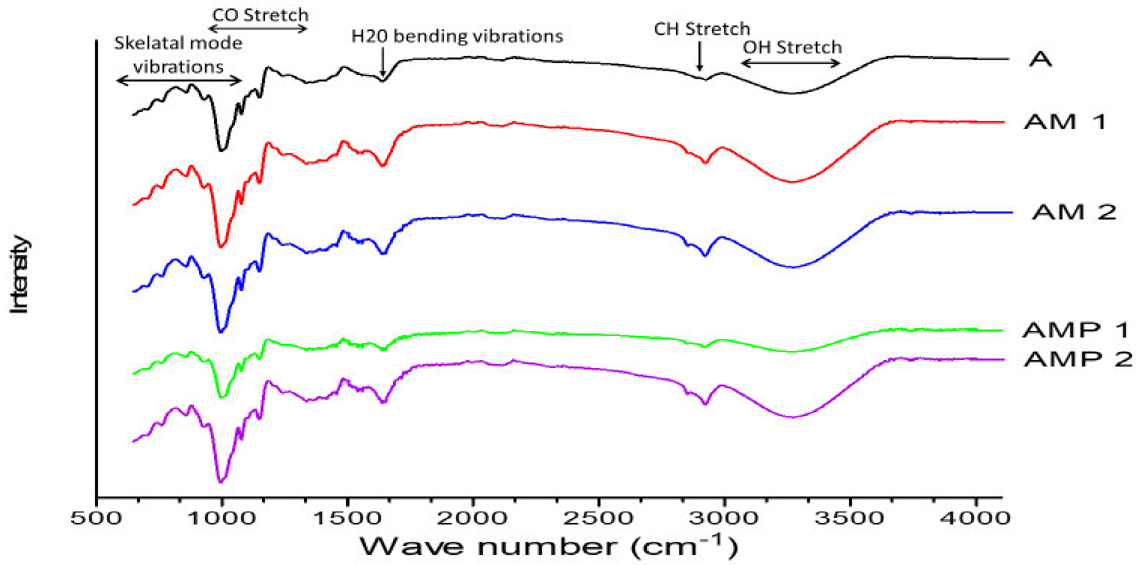

Figure 4. 1. FTIR spectra of gluten-free flour blends

4.4.3. XRD spectra of the gluten-free flour

The X-ray diffraction patterns of the gluten-free flour blends and pure amadumbe flour are shown in Figure 4.2. All samples displayed typical A-type diffraction patterns with strong single peaks at 15.01°and 22.94°(2Θ) and a doublet at 17.04°and 17.98°(2Θ). It has been established that most cereal starches display an A-type configuration, tuber starches exhibit a B-type diffraction pattern, while some seeds and roots produce a C-type pattern, which is a mix of A- and of B- type (Kowsik and Mazumder, 2018). A-type diffraction patterns result in starch granules that are more resilient and able to tolerate greater temperatures and shear conditions, given that double helices are densely packed in a crystalline matrix, forming a structure with four water molecules per cell (Boahemaa et al., 2024). According to Simsek and El (2015), A-type starches have been linked to several health benefits, including a delayed release of glucose into the bloodstream that lowers postprandial glycaemic and insulin responses. Consequently, the amadumbe starch present in the flour samples used in this study may play an important role in supporting cardiovascular, metabolic, and digestive health. (Eleazu et al., 2013).

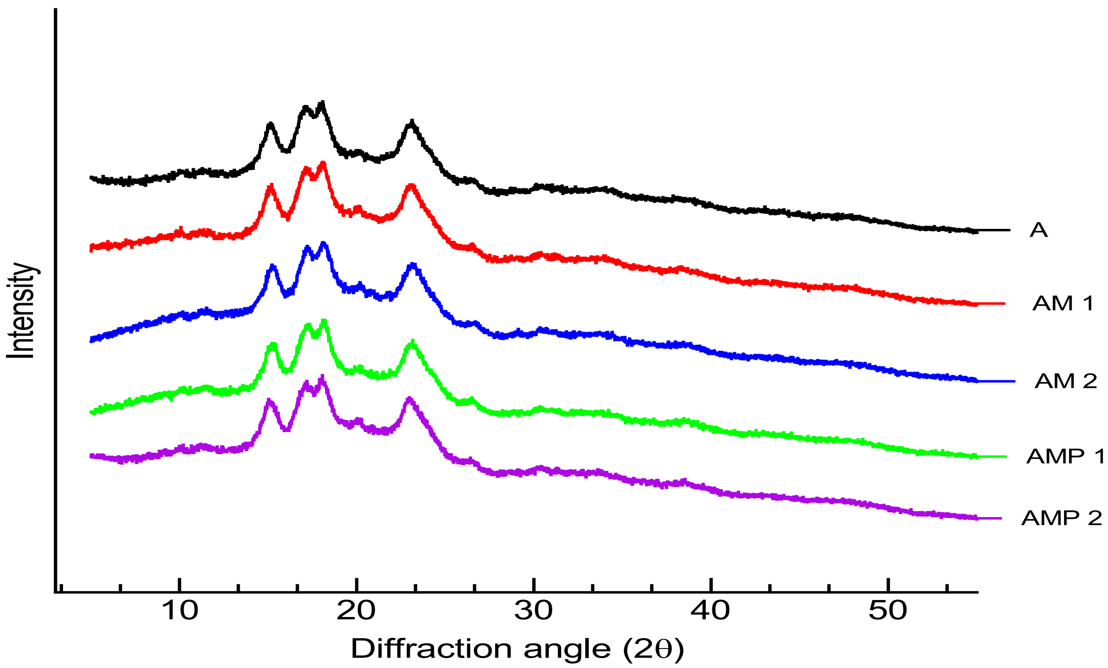

Figure 4. 2. Diffractograms of gluten-free flour blends

4.4.4. Pasting properties of gluten-free flour blends

Pasting profiles (Figure 4.3) and pasting parameters (Table 4.3) of the gluten-free flour blends showed differing patterns. It was observed that the pasting parameters of the flour blends with added psyllium husk fibre were similar to the pure amadumbe flour and differed significantly to the combination of amadumbe and millet flour only. When comparing flour blends with additional fibre to those without, all evaluated pasting parameters were higher, except for pasting temperature. The pasting temperatures of the AMP1 and AMP2 blends were around 82 °C, whereas the AM1 and AM2 blend was around 90 °C. The temperature difference is due to the presence of dietary fibre, which results in a reduced proportion of starch (Tangsrianugul et al., 2019). Previous studies have shown that the pasting temperature of amadumbe starch is typically higher relative to starches from other tubers, such as potato (64–69 °C) (Jane et al., 1999, Singh et al., 2018, Nwokocha et al., 2009) and cassava (67–68 °C) (Nwokocha et al., 2009, Srichuwong et al., 2005). Final viscosity is dependent on the starch's quality and the stability of the cooked paste—particularly its ability to solidify into different types of pastes or gels upon cooling. Complex forms among amylose-lipid or amylose-protein could be the cause of the notably raised values for the final viscosity of AMP1 and AMP2, as previously highlighted by Tangsrianugul et al. (2019). In the industry, high final viscosity of starches suggests their potential as thickeners in food applications (Oyeyinka and Amonsou, 2020). Peak viscosity assesses both the starch's power to bind water and its ability to expand freely before physical breakdown. The increased mucilage content of flour blends containing psyllium fibre may be responsible for their high peak viscosity. Typically, items in need of high gel strength and elasticity might benefit from high PV starch (Mawoyo Bruce et al., 2022).

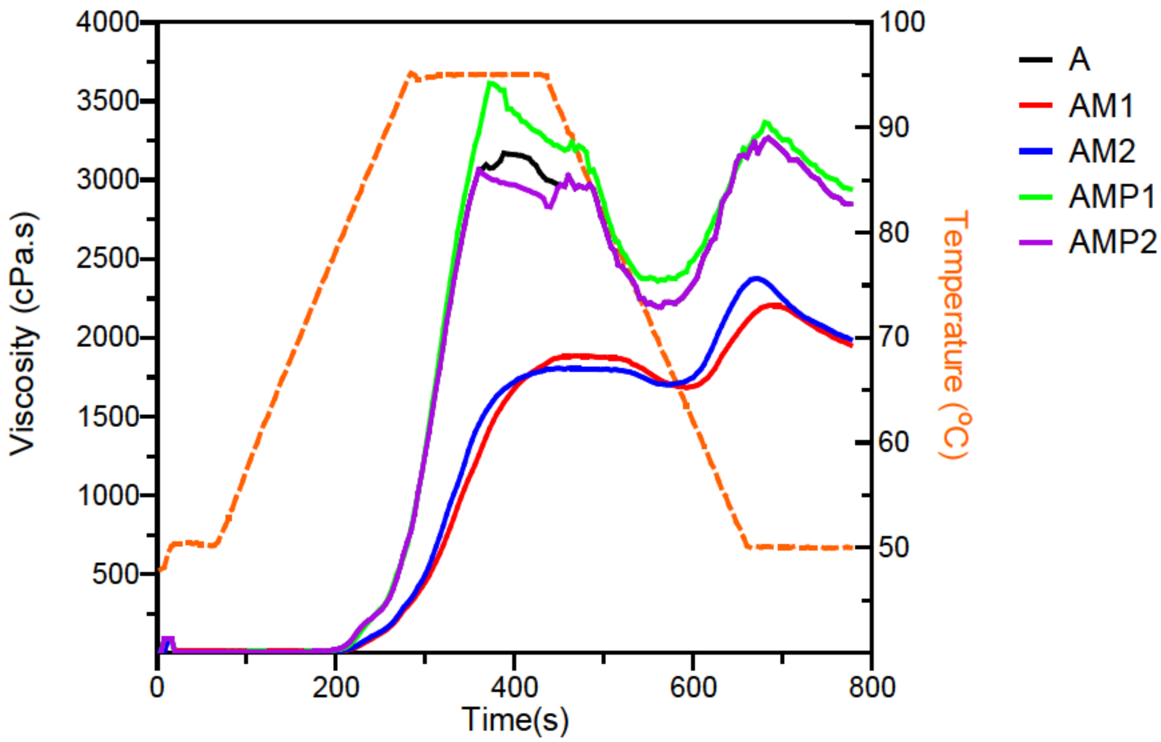

Figure 4. 3. Pasting profiles of gluten-free flour blends

Table 4. 3. Pasting parameters of gluten-free flour blends, A, AM1, AM2, AMP1 and AMP2

