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Pre-treatments and formulation for children in Mozambique

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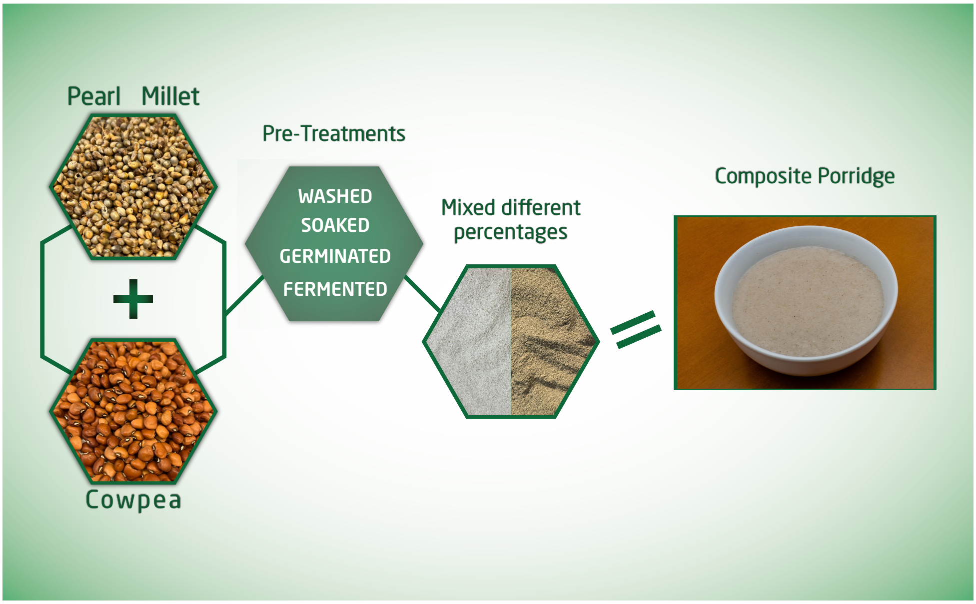

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Abstract

The aim of this thesis was to develop a composite porridge with a high nutritional value, to help combat child malnutrition in Mozambique. The research focused on the use of pearl millet and cowpea, crops that are widely cultivated and consumed in the northern region of the country, known for their climate resilience, availability and accessibility. Both grains are acknowledged for their high nutritional value and health-promoting compounds. In Mozambique, it is common for children and adults to consume the same food, which are predominantly starch-based. However, these foods are typically high in viscosity whilst low in energy and protein density and lack essential micronutrients crucial for child growth.

This thesis focused on evaluating the effects of different pre-treatments, namely soaking, germination, and fermentation, on the physicochemical and morphological properties of pearl millet and cowpea flours as well as the resulting composite porridges. The primary objective was to identify the formulation with improved nutritional quality and reduced anti-nutritional factors, while maintaining both sensory appeal and cultural acceptability. The results revealed that germination altered starch granules of pearl millet and significantly reduced a porridge's viscosity, whereas the cowpea starch granules were less affected. Pre-treatments enhanced the extractability of dietary fibre and phenolic compounds. Both fermentation and germination also promoted the enzymatic breakdown of the starch into smaller fractions. The six porridge formulations developed using fermented pearl millet exhibited rapidly digestible starch and lower levels of phytic acid. Among the developed composite porridges, those containing fermented samples, either together or combined with germinated pearl millet or cowpea were all liked by Mozambican mothers, with fermented pearl millet and cowpea combined with germinated cowpea being the most liked. Overall, the results highlight the potential of using pre-treated samples to develop affordable, culturally acceptable, and nutritionally balanced composite porridge to address protein-energy malnutrition in Mozambique and similar low-income countries.

Keywords: children undernutrition, pearl millet, cowpea, pre-treatments, composite porridges, physicochemical properties, phenolic compounds, digestible starch, sensory evaluation.