Sample	Pasting temperature (PT)	Peak Viscosity (PV)	Though Viscosity (TV)	Final Viscosity (FV)	Breakdown Viscosity (BV)	Setback Viscosity (SBV)
	°C			cPa.s[1]		
A	84.05±0.64[a]	1914±50.91[a]	1567±62.93[a]	2335±34.65[a]	347±12.02[a]	768±28.28[a]
AM1	90.45±0.04[b]	1781±14.85[b]	1313±32.53[a]	1953±84.85[b]	468±36.06[b]	640±52.33[b]
AM2	90.45±0.57[c]	1773±20.51[c]	1495±62.93[a]	1989±24.04[c]	278±41.72[c]	494±17.68[c]
AMP1	82.30±0.07[d]	3635±32.53[d]	2360±43.84[b]	2942±12.02[d]	1275±95.46[d]	582±10.61[d]
AMP2	81.55±0.07[e]	3214±6.36[e]	2194±221.3[c]	2847±42.43[e]	1020±135.76[e]	653±60.81[e]

Results are represented as mean ±SD (n=2)
Values in the same column with [a,b,c] are significantly different ($p < 0.05$)

4.4.5. Rheological characteristics of gluten-free flour blends

The viscoelastic behaviour of paste made from amadumbe flour and the flour blends, with or without the addition of psyllium fibre, was assessed in respect of storage modulus (G′), loss modulus (G″), complex modulus (G*) and tan δ values (Figure 4.4). The G′ recorded for all the flour samples was observed to be higher than the corresponding G″, and both parameters were found to increase with increasing frequency in all the flour samples. Previous studies have established the relationship between the observed behaviour and the solid-like structure of gluten-free batter preparations. In one of such studies, the G′ of gluten-free paste derived from buckwheat, rice and maize composite flour were higher than the G″ (Pruska-Kędzior et al., 2008), while the same trend was also recorded in a study on gluten-free quinoa, buckwheat, rice flour and potato starch blend (Turkut et al., 2016). Furthermore, the G′ of the flour blends, especially those supplemented with psyllium husk, were higher than that of pure amadumbe flour. According to previous studies, this might indicate the tendency of the flour blends to retain more gas and better structural integrity during proofing and baking (Hussain et al., 2021). This significant improvement in paste viscoelasticity may be due to the interactive actions of the starch, mucilage and proteins in the gluten-free flour, which mimic gluten's properties while improving dough strength (Awulachew, 2021). This has the potential to improve carbon dioxide retention during proofing (Manhivi et al., 2020). In addition, the Tan δ for all the paste samples were <1 at all frequencies, which also exhibits to a more elastic than a viscous system.

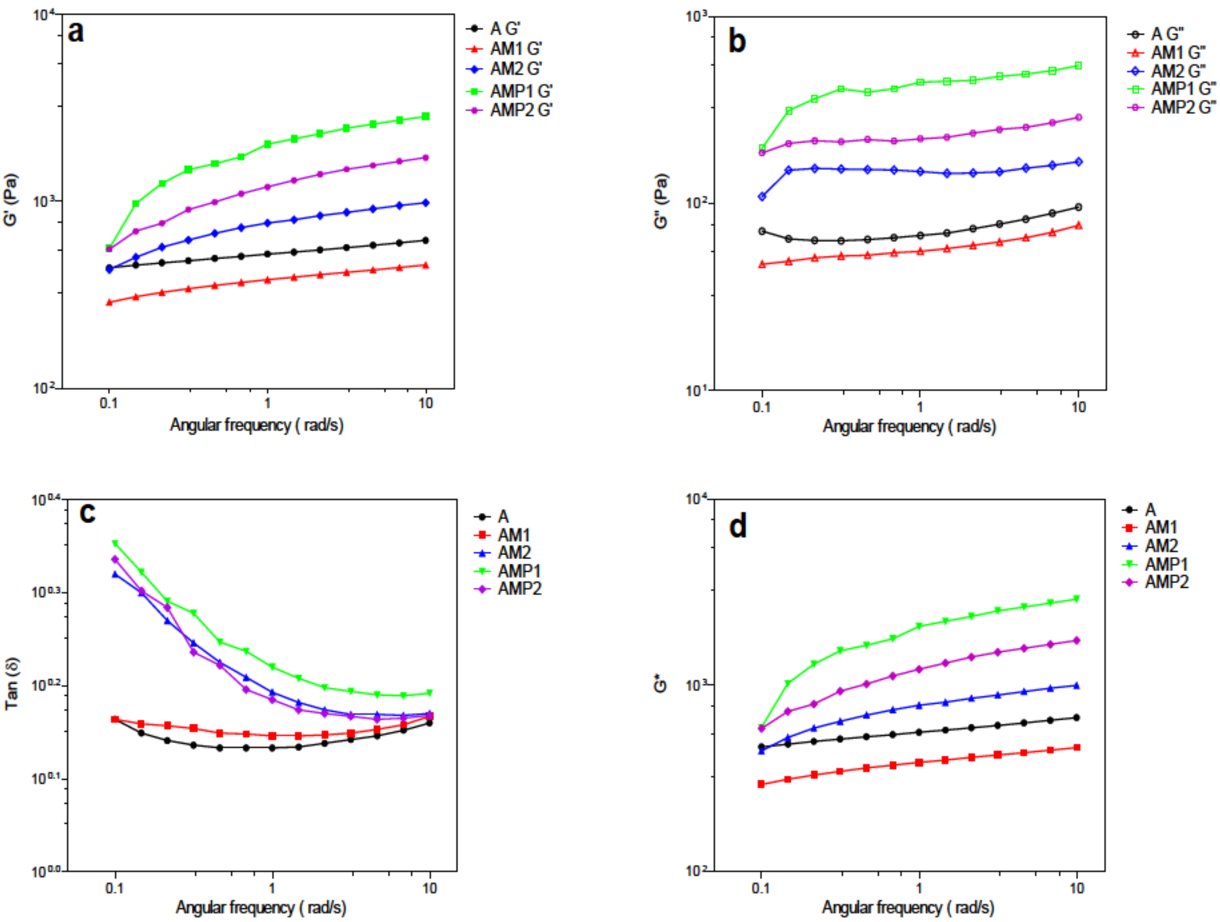

Figure 4. 4. Frequency sweep data of gluten-free flour blends: (a)storage modulus (G'), (b) loss modulus (G"), (c) dynamic loss tangent (tan δ), (d) complex modulus (G*) graphs

4.4.6. In vitro starch digestibility of gluten-free blends

The in vitro starch digestibility behaviour of the gluten-free flour blends was evaluated (Table 4.4) to determine the slowly digestible (SDS), rapidly digestible (RDS), and resistant starch (RS) fractions of the various flour formulations. The rapidly digested starch (RDS) content in food is that which causes the short-term postprandial glycaemic response, the slowly digestible starch (SDS) leads to a slow and gradual increase of the blood glucose levels, while resistant starch (RS) eludes enzymatic digestion in the small intestine and enters the large intestine where it undergoes fermentation (Sharma et al., 2017). Amadumbe flour had an RDS, SDS and RS content of 29.66, 9.07 and 61.27 %, respectively. The incorporation of millet flour had a minor effect on the starch fractions. However, adding psyllium fibre into the blends showed a significant difference in the starch fractions. RDS decreased by up to 8.52% in AMP2, whilst SDS resulted in a slight increase of up to 1.66% in AMP2, and RS showed a significant increase of up to 7.78%. This is not unexpected due to the presence of the fibre, as the cell walls, proteins, and other dietary components in fibres often form a matrix around the starch granules, thus inhibiting enzyme adsorption sites during digestion (Zheng et al., 2021). Additionally, fibrous plants tend to contain more amylose, which are more tightly packed granules, and are less accessible to digestive enzymes; their inaccessibility may also be enhanced by their complexation with fats (Sun et al., 2024, Li et al., 2021). The RS content of food depends on the processing, temperature and storage time. It is also related to starch gelatinization and retrogradation (Liu et al., 2024).

Table 4. 4. In vitro starch digestibility assay results of gluten-free flour blends

Flour blend	RDS	SDS %	RS
A	29.66±0.06[a]	9.07±0.06[a]	61.27±0.00[a]
AM1	28.27±0.07[b]	9.78±0.06[b]	61.95±0.01[b]
AM2	28.67±0.06[c]	9.52±0.05[c]	61.81±0.01[c]
AMP1	21.14±0.13[d]	9.81±0.11[d]	69.05±0.02[d]
AMP2	21.39±0.05[e]	10.73±0.05[e]	67.88±0.00[e]

Results are represented as mean ±SD (n=2)
Values in the same column with [a,b,c] are significantly different ($p < 0.05$)

4.5. Conclusion

Flour blends were formulated from amadumbe, millet and psyllium husk flour to obtain a gluten-free flour with desirable properties in food processing. The pure amadumbe, along with the flour blends, displayed competitive techno-functional properties, with the psyllium-supplemented samples exhibiting the most desirable properties in terms of total dietary fibre, viscoelasticity and resistant starch content. Significantly, the viscoelastic properties of the fibre-fortified flour blend alleviate the structural deficiency resulting from the absence of gluten. Hence, it may be concluded that AMP1 flour formulation can be efficiently incorporated into commercial gluten-free baked food without significant differences in the structural properties of the food products. In addition, psyllium husk fibre can be a very promising ingredient due to its significant effect on the rheological dough properties and nutritional value of the amadumbe-millet composite flour, which are both key factors in special requirement diets. However, future studies are essential to highlight the health benefits of the fibre-supplemented flour blends as well as consumer acceptability of the flour blends.

CHAPTER FIVE

5. Dough forming properties and baking quality of amadumbe-millet-psyllium composite flour blend.

5.1. Abstract

Developing gluten-free (GF) bread comes with many challenges, including adherence to current dietary trends, customer demands, and the rising incidence of celiac disease diagnoses. To address these challenges, new ingredients such as amadumbe, millet and psyllium have been selected as alternatives to create a GF flour blend and test the chemical interactions of these ingredients on dough forming properties and baking quality of the resultant bread. The findings indicated that incorporating psyllium husk into the GF blend (AMP1) showed a significant increase in all parameters tested on the dough. WAC and OAC showed that AMP1 increased WAC by 72.77 g/g and OAC by 7.26 g/g. For rheological analysis, AMP1 significantly increased both G′ and G″. TPA results for texture analysis revealed, AMP1 dough exhibited increased adhesiveness and decreased gumminess, cohesiveness, and hardness. To test the baking performance, texture analysis and sensory analysis were done. Texture analysis revealed an increased crumb hardness, cohesiveness and gumminess. Sensory evaluation of the AMP1 bread in reference to a commercially available GF bread showed comparable results. Starch digestibility results in a decrease in RS (7.3%) and an increase in RDS (2.98%) and SDS (4.32%). Findings from this study show that AMP1 dough showed substantial development potential for GF and nutritionally enriched bread products. The AMP1 dough not only meets the functional requirements for bread making but also offers potential health benefits due to the high fibre content of psyllium husk and the nutritional profile of amadumbe and millet.

5.2. Introduction

GF bread has been the subject of extensive research and development due to current dietary trends, the growing diagnosis of coeliac disease, and increased awareness of the condition. The market dynamics of GF products, which are expanding significantly, reflect this trend. The market is projected to increase at a yearly rate of 9.8% from 2023 to 2030, based on a net valuation of USD 6.45 billion in 2022 (Size, 2023). Low bread volume, poor dough tolerance, high hardness, poor crust quality and appearance, and short shelf life are only a few of the nutritional and technological limitations of GF products, despite their growing popularity (Šmídová and Rysová, 2022). Because the GF diet pattern is frequently characterised by an excessive intake of fats and a decreased intake of complex carbohydrates, dietary fibre, vitamins, and minerals, there is rising concern regarding the nutritional quality of this type of diet (Capriles et al., 2021).

In addition to sensory acceptability, research on gluten-free food, especially bread, has focused on enhancing technological factors (loaf volume, crumb hardness). The crumb structure and loaf shape of GF products are influenced by the hydrocolloids that are combined with native and modified starches. Finding alternatives to replicate the viscoelastic characteristics of the gluten network—particularly hydrocolloids, enzymes, and emulsifiers —is the most preferable option (Cappelli et al., 2020). Incorporating other protein sources like eggs, dairy products, and insects (Masure et al., 2019, Cappelli et al., 2020) as well as pulses (Crockett et al., 2011) is also a novel method to improve GF dough and bread. However, the use of soluble dietary fibre is one of the most novel ways to improve GF bread and dough (Fratelli et al., 2021).

It has been demonstrated that adding soluble dietary fibres to GF bread enhances its nutritional value, shelf life, sensory acceptability, physical characteristics, and glycaemic response (Capriles et al., 2016, Bender and Schönlechner, 2020). The husks of *Plantago ovata* seeds contain psyllium, a naturally occurring bioactive soluble fibre that is a hydrocolloid which can help with intestinal transit, cholesterol management, satiety (Fratelli et al., 2018), and glycemia (Franco et al., 2020). Psyllium possesses water-binding, gelling, and structural qualities that enhance the structure, texture, appearance, acceptance, and shelf life of gluten-free bread, in addition to its beneficial effects on health. These properties can enhance the dough's viscosity, reinforce the boundaries of the expanding cells, enhance gas retention during baking, and boost volume. Additionally, psyllium can reduce the loss of crumb moisture, softness, cohesiveness, and springiness during storage (Fratelli et al., 2018, Mancebo et al., 2015, Ziemichód et al., 2019, Santos et al., 2020a, Santos et al., 2020b), together with fibre supplementation, which reduces the glycemic index (Fratelli et al., 2018).

Most of the functionality targets have been met by using new processing technologies when combined with ingredients that have a strong film-forming capacity, water-binding ability, structure-forming capacities, and emulsifiers (Bender and Schönlechner, 2020). High-quality products can be developed by combining components and additives with different qualities in these formulations; however, the presence of unknown ingredients on the label may have a detrimental impact on the opinions and preferences of consumers. Consumers' current concern about the potential harm that all these interventions could cause to their health and the environment makes them especially interested in the constituents included in foods, together with the applied technology and processing techniques used in their manufacture (Asioli et al., 2017).

Consumers are therefore moving toward more specialized foods that, in their opinion, are made with healthy ingredients and do not include chemical components or unidentified compounds. This "clean label" idea has become more popular in recent years (Jamaluddin et al., 2022, Maruyama et al., 2021, Montemurro et al., 2021). Consumers and food manufacturers have created and defined the phrase "clean label," even though there is presently no official definition or specific law to define it (Asioli et al., 2017, Varela and Fiszman, 2013). Although the phrase is still subjective, a "clean label" food is usually linked to a few well-known ingredients, little processing from natural raw materials, and most certainly contains no artificial or synthetic substances (Aschemann-Witzel et al., 2019, Asioli et al., 2017, Bender and Schönlechner, 2020). Customers who omit gluten as a food component look for gluten-free items that might adhere to the current "clean label" trend in an effort to elevate their health (Kajzer and Diowksz, 2021).

This study aimed to create a dough using minimally processed flours from millet, amadumbe and psyllium husk fibre, evaluate the dough forming properties and test its baking quality.

5.3. Materials and Methods

5.3.1. Water (WAC) and oil absorption capacity (OAC)

Water absorption capacity was determined according to the method described by Falade and Okafor (2015) with slight modifications. Two grams of the flour sample were measured into a dry, clean, pre-weighed centrifuge tube. Ten millilitres of water were placed into the tube and thoroughly mixed with a vortex mixer. The suspension was left to stand for 30 minutes at ambient temperature, followed by centrifugation at 3500 x g for 15 minutes. The supernatant was removed, and the tube containing the sediment was weighed again. The identical process was replicated for oil absorption capacity by substituting water with oil. The subsequent equation was employed to determine water/oil absorption capability:

$$\text{Water/ Oil Absorption [g } H_2O\text{/ oil/g of dry sample weight]} = \frac{\text{Wet sample weight} - \text{Dry sample weight}}{\text{Dry sample weight}}$$

5.3.2. Total starch determination

The total starch content of the gluten-free flour blends was determined using a total starch test kit (Megazyme International Ireland, Wicklow, Ireland).

5.3.3. Formulation of dough for breadmaking

For the preparation of bread, a simple bread recipe was used, comprising AMP1 (amadumbe-millet-psyllium blend) blend 400 g, warm water 250 g, sugar 20 g, salt 6.8 g, yeast 6 g and oil 10g. The ingredients were placed in the mixing bowl of the Kitchen mixer (Kitchen Aid, Model 5KSSS, Michigan, USA) and mixed for 5 min for yeast activation. Thereafter, it was proofed for 60 minutes in a proving oven (Svenka AB Bagerimaskiner, Sweden) at 45°C, then baked at 190°C for 45 minutes using a baking oven (Mac Adams, Kestrel 1 ,South Africa).

5.3.4. Determination of the rheological properties of dough

The rheological parameters of the dough were evaluated using the procedure outlined by Zhang et al. (2017), with minor adjustments. The linear viscoelasticity area was evaluated using a strain sweep test, with a strain range of 0–100%. A strain value of 0.05% was chosen to ensure that the strain of the components evaluated for rheological analysis remained within the linear viscoelastic area. The rheology of dough was examined using dynamic oscillatory and creep-recovery tests conducted with an MCR102 Rheometer from Anton Paar, Austria. Before analysis, the dough was allowed to rest for 10 minutes in a sealed container, subsequently undergoing 5 minutes of relaxation on the apparatus plates at 25 °C. Frequency sweep tests were performed on the dough from 0.1 to 10 rads^{-1} at a strain of 0.05% and a temperature of 25 °C to determine G′ and G″ as functions of frequency.