Gröt av pärlhirs och ögonböna

Sammanfattning

Syftet med denna avhandling var att utveckla en sammansatt gröt med högt näringsvärde, avsedd att bekämpa barnundernäring i Moçambique. Forskningen fokuserade på användningen av pärlhirs och ögonböna som odlas och konsumeras i stor utsträckning i den norra delen av landet och är kända för sin klimattålighet, tillgänglighet och åtkomlighet. Båda grödorna är erkända för sitt höga näringsinnehåll och sina hälsofrämjande egenskaper. I Moçambique är det vanligt att barn äter samma mat som vuxna, vilken huvudsakligen består av stärkelserika livsmedel. Dessa har dock ofta hög viskositet, lågt energi- och proteininnehåll samt brist på viktiga näringsämnen som är avgörande för barns tillväxt.

Studien syftade till att utvärdera effekterna av olika förbehandlingar, såsom blötläggning, groddning och fermentering, på de fysikalisk-kemiska och morfologiska egenskaperna hos pärlhirs och ögonböna samt på de resulterande grötarna. Målet var att identifiera den sammansättning som gav förbättrad näringskvalitet och minskade nivåer av antinutritionella faktorer hos en sensoriskt och kulturellt accepterad produkt. Resultaten visade att groning förändrade stärkelsegranulerna i pärlhirs och avsevärt minskade grötens viskositet, medan stärkelsen i ögonböna påverkades i mindre grad. Förbehandlingarna förbättrade även frisättningen av kostfiber och fenolföreningar. Både fermentering och groning främjade enzymatisk nedbrytning av stärkelsen till mindre fragment. De sex sammansättningarna som utvecklades med fermenterad pärlhirs uppvisade snabbt nedbrytbar stärkelse och lägre nivåer av fytinsyra. Grötarna som innehöll fermenterad pärlhirs, grodd pärlhirs samt fermenterad ögonböna uppskattades bland moçambikiska mödrar, där den mest omtyckta kombinationen var fermenterad pärlhirs och ögonböna med tillsatt grodd ögonböna. Resultaten visar på potentialen att använda förbehandlade ingredienser för att utveckla prisvärda, kulturellt accepterade och näringsmässigt balanserade grötar för att motverka protein- och energibrist hos barn i Moçambique och andra låginkomstländer.

Nyckelord: barnundernäring, pärlhirs, ögonböna, förbehandlingar, sammansatta grötar, fysikalisk-kemiska egenskaper, fenolföreningar, nedbrytbar stärkelse, sensorisk utvärdering.

Papas compostas de mexoeira e feijão-nhemba

Resumo

A presente tese teve como objetivo desenvolver uma papa composta de elevado valor nutricional, destinada ao combate da desnutrição infantil em Moçambique. A pesquisa centrou-se no aproveitamento da mexoeira e do feijão-nhemba, culturas tradicionalmente cultivadas e consumidas na região norte do país, reconhecidas pela sua resistência climática, ampla disponibilidade e acessibilidade. Ambos os grãos apresentam perfil nutricional favorável, incluindo compostos bioativos com propriedades benéficas à saúde. Em Moçambique, é comum que as crianças consumam os mesmos alimentos que os adultos, à base de amido. Esses alimentos, contudo, apresentam elevada viscosidade, baixa densidade energética e proteica, além de serem deficientes em micronutrientes essenciais para o crescimento infantil.

Esta investigação avaliou os efeitos de diferentes pré-tratamentos: imersão, germinação e fermentação, sobre as propriedades físico-químicas e morfológicas das farinhas de mexoeira e feijão-nhemba, bem como nas papas resultantes. O objetivo principal foi identificar a formulação com melhor qualidade nutricional e menores níveis de fatores anti-nutricionais, assegurando a aceitabilidade sensorial e cultural. Os resultados revelaram que a germinação alterou significativamente os grânulos de amido da mexoeira, reduzindo a viscosidade das papas. Os pré-tratamentos favoreceram extração de fibra alimentar e compostos fenólicos. A germinação e a fermentação também promoveram a degradação enzimática do amido em frações mais simples. As seis formulações com mexoeira fermentada apresentaram rápida digestão do amido e redução dos níveis de ácido fítico. Entre as papas desenvolvidas, aquelas com amostras fermentadas e adição de farinha germinada destacaram-se pela boa aceitação por parte das mães moçambicanas, sendo a mais preferida, a formulação com amostras fermentadas e feijão-nhemba germinado. De modo geral, os resultados evidenciam o potencial de uso de amostras pré-tratadas para desenvolver papas nutricionalmente balanceadas, constituindo uma estratégia promissora no combate da desnutrição energético-proteica em Moçambique e em outros contextos de baixa renda.