Creep and recovery tests were conducted, with the creep phase documented at a shear stress of 250 MPa, passing the LVR for 300 seconds, followed by a recovery phase of 300 seconds at a stress of 0 MPa. Tests were conducted at 25 °C. Creep and recovery curves were recorded and examined. All rheological measurements were conducted in duplicate.

5.3.5. Texture analysis of dough and bread

Texture analysis of the bread samples was carried out using a method by Qi et al. (2024)Qi, Cao and Li (2024), with slight modifications. The gluten-free bread was baked and rested for 3 hours before it was cut into square pieces with dimensions of 2.5 × 2.5 × 5.0 cm for analysis. Texture profile analysis (TPA) of the samples was carried out using a Shimadzu Texture Analyzer Ez SX, Kyoto, Japan) loaded with a 500N load cell. For the dual compression test (force and distance), A cylindrical plate probe with a diameter of 75 mm was utilised, programmed with the following parameters: measurement speed set to 1 mm/s, compression pressure maintained at 50%, and a 5-second interval established between compressions. Hardness, chewiness, springiness, resilience, and cohesiveness measurements were extracted from the data profile generated using Trapezium software. Five texture measurements were obtained for each dough and bread sample.

5.3.6. In vitro starch digestibility of bread

The bread samples were subject to analysis for simulated starch digestion based on methods used by Ren et al. (2016) and Englyst et al. (1992) with modifications. Accurately, 0.6 g of sample was dissolved in 10.0 mL of freshly prepared pepsin solution (5.0 g L−1 pepsin and 5.0 g L−1 guar gum in 0.05 mol L−1 HCl) and transferred to 50 mL centrifuge tubes. The tubes were then incubated in a water bath at 37 °C for 30 minutes to aid in protein hydrolysis. Subsequently, 5 mL of 0.5 M sodium acetate buffer solution (pH 5.2 ± 0.2) and five glass balls (15 mm diameter) were placed into each tube. Then, 5.0 mL of the enzyme mixture was incorporated to start starch digestion. The enzyme mixture was prepared by suspending 3.0 g of pancreatin in 20.0 mL of a 0.1M CaCl2 solution by stirring with a magnetic stirrer for 10 minutes. The mixture was then centrifuged at 2500 rpm for 10 minutes to collect 15.0 mL of pancreatin supernatant. Next, 1.33 mL of amyloglucosidase and 0.5 mL of invertase were added. The samples underwent digestion at a temperature of 37 °C for a duration of 2 hours in a horizontal shaking water bath set to 160 rpm. At precisely 20 and 120 minutes of digestion, 0.2 mL of each sample was extracted from the tubes and mixed with 4.0 mL of absolute ethanol. The mixture was thoroughly stirred to isolate the glucose fraction for the 20-minute (G20) and 120-minute (G120) intervals.

Following the collection of 0.2 ml of the G120 samples, the tubes underwent vigorous vortex mixing. Following a 30-minute incubation in boiling water, the contents were cooled to 0 °C using an ice bath and then mixed with 10.0 mL of 7.0 mol L^{-1} potassium hydroxide. Following a 30-minute incubation in ice water, 0.2 mL of each sample was combined with 1.0 mL of 1.0 M acetic acid that contained 40.0 μL of amyloglucosidase (diluted 1:7). The mixture was then placed in a water bath at 70 °C for 30 minutes, followed by a boiling water bath for 10 minutes. After cooling to room temperature, 20.0 mL of water was added to achieve the total glucose portion (TG).

All samples (G20, G120, and G0) were centrifuged at 4000 rpm for 5 minutes. The glucose concentration in the supernatant was quantified by using the glucose oxidase-peroxidase method, using a GOD-POD testing kit. The quantitative determination of the percentages of RDS, SDS, and RS in the starch samples was conducted in accordance with their definitions by Englyst, Kingman and Cummings (1992). The equations were as follows:

$$\text{RDS (\%)} = (\text{G20} - \text{G0}) \times 0.9 \times 100$$

$$\text{SDS (\%)} = (\text{G120} - \text{G20}) \times 0.9 \times 100$$

$$\text{RS (\%)} = \text{TS} - \text{RDS} - \text{SDS}$$

where G20 and G120 were the contents of glucose released after 20 and 120 min, respectively; G0 was the content of free glucose. TS was the total starch in the sample. The percentage of hydrolysed starch was calculated by multiplying a factor of 0.9 by the glucose content. Each sample was analysed in duplicate.

5.3.7. Sensory evaluation

Sensory evaluation of the gluten-free bread was conducted using a 70-member consumer acceptance panel. Ethical Clearance number IREC 130/24 (Appendix 3) for the study was obtained through the Durban University of Technology IREC department, following their procedures and protocols. The sensory panel used was untrained and included students and staff from the student population of the Durban University of Technology, with most panelists from the Department of Biotechnology and Food Science. The sensory evaluation was carried out by panellists who frequently ate wheat and/or gluten-free bread. Evaluation of samples was based on a 9-point hedonic scale, where 1 represented the preference “dislike extremely”, 5 “neither like nor dislike”, and 9 “like extremely” (Appendix 4). The sensory sessions were all performed in the Food Science Laboratory. A standard control of commercial gluten-free bread (containing maize flour) was purchased from a local supermarket. The amadumbe-millet bread was freshly baked (2h prior to evaluation). The samples for taste testing were prepared prior to the evaluation in the following manner: The breads, specifically the commercial control and amadumbe millet test samples, were sliced into 2 cm portions. Only the central slices were selected and transferred into containers. Each sample was designated a unique three-digit code. The samples were distributed randomly to the panellists, who were instructed to rinse their mouths with water prior to testing each sample. Panelists were asked to evaluate the sensory characteristics such as colour, texture, mouth feel and taste of the amadumbe-millet gluten-free bread against a commercially available gluten-free bread.

5.3.8. Statistical analysis

All the results were presented as mean ± standard deviation with triplicate experiments unless otherwise specified. Analysis of variance (ANOVA) was used to analyse the data, and Fischer's Least Significant Differences Test was used to compare means ($p < 0.05$). Statistical analysis was done using GraphPad Prism (V5).

5.4. RESULTS

5.4.1. WAC, OAC and total starch content of flour blends

Figure 5.1 shows the results obtained for WAC, OAC and WAC showed a significant ($p > 0.05$) difference between the samples. Sample A (153,01g/g) had the lowest WAC. Amadumbe flour blended with millet flour (50/50) resulted in a 9.84 g/g increase, and the most significant increase was observed with the same blend supplemented with 3% fibre (54.77 g/g). This increase in WAC for fibre supplementation of blends may be a result of improved interaction with water molecules brought on by the addition of fibre. A food product's WAC measures the amount of starch gelatinisation by measuring the starch's ability to hold onto water after swelling, which in turn translates into the weight of the gel that forms (Godswill, 2019). It is dependent on the macromolecules' ability to form gels as well as the presence of the hydrophilic groups that bind water molecules (Tenagashaw et al., 2016)

OAC showed no significant differences among all samples. Sample A recorded the lowest oil absorption capacity 123.94 g/g, followed by AM1 126.95 g/g and AMP1 131.20 g/g. OAC is the term for the binding of fat by the non-polar side chain of proteins (Awuchi et al., 2019). The primary cause of OAC is the physical trapping of oils. It provides information on how quickly proteins bind to fat in food formulations (David et al., 2015). The ability of flour to absorb oil improves the flavour and mouthfeel in food preparations (Desalegn, 2015).

The total starch content decreased slightly from A > AM1 >AMP1. Sample A had the highest starch content at 73.73, whereas AM1 and AMP1 exhibited reduced values of 1.39% and 2.15% respectively. This reduction could be linked to partial hydrolysis or leaching during processing. These trends suggest structural and compositional changes in the starch matrix due to the increased fibre content.

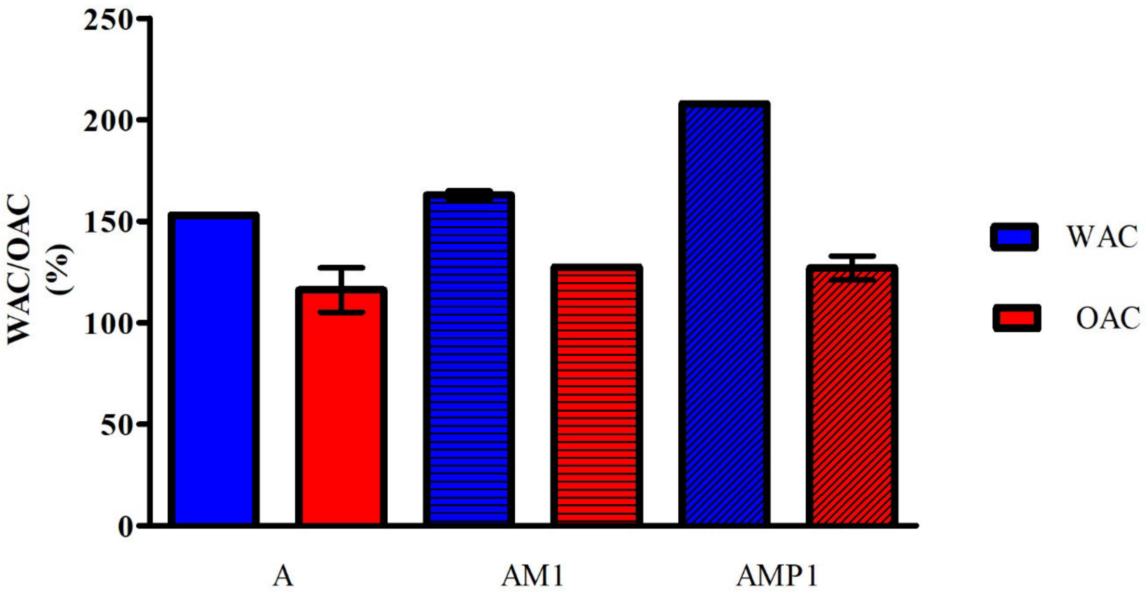

Figure 5. 1. WAC and OAC of gluten-free blends

A=100% amadumbe flour, AM1=70:30 amadumbe: millet, AMP1= 70:30:3 amadumbe: millet: psyllium,