Palavras-chave: desnutrição infantil, mexoeira, feijão-nhemba, pré-tratamentos, papas compostas, propriedades físico-químicas, compostos fenólicos, digestão do amido, avaliação sensorial.

Preface

This thesis aimed to develop a nutrient-dense composite porridge made from pearl millet and cowpea to combat protein-energy malnutrition among children in Mozambique. These locally available and affordable seeds are known for their essential nutrients and health-promoting compounds. The study explored the use of different traditional pre-treatments such as soaking, germination, and fermentation to enhance nutritional value and reduce anti-nutritional factors. Overall, this work contributes to the development of culturally acceptable, affordable, and nutritionally improved composite porridge. It also provides insights that can help in developing community-based nutrition programmes and support government policy intervention in Mozambique.

Dedication

To my family, who shaped my foundation, and to the family I have built and to my country, Mozambique, may this work be a step toward change.

“Be the change you wish to see in the world”
Mahatma Gandhi

Contents

List of publications

This thesis contains the following papers, referred to by Roman numerals in the text:

I. **Sunera Nurmomade***, Santanu Basu, Irene de Carvalho, Maria Eduardo, Roger Andersson (2024). Effect of pre-treatment on physicochemical, microstructural and pasting properties of pearl millet and cowpea. LWT, 198, 115951. https://doi.org/10.1016/j.lwt.2024.115951

II. **Sunera Nurmomade***, Santanu Basu, Irene de Carvalho, Maria Eduardo, Roger Andersson. Effect of pre-treatments on dietary fibre components, phenolic compounds and minerals of pearl millet and cowpea. (Submitted)

III. **Sunera Nurmomade***, Santanu Basu, Irene de Carvalho, Maria Eduardo, Mehdi Abdollahi, Roger Andersson. Impact of pre-treated pearl millet and cowpea on porridge quality. (Manuscript)

IV. **Sunera Nurmomade***, Janicka Nilsson, Irene de Carvalho, Maria Eduardo, Santanu Basu, Roger Andersson. Effect of pearl millet and cowpea pre-treatments on rheology, digestible starch and molar mass distribution of extractable polysaccharides in a composite porridge. (Submitted)

V. **Sunera Nurmomade***, Maria Eduardo, Santanu Basu, Irene de Carvalho, Roger Andersson, Karin Wedin (2025). Sensory evaluation of composite porridges based on pearl millet and cowpea for Mozambican children under five years old. International Journal of Gastronomy and Food Science, 41, 101271. https://doi.org/10.1016/j.ijgfs.2025.101271

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The contribution of Sunera Zulficar Nurmomade to the papers included in this thesis was as follows:

I. Designed the experiment with the supervisor's team, performed the majority of experiments, conducted the statistical analysis, evaluated the results, wrote, and revised the manuscript.

II. Designed the experiment with the supervisors, performed the experiments, conducted the statistical analysis, evaluated the results, wrote, and revised the manuscript.

III. Designed the experiment with supervisors, performed the majority of the experiments, conducted the statistical analysis, evaluated the results, wrote, and revised the manuscript.

IV. Designed the experiment with supervisors, performed the majority of the experiments, conducted the statistical analysis, evaluated the results, wrote, and revised the manuscript.

V. Designed the study together with co-authors, compiled the ethical application, conducted the sensory experiments in Mozambique as well as the laboratory experiments in Uppsala, analysed the evaluation forms and the questionnaire, conducted the statistical analysis, evaluated the results, wrote, and revised the manuscript.

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Abbreviations

ANCs	Anti-nutritional compounds
ANOVA	Analysis of variance
DF	Dietary fibre
DM	Dry matter
FAO	Food and Agricultural Organization
FCP	Fermented cowpea
FGMFP	55% fermented pearl millet + 5% germinated pearl millet + 40% fermented cowpea
FGMSP	55% fermented pearl millet + 5% germinated pearl millet + 40% soaked cowpea
FMFGP	60% fermented pearl millet + 35% fermented cowpea + 5% germinated cowpea
FMFP	60% fermented pearl millet + 40% fermented cowpea
FMSGP	60% fermented pearl millet + 35% soaked cowpea + 5% germinated cowpea
FMSP	60% fermented pearl millet + 40% soaked cowpea
FPM	Fermented pearl millet
GCP	Germinated cowpea
GPM	Germinated pearl millet
HPIC	High performance ion chromatography
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
ICF	International company for implementing the project
ICP-MS	Inductively coupled plasma mass spectrometer
INE	Instituto Nacional de Estatística
LVR	Linear viscoelastic region

MALS	Multi angle light scaterring
PCA	Principal component analyses
PEM	Protein energy malnutrition
RDS	Rapid digestible starch
RI	Refractive index
RS	Resistant starch
RVA	Rapid visco analyser
SCP	Soaked cowpea
SDG	Sustainable Development Goal
SDS	Slow digestible starch
SEM	Scanning electron microscopy
SGMFP	55% soaked pearl millet + 5% germinated pearl millet + 40% fermented cowpea
SGMSP	55% soaked pearl millet + 5% germinated pearl millet + 40% soaked cowpea
SMFGP	60% soaked pearl millet + 35% fermented cowpea + 5% germinated cowpea
SMFP	60% soaked pearl millet + 40% fermented cowpea
SMSGP	60% soaked pearl millet + 35% soaked cowpea + 5% germinated cowpea
SMSP	60% soaked pearl millet + 40% soaked cowpea
SPM	Soaked pearl millet
TDF	Total dietary fibre
TDS	Total digestible starch
WCP	Washed cowpea
WHO	World Health Organisation
WPM	Washed pearl millet

1. Introduction

Child malnutrition is a significant issue in many low- and middle-income countries, including Mozambique (Ganhão et al., 2017; Tasnim, 2018). There, approximately 37% of children under five years of age experience chronic malnutrition (INE and ICF, 2023). Protein-energy malnutrition (PEM) is a serious health concern and contributes to childhood morbidity and mortality in low income countries (Deng et al., 2024; Walker, 1990). Persistent socioeconomic inequalities, inadequate access to food, and insufficient health services affect a child's physiological and intellectual development (WHO, 2018). Despite various efforts to eliminate hunger, the Sustainable Development Goal (SDG) of "Zero Hunger" has not been achieved globally, notably in many African countries.

In Mozambique, diets primarily revolve around staple cereals that often lack animal protein, similar to other parts of Africa (Oladiran & Emmambux, 2022; Temba et al., 2016). The porridge prepared for children is typically cereal-based, thick, high in viscosity, and low in both energy and protein density. This deficiency in energy and protein significantly contributes to high rates of PEM among children.

Formulating composite porridges that combine cereals with legumes, specifically pearl millet and cowpea, has emerged as a promising strategy to tackle the problem of children malnutrition. This can be accomplished by using pre-treated flours. Traditional pre-treatments include soaking, germination, and fermentation. These treatments are known to enhance nutritional quality, reduce anti-nutritional compounds, decrease porridge viscosity without compromising energy density, and improve the bioavailability and digestibility of nutrients (Kumari & Platel, 2020; Mtebe et al., 1993; Nkhata et al., 2018; Nout & Ngoddy, 1997).

Both pearl millet (*Pennisetum glaucum* (L.) R. Br.) and cowpea (*Vigna unguiculata* (L.) Walp) are acknowleded for their high nutritional value and various health-promoting compounds (Jayathilake et al., 2018; Jukanti et al., 2016; Phillips et al., 2003; Taylor et al., 2014). They are widely consumed and produced due to their affordability and adaptability to harsh environmental conditions. Indeed, both crops are climate-resilient and can be cultivated in regions facing drought. Pearl millet is a source of energy and essential nutrients, whilst cowpea offers an affordable source of protein. The main reason for combining these two seeds is that together they provide a balanced amino acid and essential nutrient profile, which can help address the critical nutritional gaps in children's diets (Abebe & Alemayehu, 2022; Kumari & Sangeetha, 2017).

Previous studies have shown promising results when combining available and affordable cereals and legumes to reduce PEM (Adeyanju et al., 2025; Makame et al., 2020; Ogunniran et al., 2024). However, it must be noted that when formulating a newly developed product, its adoption and replication within the communities should be considered. This thesis explores the use of pre-treated pearl millet and cowpea seeds to develop an affordable, nutrient-dense, and culturally acceptable composite porridge for children in Mozambique. This project evaluates the effects of pre-treatments on the physicochemical properties, anti-nutritional factors, microstructural characteristics, and rheological behaviour of flours and composite porridges. It also investigates the enzymatic breakdown of the starch and assesses the sensory attributes of the porridges among mothers in Mozambique.