5.4.2. Rheological properties of dough

Dough sample frequency sweep tests (Figure 5.2) reveal that across the frequency range (0–10 rads/s), the G' (elastic/storage modulus) was consistently higher than the G" (viscous/loss modulus). These findings imply that the dough systems behaved mostly in an elastic manner. Both G′ and G″ significantly increased in the dough sample that had fibre added. Studies undertaken by Ren et al. (2020a), Mancebo et al. (2015), Farahnaky et al. (2010) have noted that the presence of psyllium in the dough results in a weak gel. In these studies, it was observed that G' was always larger than G" with both moduli being almost parallel to each other across the frequency range measured, and this trend increased with an increase in the level of psyllium added. Studies conducted by Mei et al. (2024) found that modified dietary fibre significantly improves interactions with dough's starch molecules, resulting in a strong and cohesive network. However, excessive fibre significantly decreases both the storage modulus (G′) and loss modulus (G″), which could cause a breakdown of disulfide bonds, decreasing some of the gluten crosslinking and affecting the dough's elasticity and flow (Wang et al., 2022).

Creep recovery data indicate that the addition of dietary fibre to the GF blend (Figure 5.3) resulted in a decrease in the creep strain. This could be due to the increased moisture content of the fibre-supplemented GF dough. The reduced creep strain suggests improved deformation resistance in fibre-supplemented GF doughs (Wang et al., 2022). Creep strain correlates with the moisture level of the dough; doughs with increased moisture levels demonstrate a more pronounced maximum creep strain. The study indicated that the reduction in creep strain reflects enhanced deformation resistance in the doughs enriched with dietary fibre. Modified dietary fibres may be able to improve the dough's gel-forming qualities by promoting the formation of hydrogen bonds (Dangi et al., 2020).

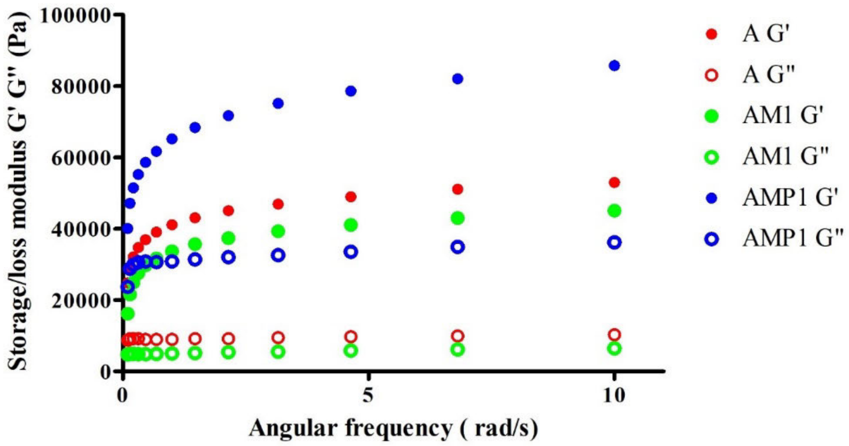

Figure 5. 2. Frequency sweep graph of dough of the gluten-free flour blends A, AM1 and AMP1

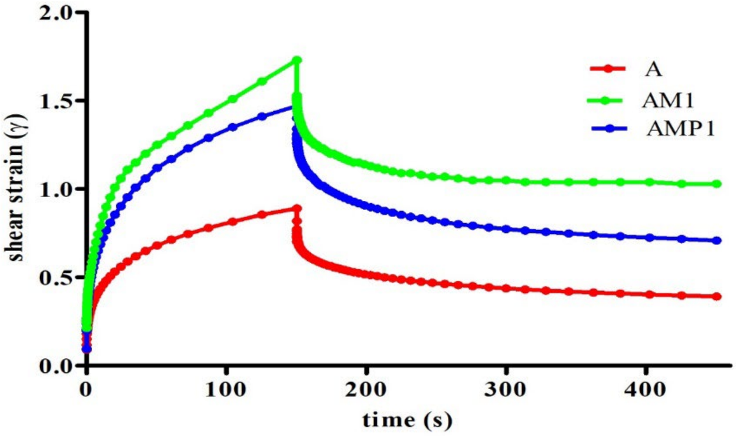

Figure 5. 3. Creep recovery data of dough from gluten-free flour blends A, AM1, AMP1

5.4.3. Texture profile of dough and bread

From the results obtained (Table 5.1), fibre addition to the dough led to a decrease in hardness, cohesiveness and gumminess and an increase in adhesiveness. This pattern is demonstrated by the competition for water between the proteins and dietary fibre, which prevents the network from forming (Zhao et al., 2025). The absence of gluten protein weakened the dough texture, while dietary fibre (DF) improved the dough texture characteristics (Li et al. 2017). DF crosslinks with the proteins when it swells and absorbs water, creating a finer, harder shape that increases the dough's cohesiveness and elasticity. This outcome aligns with Jiang et al. (2019) findings. However, excessive addition of DF may lead to confined water loss, potentially compromising the dough's elasticity and cohesiveness, lowering the protein content, and making it more difficult for the gluten proteins to absorb water (Curti et al., 2016). Cohesiveness values were similar for all samples and showed no significant difference ($p > 0.05$).

For the bread sample, hardness, adhesiveness, and gumminess increased whilst cohesiveness decreased. Hardness (19.40 N) of the bread was far higher than that of the dough samples, which resulted from the baking process. Adhesiveness (0.04 N) was a positive value as compared to the dough samples, and this was due to the baking process.

Table 5. 1. Texture properties of Gluten-free dough with and without fibre and bread

Samples	Hardness (N)	Cohesiveness	Gumminess (N)	Adhesiveness (N)
Dough				
A	12.66±2.23^{a}	0.05±0.07^{a}	0.58±0.80^{a}	-0.70±0.45^{a}
AM1	10.20±0.68^{a}	0.04±0.05^{a}	0.44±0.60^{a}	-0.85±0.09^{a}
AMP1	5.40±0.39^{b}	0.06±0.05^{a}	0.32±0.29^{a}	-0.52±0.06^{a}
Bread				
Bread AMP1	19.40±2.64^{c}	0.03±0.09^{a}	0.73±1.64^{b}	0.04±0.04^{b}

Mean ±SD (n=5) reported
Values in the same column with ^{a,b,c} are significantly different ($p < 0.05$)
A=100% amadumbe flour, AM1=70:30 amadumbe: millet, AMP1=70:30:3 amadumbe: millet: psyllium, BREAD AMP1 = 70:30:3 amadumbe: millet: psyllium.

5.4.4. Starch digestibility of gluten-free bread

In vitro starch digestion data (Table 5.2) were assessed to categorize the digested starch into 3 fractions, namely rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS). The digestibility profile for bread showed an increase in RDS and SDS of 2.98% and 4.32% respectively, and a decrease in RS of 7.3%. This increase in RDS fraction could be a result of starch gelatinization during the baking process (Shumoy et al., 2018), which resulted in starch degrading rapidly. Due to increased fibre addition, the water content of the formulation increased, and this led to an increase in starch gelatinization. Starch gelatinization is increased by higher water content, which may result in an increased level of RDS. During the baking process, extra water causes the starch granules to swell and break down more thoroughly, increasing their accessibility to digestive enzymes (De La Hera et al., 2014). SDS showed a significant difference of 4.32% and this could be due to the increased moisture of the dough and resultant bread. In a study conducted by De La Hera et al. (2014), it was found that increasing water content maintained SDS fractions due to a more consistent gelatinization process. This led to a more controlled glucose release during digestion. Temperature, food processing, starch gelatinization, and storage conditions all influence the RS content of starch. RS results in this study showed a decrease of 7.3 %, which could have been due to the high baking temperature (190 ◦C) and time (45 min). In comparison to bread made under standard conditions (200◦C for 45 min), a product baked at a low temperature (120 ◦C) for an extended period of time (20 hrs) had significantly higher RS (Amaral et al., 2016).