2. Background

2.1 Protein-energy malnutrition in children

PEM remains a significant global health issue and, although influenced by a variety of factors, it predominantly arises from an insufficient intake of protein and calories (Deng et al., 2024; Walker, 1990). PEM is associated with a lack of food, socioeconomic inequalities, and poor health. Most Africans live on a diet based on one to three staple food groups, such as cereals, roots, and tubers, with minimal or no protein of animal origin whatsoever (Temba et al., 2016). This largely preventable condition is responsible for over half of all childhood deaths. Undernutrition in early childhood may also cause impaired psychological and intellectual development (Deng et al., 2024; Tasnim, 2018; WHO, 2018). As a result, these children start life at a significant disadvantage. According to the UNICEF, the WHO, and the World Bank Group (2023) more than half of all children under the age of five affected by stunting in 2020 resided in Asia (51%), whilst two out of five (41%) resided in Africa.

In Mozambique, 37% of children under the age of five suffer from chronic malnutrition (INE and ICF, 2023). The northern region is the most affected, particularly Nampula (47%), Cabo Delgado (45%), and Zambézia (44%). As stated by Ganhão et al. (2017) and Lusambili et al. (2020), the elevated rates of undernutrition in the northern region may also stem from a general lack of education. This problem requires an immediate intervention approach, which could include promoting traditional knowledge and incorporating technologies to create food from locally available nutrient-rich indigenous crops. Ogunniran et al. (2024) highlighted the importance of using locally available and affordable crops to prepare composite porridges for children and their influence on preventing different forms of malnutrition. Furthermore, according to de Onis et al. (2013), nutrition interventions alone are insufficient, and a multisectoral approach is also essential to effectively reduce chronic malnutrition. This multisectoral approach should also involve improvements in education, water quality, sanitation, hygiene, poverty reduction, and maternal education.

2.2 Governmental strategies for addressing undernutrition in Mozambique

The National Directorate of Public Health (2010) emphasises that chronic malnutrition must be urgently addressed and various recommendations have been suggested. Among the seven strategic objectives outlined in the multisectoral action plan to address chronic malnutrition, the most crucial measure is enhancing nutritional initiatives targeted at children. This strategy aims to boost nutrition and health whilst educating communities about improved food preparation methods.

The government of Mozambique encourages Mozambican researchers to study available and affordable agricultural products, including nutritious wild foods, as strategies to combat undernutrition (National Directorate of Public Health, 2010). Moreover, the government of Mozambique is committed to support the Sustainable Development Goals, specifically Goal Two, which aims to "reduce hunger, achieve food security and nutrition, and promote sustainable agriculture" (Republic of Mozambique, 2020).

This thesis attempts to create new scientific insights into accessible, nutritious food for children and targets the broader goal of achieving nutritional security for children in Mozambique.

2.3 Traditional food for children in Mozambique and their limitations

In developing countries such as Mozambique, low-income families primarily rely on food that is locally available and affordable. Thus, their diets typically consist of cereals and other starchy crops such as maize, millets, cassava, and rice. Although these crops are often used to prepare porridge for children, they are frequently characterised by high viscosity, low energy and protein density, poor protein quality, and micronutrient deficiencies (Oladiran & Emmambux, 2022; Temba et al., 2016). Such characteristics can contribute to the development of PEM.

In the initial phase of this PhD project, fieldwork was conducted to gain a better understanding of the dietary habits and food preparation practices within local communities. The aim was to identify the types of porridges commonly consumed, the traditional pre-treatments methods used, and the crops that are locally accessible and affordable. Although this fieldwork was not included in any scientific publication, it provided insights into the cultural aspects of food preparation. This preliminary understanding played a pivotal role in screening the raw material to develop culturally acceptable porridge. Researchers in Africa have explored the potential of using locally available crops to develop composite foods for children to reduce PEM (Ogunniran et al., 2024). Different strategies have been implemented, including the employment of traditional processing methods that can be utilised within the communities. These processing techniques aim to enhance energy and protein levels, improve protein quality, and increase the availability and digestibility of both macro and micronutrients (Nkhata et al., 2018; Ogunniran et al., 2024; Oladiran & Emmambux, 2022). Ultimately, the goal is to boost nutritional status, reduce PEM, and improve overall health.