Table 5. 2. In vitro starch digestibility profile of AMP1 bread

Sample	RDS	SDS %	RS
Bread AMP1	17.58±0.19	10.11±0.19	44.20±0.00

Mean ±SD (n=3) reported
RDS = Rapidly digestible starch, SDS = Slowly digestible starch, RS = Resistant starch, Bread AMP1 = 70:30:3 amadumbe: millet: psyllium,

5.4.5. Sensory acceptability of bread

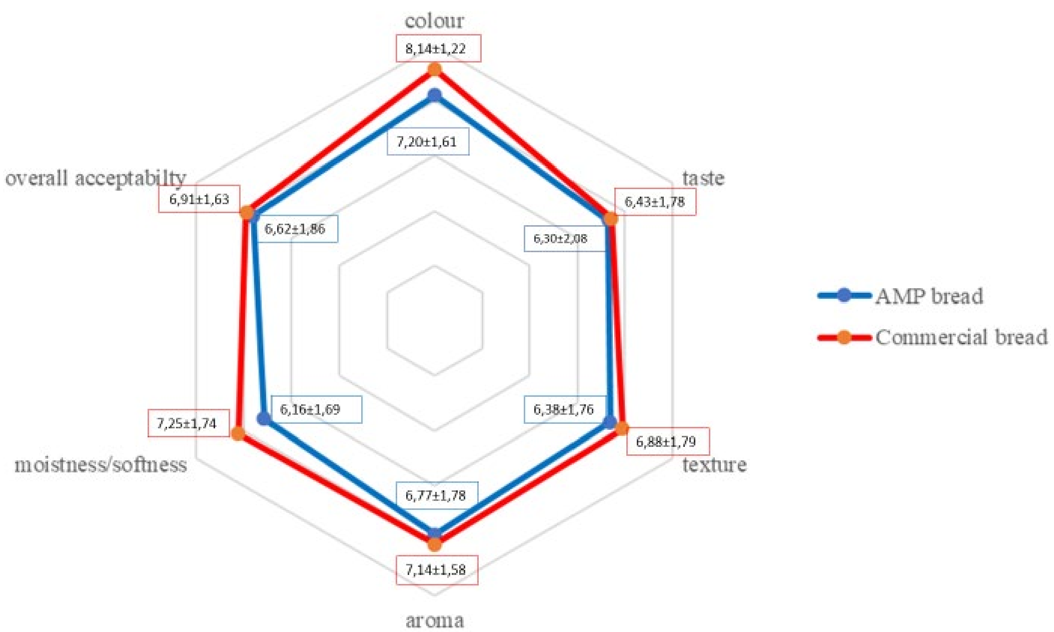

Figure 5. 4. Sensory evaluation radar chart of AMP1 bread vs commercial GF bread.

Sensory acceptability of the AMP1 bread was rated against a commercially available GF bread made up of maize starch. Sensory attributes with respect to colour, texture, taste, aroma, moistness/softness and overall acceptability of the breads were assessed. Results (Figure 5.4) showed that there was a significant difference between the colour and moistness/softness of the breads; however, taste, texture, aroma, and overall acceptability showed no significant difference ($p > 0.05$). Overall, the commercial GF bread rated higher in all sensory attributes, but by a very narrow margin. Evaluation of colour and moistness/softness of the commercial bread (7,20±1,61) vs AMP1 bread (8,14±1,22), showed that the light colour and the softness of the bread, which resembled more of a cake texture, were more preferred by the panelists. The darker colour of the bread may be attributed to the inherent pigmentation of millet flour, as well as to non-enzymatic browning reactions, including Maillard reactions and caramelisation. Additionally, oxidation of phenolic compounds may have contributed, particularly as the flour was milled and used directly for baking without further processing (Li et al., 2015). The addition of psyllium fibre could have led to a decrease in the final moistness of bread. The texture (6,38±1,76), taste (6,30±2,08) and overall acceptability (6,62±1,86) of the AMP1 bread were rated very close to the commercial GF bread at (6,88±1,79), (6,43±1,78) and (6,91±1,63), respectively. Most panelists liked that the AMP1 tasted and had the texture of normal wheat-based bread. A few panellists felt that the AMP1 bread had an earthy aroma, due to the amadumbe flour; however, most panelists stated it smelled of freshly baked bread. The primary factor influencing the acceptability of most foods is taste. The formulation of amadumbe, millet and psyllium fibre flours blended resulted in a bread of desirable flavour, which was perceived well by most panellists and showed much potential. The mean scores for the AMP1 bread were greater than 6, indicating their sensory attributes were acceptable.

5.5. Conclusion

In this study, fibre supplementation significantly improved the characteristics of the dough. The addition of fibre to the dough improved the functional properties by increasing WAC, thereby increasing starch gelatinization and improving the interaction of water molecules. The viscoelastic properties improved, suggesting a stronger, more cohesive network. Texture analysis revealed decreased dough firmness, which may be attributed to competition between dietary proteins and water molecules, thereby restricting the development of a cohesive structural network. In evaluating the baking performance of the bread, analysis of textural properties showed an increase in the crumb firmness. Sensory analysis indicated that the bread made from the composite blend was rated very closely to the commercially available GF bread in terms of aroma, taste and texture. Starch digestibility results showed a decrease in the RS content, which could be due to high baking temperatures and increased baking times.

Maintaining or improving the sensory attributes (taste, aroma, texture) of bread made from alternative ingredients is essential for consumer acceptance. Balancing these attributes with nutritional benefits is crucial. Whilst alternative ingredients can enhance the nutritional profile of bread, ensuring that these benefits are retained through the baking process and are bioavailable to consumers is important. This composite blend satisfies most of the demands for a nutritionally enriched GF bread, creating healthier and more diverse options in the bakery industry.

CHAPTER SIX

6. General Discussion

In response to the rising prevalence of gluten sensitivities and celiac disease, the demand for high-quality gluten-free baked goods has surged. However, formulating GF dough mixes that replicate the desirable characteristics of their gluten-containing counterparts remains a significant challenge (Cappelli et al., 2020, Siminiuc and Ţurcanu, 2023). This required a multifaceted approach, including selecting alternative ingredients that mimic gluten's viscoelastic properties and modifying starch to control its digestibility.

The efficacy of hydrothermal modification in modifying starch characteristics is well-documented, highlighting the necessity of precisely calibrating treatment parameters to attain the desired level of modification or improvement (Subroto et al., 2022). Psyllium fibre further enhanced the impact of hydrothermal treatment on maize starch by promoting the formation of resistant starch and slowly digestible starch fractions while reducing the amount of rapidly digestible starch (Mancebo et al., 2015). The incorporation of psyllium fibre introduces complexities owing to its significant water-binding capacity and possible interactions with starch molecules, which can lead to alterations in viscosity and gelation behaviour. The synergistic interaction between psyllium and hydrothermal treatment creates a matrix within the starch structure, modulating enzyme accessibility and slowing down the digestion rate. The influence of hydrothermal methods on dough quality depends on the flour type, where increasing native starch concentration reduces the dough storage modulus in multi-cereal bread formulations; pre-gelatinized starch increases the linear viscoelastic interval of the dough, indicating enhanced viscoelastic properties (Siminiuc & Ţurcanu, 2023).

Structural analysis using FTIR and XRD analyses revealed subtle structural changes in starch following hydrothermal treatment. FTIR analysis of both native and hydrothermally treated starches exhibited characteristic starch peaks, primarily in the carbohydrate region (900-1200 cm^{-1}) and, to a lesser extent, in the protein region (1500-1750 cm^{-1}), with no significant peak shifts observed. XRD analysis indicated that native waxy and normal maize starches displayed A-type crystallinity, while high amylose maize starch showed B-type crystallinity.

FTIR and XRD analyses indicate that hydrothermal treatment induces only minimal molecular rearrangements without fundamentally altering the starch structure, thereby preserving the native crystalline polymorph of each starch type. Functionally, this translates into improved thermal stability and paste strength while largely maintaining rheological behaviour. In addition, the treatment confers nutritional advantages—particularly in high-amylose starch—by preserving B-type crystallinity and reducing digestibility.

Pasting properties of native, HMT and ANN-treated, and psyllium-supplemented HMT and ANN-treated starches demonstrated variations in pasting parameters. HMT-treated starches exhibited significantly ($p \leq 0.05$) lower peak, final, trough, breakdown, and setback viscosities, alongside higher pasting temperatures. This suggests a restricted swelling capacity and increased granular stability, potentially due to increased interactions between starch chains (Siminiuc and Ţurcanu, 2023). In contrast, ANN-treated starches generally showed higher viscosities across all parameters (peak, final, trough, breakdown, and setback), coupled with lower pasting temperatures. This indicates enhanced swelling and a greater degree of starch gelatinization (Siminiuc and Ţurcanu, 2023). The addition of psyllium to HMT-treated starches resulted in lower viscosities compared to the native starch, but higher viscosities than their HMT-only counterparts. This suggests that psyllium partially counteracts the viscosity-reducing effects of HMT, likely due to its water-binding capacity (Mancebo et al., 2015). For psyllium-supplemented ANN-treated starches, a general decrease in peak, final, setback, and trough viscosities was observed for normal corn starch, whereas for waxy corn starch, only peak and breakdown viscosities increased, with an overall decrease in pasting temperature. The contrasting behaviour between NCS and WCS likely reflects differences in their amylose/amylopectin ratios and their interactions with psyllium. The higher temperature (over 100 °C) required for paste formation, as indicated by HACS, influenced all subsequent parameters. The addition of psyllium to gluten-free doughs results in reduced pasting temperature, in addition to increasing elastic and viscous moduli values (Mancebo et al., 2014).

Rheological analysis using the frequency sweep analysis revealed that all starch pastes exhibited a predominantly "solid-like" gel behaviour, characterized by a storage modulus exceeding the loss modulus across all samples. Hydrothermally treated starches displayed higher G' values compared to their psyllium-supplemented counterparts. Among all the samples, NCS with psyllium supplementation exhibited the highest G' values when compared to WCS and HACS.

Findings from rheological analysis indicate that both, type of starch and psyllium supplementation significantly alter the viscoelastic properties of starch pastes. Hydrothermal treatment improves elastic strength by strengthening starch molecular interactions, while psyllium influences network formation by water retention and structural disruption. The enhanced efficacy of psyllium-supplemented NCS indicates that starches with a balanced amylose composition may provide optimal performance in fiber-enriched systems, especially when a balance between structural integrity and textural flexibility is required.

Hydrothermal treatment significantly altered the digestibility of the maize starches, generally increasing resistant starch and slowly digestible starch content while slightly decreasing rapidly digestible starch. Normal corn starch exhibited the most consistent increase in RS and SDS content following hydrothermal treatments, both with and without 3% psyllium fibre. Psyllium supplementation further enhanced this effect, resulting in a 24.87% and 14.88% increase in SDS + RS content for HMT-treated and ANN-treated NCS, respectively. The alteration of native starch through hydrothermal modification and, to a smaller extent, Annealing, induces significant changes in the molecular structure and overall design of the starch, consequently elevating the content of resistant starch (Zdybel et al., 2021). These modifications collectively contribute to an increase in both resistant starch and slowly digestible starch fractions, thereby substantially diminishing the proportion of rapidly digestible starch (Siminiuc and Ţurcanu, 2023). This reduction in digestibility, achieved through synergistic action of the modifications, suggests a potential pathway towards healthier starch alternatives with a lower glycemic response (Ge et al., 2021).

Amadumbe, millet, and psyllium husk flour blends (AM1, AM2, AMP1 and AMP2) presented a viable opportunity for crafting gluten-free flours with enhanced functional attributes, particularly for specialized dietary needs. The absence of gluten in food formulations often leads to structural and textural deficiencies, necessitating the exploration of alternative ingredients and techniques to replicate its viscoelastic properties (Cappelli et al., 2020).

The functional properties exhibited by pure amadumbe flour (A) and the formulated blends (AM1, AM2, AMP1, AMP2) demonstrate their competitive potential, with psyllium supplementation (AMP1, AMP2) yielding the most promising results in terms of total dietary fibre, viscoelasticity, and resistant starch content. The total dietary fibre content increased by 3.4% for AM → AMP1 and 2.84% for the AM2→AMP2 blends, respectively. The incorporation of psyllium husk fibre emerges as a pivotal strategy, effectively compensating for the structural losses typically associated with gluten absence by imparting desirable viscoelastic characteristics to the flour blend (Ren et al., 2020a). The rheological properties of gluten-free doughs are critical indicators of end-product quality, providing valuable insights for food technologists in selecting appropriate ingredients to optimize the final product.

Starch structural analysis (FTIR and XRD) revealed no major structural changes in the flour blends, with similar patterns observed across all samples. Specifically, FTIR analysis showed variations in peak intensities but no shifts in peak positions, and XRD analysis indicated typical A-type diffraction patterns for all flour blends. Despite the similarities in basic structure, pasting properties were significantly influenced by psyllium supplementation ($p \leq 0.05$), with psyllium-supplemented blends exhibiting nearly double the viscosity (of all parameters assessed) of blends without psyllium, although pasting temperatures decreased with psyllium addition.

Rheological analysis indicated a strong viscoelastic behaviour in all flour blends (with and without psyllium), as evidenced by a storage modulus (G') exceeding the loss modulus (G"). This is consistent with previous research suggesting that psyllium's water-binding capacity and interactions with starch molecules can significantly alter viscosity and gelation behaviour (Pejcz and Burešová, 2022). The changes observed in pasting and rheological properties, despite the lack of major structural changes detected by FTIR and XRD, underscore the complex interactions between starch, fibre, and water in these systems, as well as the limitations of FTIR and XRD in detecting subtle structural differences. The quality of dough and bread is significantly influenced by hydration-based interactions involving starch, fibre, and water, which can significantly affect rheology and processing quality, without observable structural modifications. These interactions regulate dough stability, gas retention, crumb texture, and shelf life, highlighting the significance of functional characterisation in the formulation of fiber-enriched and hydrothermally modified starch-based breads.

The strategic use of psyllium husk fibre in amadumbe-millet composite flour not only enhances the rheological dough properties but also significantly improves the nutritional quality with respect to its dietary fibre content, addressing key considerations for individuals with specific dietary requirements (Mancebo et al., 2015). Psyllium's inclusion in gluten-free formulations contributes to a more rigid polysaccharide chain conformation, enhancing dough stability. Understanding the viscoelastic parameters, such as elasticity modulus and viscosity coefficients, is essential for assessing the textural attributes of doughs, which can be significantly influenced by the addition of alternative flours. Various studies on different flour dough systems have mostly used standard oscillatory parameters (G′ and G″). However, this study, focused on the use of elasticity moduli and viscosity coefficients to give a more structural explanation of dough structure. Providing information on the difference between instantaneous elastic reaction and time-dependent viscous flow. This gives us a better understanding of how different flours change the behaviour of dough in ways other than gluten containing doughs.

Starch digestibility analysis revealed significant differences in the starch fractions of psyllium-supplemented flour blends. Specifically, the addition of psyllium resulted in a statistically significant ($p \leq 0.05$) increase in resistant starch (RS) and slowly digestible starch (SDS) by 8.05% and 6.36% in the AMP1 and AMP2 blends, respectively. The observed increase can be attributed to the ability of psyllium to interact with starch molecules, thereby modulating enzyme accessibility and reducing the rate of digestion. This also aligns with other studies that demonstrate psyllium's capacity to promote the formation of resistant starch and slowly digestible starch fractions (Pejcz and Burešová, 2022). In gluten-free bread production, psyllium is often added to enhance the structure and improve the mouthfeel of the bread (Ren et al., 2020a). In this study, the supplementation of psyllium significantly modified the viscoelastic profile of gluten-free doughs, as evidenced by increased elasticity moduli and viscosity coefficients. These changes indicate the formation of a stable, hydrated polysaccharide network capable of mimicking gluten functionality by enhancing both recoverable deformation and resistance to time-dependent flow.

The development of gluten-free (GF) bread comes with many challenges, including adherence to current dietary trends and customer demands for healthier 'clean label' products. While findings from the preceding objective suggest the potential of AMP1 flour formulation for gluten-free baked goods, further investigations were needed to fully elucidate the texture, sensory acceptance and quality of fibre-supplemented flour blends.

The incorporation of fibre into dough matrices elicits a cascade of changes in the dough's fundamental characteristics, influencing its functional attributes and ultimately affecting the quality of the resultant baked product (Mesta-Corral et al., 2024). The improvement of water absorption capacity upon fibre addition signified a pivotal modification, as it directly impacted the extent of starch gelatinization (Ren et al., 2020a). This phenomenon arises from the increased interaction of water molecules with the starch granules, facilitating their swelling and subsequent disruption of the crystalline structure (Mancebo et al., 2015).

The observed improvement in viscoelastic properties suggested the formation of a more robust and cohesive network within the dough, attributable to the intricate interplay between fibre, starch, and protein components (Ren et al., 2020a). This enhancement in dough handling and gas retention during proofing is crucial, especially in gluten-free formulations where the absence of gluten necessitates alternative network-forming agents (Manhivi et al., 2018). Texture analysis revealed a decrease in dough hardness, which may be attributed to the competition between dietary fibre and protein for available water molecules, thereby hindering the formation of extensive protein networks. The modification of the water distribution within the dough matrix by fibre impacts the interactions between gluten-free flour components, influencing dough development and gas retention, which are both essential to the final product (Siminiuc and Ţurcanu, 2023).

The evaluation of bread quality, particularly in terms of textural properties, demonstrated an increase in crumb firmness in bread supplemented with fibre. The competition of fibre with starch and gluten for water can limit starch gelatinization during baking, leading to a firmer crumb texture upon cooling (Atzler et al., 2021). Sensory evaluation indicated that the composite blend closely resembles commercially available gluten-free bread in aroma, taste, and texture, which is of paramount importance for consumer acceptance. Starch digestibility results displayed a decrease in resistant starch content (from 69% for the composite flour blend to 44% for the bread), potentially due to high baking temperatures and prolonged baking times that support starch conversion into more digestible forms. Mitigating RS loss in baked bread requires strategies that promote the formation of more thermally stable resistant starch fractions. Approaches such as optimising baking time–temperature profiles, incorporating lipids to encourage amylose–lipid complex formation, and utilising high-amylose components to enhance post-baking retrogradation may help preserve or partially recover resistant starch content. The optimization of baking parameters is critical to maintaining the nutritional benefits of fibre supplementation while preserving the desired sensory attributes. Maintaining or improving the sensory attributes of bread made from alternative ingredients is essential for consumer acceptance and reformulation design. Balancing these attributes with nutritional benefits is crucial in the development of functional food products. Ensuring that these benefits are retained through the baking process and are bioavailable to consumers is of paramount importance (Siminiuc and Ţurcanu, 2023). The use of fibre-rich ingredients not only contributes to improved dough handling and texture but also introduces valuable prebiotic effects, promoting gut health and overall well-being (Gobbetti et al., 2018).

CHAPTER SEVEN

7. Conclusion and Recommendations

7.1. Conclusion

Hydrothermal modification of maize starch, particularly when synergized with psyllium fibre, presents a complex yet promising avenue for tailoring starch characteristics and enhancing dough quality. The care required in regulating treatment parameters during hydrothermal modification underscores the sensitivity of starch to these processes, influencing its digestibility and functionality. The synergistic interaction between psyllium and hydrothermal treatment organizes a structural rearrangement within the starch matrix, modulating enzyme accessibility and slowing down the digestion rate, which has implications for glycemic control in food applications.