2.4 Composite porridges based on cereals and legumes

Different approaches to combining cereals and legumes, along with the employment of traditional pre-treatments, have been explored. The main motivation for mixing starchy crops with legumes is that legumes have a significantly higher protein content and are excellent sources of dietary nutrients (Abebe & Alemayehu, 2022; Kumari & Sangeetha, 2017; Temba et al., 2016). Moreover, legumes are often referred to as the “poor man’s meat”. Cereals generally have low levels of the essential amino acid lysine, which is abundant in legumes. On the other hand, cereals are rich in methionine, whilst legumes contain lower amounts. Therefore, combining cereals and legumes in foods can enhance protein quality and improve health whilst reducing PEM (Iqbal et al., 2006; Temba et al., 2016).

In this study, pearl millet and cowpea were selected due to their affordability and availability in Mozambique. Cowpea is a key crop in Africa and in Mozambique (Chiulele, 2010; Phillips et al., 2003; Singh et al., 2003), where both its grain and leaves often serve as the primary source of protein for low-income families. Pearl millet is also a significant source of energy within Africa, and in Mozambique, it is most cultivated in the northern region. Recently, millets have gained attention in the development of new food formulations due to their health benefits and nutrient content (Hema et al., 2022; Jukanti et al., 2016; Kaur et al., 2019; Saleh et al., 2013). Processing cereals and legumes is essential to preserve and enhance their nutritional value before consumption.

2.5 Effect of traditional pre-treatments

Traditional pre-treatments are important low-cost techniques that can be applied to seeds prior to consumption to enhance their nutritional value, reduce the levels of anti-nutritional factors, and improve their sensory attributes. These pre-treatments can be easily carried out at the household level in the villages. This thesis explores the impact of different traditional pre-treatments applied to seeds: soaking, germination, and fermentation followed by cooking.

2.5.1. Soaking

Soaking involves steeping the grains in water. It induces the leaching out of water-soluble components, softening the seeds and reducing the cooking time (Ocheme & Chinma, 2008). El-Adawy et al. (2000) reported several nutritional benefits from soaking legume seeds, including decreasing components that cause flatulence and other anti-nutritional factors.

2.5.2. Germination

Germination refers to the process of soaking the seeds in water and allowing them to germinate prior to consumption. During this process, various endogenous enzymes are activated which break down starch into dextrin and simple sugars. This generates biochemical changes that enhance the sensory and nutritional characteristics of the food (Nkhata et al., 2018; Yang et al., 2021).

Similar to soaking, germination improves the nutritional quality of seeds by reducing anti-nutritional factors (James et al., 2020; Sharma et al., 2016). Additionally, it serves as an effective pre-treatment for reducing the viscosity thereby increasing energy density without requiring as much water in the formulation (Alexander, 1983; Mtebe et al., 1993). Furthermore, germination significantly affects the physicochemical properties and in vitro digestibility of starch and protein in flours (Ghavidel & Prakash, 2007; Khetarpaul & Chauhan, 1990; Yang et al., 2021).

2.5.3. Fermentation

Fermentation is a biochemical process carried out by enzymes produced by microbes (Nkhata et al., 2018). It is one of the most used traditional pre-treatments worldwide, as well as in Mozambique. Fermentation has been shown to improve nutrient profile, reduce anti-nutrients, and improve sensory attributes of food products (Chaves-López et al., 2014; Gabaza et al., 2017; Nout & Ngoddy, 1997; Oladiran & Emmambux, 2022). Various studies have reported that fermentation increases in vitro starch digestibility whilst simultaneously decreasing the total amount of starch (Elkhalifa et al., 2004; Khetarpaul & Chauhan, 1990; Osman, 2011).

2.5.4. Cooking

Cooking generally provides physical and chemical advantages for food. It helps with mastication and digestion (Carmody & Wrangham, 2009; Singh et al., 2010). Additionally, cooking enhances food safety and its sensory attributes whilst reducing anti-nutrients (Rehman & Shah, 2005). It has been shown that cooking at high temperatures affects the pasting properties and increases the starch and protein digestibility (Almeida-dominguez et al., 1993; Rehman & Shah, 2005; Singh et al., 2010).