The strategic formulation of gluten-free flour blends utilizing amadumbe, millet, and psyllium fibre presents a viable pathway towards addressing the inherent functional limitations often encountered in gluten-free baking and food product development. The incorporation of psyllium fibre emerges as a particularly compelling approach, effectively compensating for the structural deficits typically associated with the absence of gluten by providing desirable viscoelastic properties to the flour blend. The enhanced viscoelasticity achieved through psyllium supplementation not only improves the dough handling characteristics but also contributes to the overall textural quality of the final baked product. This improvement is crucial, as gluten-free formulations often struggle to replicate the structural integrity and mouthfeel of their gluten-containing counterparts.

7.2. Recommendations

- There should be further exploration to evaluate the potential of sourdough fermentation as a biotechnological strategy to enhance both the technological and nutritional properties of fibre-enriched gluten-free bread. Sourdough fermentation has demonstrated promising results in improving the nutritional aspects of gluten-free diets. The implementation of specific cultures as starters in sourdough processes can substantially diminish fructan levels, yielding a low-FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols) bread alternative, which is particularly beneficial for individuals suffering from Irritable Bowel Syndrome, while also boosting both the nutritional value and technological attributes of the bread.
- Future research into the bioavailability of nutrients in fibre-enriched gluten-free bread, investigating the impact of fibre on the absorption and utilization of vitamins, minerals, and antioxidants, is essential for substantiating health claims and promoting the nutritional benefits of these products.

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APPENDICES

APPENDIX 1: Publication of Manuscript

Starch - Stärke

WILEY-VCH
Starch

RESEARCH ARTICLE

Modulating Starch Properties Through Hydrothermal Treatment and Psyllium Fibre Supplementation: Influence of Amylose Contents

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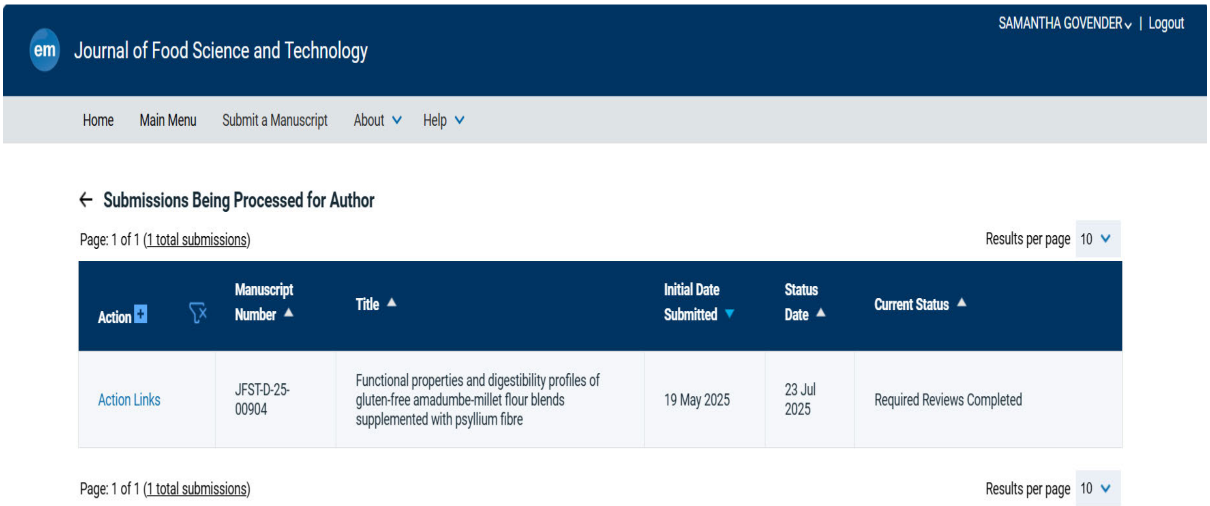
Funding: This study was supported by the National Research Foundation of South Africa (Unique Grant Number 141983).

ABSTRACT

Although the individual effects of hydrothermal treatment, psyllium fiber, and amylose content on starch properties are well understood, their combined interactions remain largely unexplored. This study investigated the impact of heat moisture treatment (HMT) and annealing (ANN), combined with 3% psyllium husk fiber (PHF), on the structural, rheological, and digestibility properties of maize starch with varying amylose content. The starch samples were analyzed for crystallinity, viscosity, and in vitro digestibility. Frequency sweep and FTIR spectra were also determined. Waxy and normal maize starch showed typical A-type diffraction pattern, while high-amylose starch exhibited B-type diffraction pattern. HMT-treated starches had significantly ($p \leq 0.05$) lower peak and final viscosities, while ANN-treated starches exhibited higher viscosities. HMT and ANN treatments generally increased G′, regardless of fiber addition, except for waxy maize starch. Hydrothermal treatments alone had minimal impact on slowly digested starch (SDS) and resistant starch (RS) content, but psyllium significantly increased SDS and RS levels. Findings from the study suggest that combining HMT and ANN with PHF supplementation can significantly modulate starch digestibility, making them suitable for functional food applications with potential health benefits such as diabetes management and improved gut health. Future research should focus on the application of the starch blends in product formulations, as well as the evaluation of their shelf life and sensory properties in the newly developed products.

APPENDIX 2: Manuscript under review in Journal of Food Science and Technology



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Submissions Being Processed for Author

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Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links	JFST-D-25-00904	Functional properties and digestibility profiles of gluten-free amadumbe-millet flour blends supplemented with psyllium fibre	19 May 2025	23 Jul 2025	Required Reviews Completed

Page: 1 of 1 (1 total submissions) Results per page 10

APPENDIX 3: Ethical Approval

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
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Durban University of Technology

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Tel: 031 373 2375
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www.dut.ac.za

17 October 2024

Prof E o Amosou
Department of Biotechnology and Food Science
Faculty of Applied Sciences
Durban University of Technology

Dear Prof Amosou

Characterisation of gluten-free dough mixes with reduced starch digestibility for baking applications

I am pleased to inform you that **PROVISIONAL APPROVAL** has been granted to your proposal subject to:

- Obtaining and submitting the necessary gatekeeper permission/s to DUT-Institutional Research Ethics Committee (DUT-IREC).

PLEASE NOTE THAT THIS IS NOT A FINAL APPROVAL LETTER. KINDLY SUBMIT THE ABOVE MENTIONED DOCUMENTS WITHIN THREE MONTHS TO THE DUT-IREC OFFICE. DATA COLLECTION CAN ONLY COMMENCE WHEN DUT-IREC ISSUES FULL APPROVAL

The Proposal has been allocated the following Ethical Clearance number **IREC 130/24**. Please use this number in all communication with this office.

Approval has been granted for a period of **ONE YEAR**, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the DUT-IREC. This form must be submitted to the DUT-IREC at least 3 months before the ethics approval for the study expires.

It is compulsory for a student or researcher to apply for recertification on an annual basis. The failure to do so will result in withdrawal of ethics clearance. It is the responsibility of the researcher and the supervisor to apply for recertification.

Yours Sincerely

Professor P Mashau
Chairperson: DUT-IREC

APPENDIX 4: Sensory evaluation guidelines, instructions and scorecard

DEPARTMENT OF BIOTECHNOLOGY AND FOOD SCIENCE

DURBAN UNIVERSITY OF TECHNOLOGY

SENSORY EVALUATION – GLUTEN-FREE BREAD

SENSORY EVALUATION GUIDELINES:

1. Please rinse your mouth with water before tasting the bread.
2. There are 2 samples to evaluate
3. Please rinse your mouth with water after tasting each sample.
4. Please taste each sample of bread in the order presented to you from left to right.
5. Please rate the colour, taste, texture (mouthfeel), aroma and moistness/softness by placing a cross in line with the attribute you are scoring that best describes the sample.
6. You may re-taste the sample if you wish.

INSTRUCTION ON HOW TO EVALUATE SENSORY ATTRIBUTES:

Appearance/colour/visual appeal: Observe the samples visually and rate them for these attributes.

Odour: Smell using small sniffs and rate the odour.

Taste: Taste the bread, chew for a short while, swirl on the tongue and then swallow, and rate the taste perceived

Texture: Taste the bread piece, chew for a while, swirl on the tongue and swallow, rate the texture

Moistness/ softness: observe the bread, cut a small portion and taste it using a spoon to rate the moistness, or you can feel the sample in your hand by pressing it between your fingers to rate softness.

PLEASE TURN OVER

SAMPLE CODE:________________

	COLOUR	TASTE	TEXTURE (MOUTHFEEL)	AROMA	MOISTNESS/ SOFTNESS	OVERALL ACCEPTABILITY
LIKE EXTREMELY (9)						
LIKE VERY MUCH (8)						
LIKE MODERATELY (7)						
LIKE SLIGHTLY (6)						
NEITHER LIKE NOR DISLIKE (5)						
DISLIKE SLIGHTLY (4)						
DISLIKE MODERATELY (3)						
DISLIKE VERY MUCH (2)						
DISLIKE EXTREMELY (1)						

SAMPLE CODE:________________

	COLOUR	TASTE	TEXTURE (MOUTHFEEL)	AROMA	MOISTNESS/ SOFTNESS	OVERALL ACCEPTABILITY
LIKE EXTREMELY (9)						
LIKE VERY MUCH (8)						
LIKE MODERATELY (7)						
LIKE SLIGHTLY (6)						
NEITHER LIKE NOR DISLIKE (5)						
DISLIKE SLIGHTLY (4)						
DISLIKE MODERATELY (3)						
DISLIKE VERY MUCH (2)						
DISLIKE EXTREMELY (1)						