2.6 Characterisation of pearl millet and cowpea

2.6.1 Classification and taxonomy

Pearl millet and cowpea are climate-resilient crops. They are resistant to abiotic stress, native to Africa, and are cultivated in arid and semi-arid regions, including Mozambique. They are considered environmentally friendly crops due to their water use efficiency and nutrient absorption when intercropped (Boukar et al., 2019; Jukanti et al., 2016; Punia et al., 2021; Shrestha et al., 2023).

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) belongs to the Poaceae family, is a robust annual grass, and one of the most recently evolved millets (Punia et al., 2021). Cowpea (*Vigna unguiculata* (L.) Walp) belongs to the Fabaceae family and is an herbaceous annual crop (Boukar et al., 2019).

Figure 1: Pearl millet (left) and cowpea (right)

2.6.2 Grain chemical composition

The proximate composition of pearl millet and cowpea can vary due to the genotype, location, soil, water accessibility, and other growth-limiting factors (Dias-Martins et al., 2018; Giami, 2005; Serna-Saldivar & Espinosa-Ramírez, 2019).

Pearl millet has a higher protein and fat content compared to other millet types (Serna-Saldivar & Espinosa-Ramírez, 2019). Its protein content ranges from 8.6 to 19.4g/100g on a DM basis, whilst the fat content is approximately 1.4 to 7.3g/100g on a DM basis. The ash content varies from 1.6 to 3.6g/100g on a DM basis. The carbohydrates composition includes starch, soluble sugars, and dietary fibre, with a total carbohydrate content at around 55 to 85g/100 g on a DM basis (Jukanti et al., 2016). Starch is the most abundant carbohydrate in pearl millet, followed by dietary fibre and soluble sugars (Jukanti et al., 2016; Nambiar et al., 2011).

Cowpeas are a good source of protein, but they have a relatively low fat content (Abebe & Alemayehu, 2022; Giami, 2005). The protein content in cowpea ranges from 21 to 30g/100 g on a DM basis, making them high in protein and a cost-effective source of nutrition (Abebe & Alemayehu, 2022; Giami, 2005; Jayathilake et al., 2018). Cowpea boost the quality of cereal-based diets by providing various essential amino acids (Abebe & Alemayehu, 2022; Lambot, 2002). The fat content of cowpea is between 0.5 to 3.9g/100g on a DM basis, which is lower than that of other legume grains (Jayathilake et al., 2018). The carbohydrate content is approximately 50 to 60g/100g on a DM basis.

Both pearl millet and cowpea serve as affordable sources of carbohydrates, proteins, minerals, vitamins, and health-promoting bioactive compounds beneficial for children's growth and development. Processing can generate changes in the chemical composition, leading to increases or decreases in specific nutrients, depending on the type of seed and the processing method used (Nkhata et al., 2018). Nevertheless, processing the seeds is important for improving starch digestibility (Annor et al., 2017; Sá et al., 2020).

2.6.3 Starch, dietary fibre components and other compounds

Starch

Starch is a major energy source. It is widely found in various food products and has a significant impact on the texture of cooked products (Punia et al., 2021; Suma & Urooj, 2015). Starch consists of two main components, namely amylose and amylopectin. Amylose is a linear molecule, whereas amylopectin has a highly branched structure (Buléon et al., 1998). Starches are classified based on their amylose content as follows: waxy (0.2% amylose), very low (5-12% amylose), low (12-20% amylose), intermediate (20-25% amylose), and high (above 25% amylose) (Juliano, 1972).

In pearl millet, the starch content varies among different genotypes, ranging from 63 to 79 g/100g on a DM basis (Punia et al., 2021; Serna-Saldivar & Espinosa-Ramírez, 2019; Suma & Urooj, 2015). The amylose content in millet starch varies between 29 to 34 % of total starch (Annor et al., 2014). In cowpea, the starch content varies between 11 to 66%, with the amylose content ranging from 19 to 50% of the total starch (Oyeyinka et al., 2021).

Dietary fibre

Dietary fibre (DF) is an indigestible carbohydrate found in plant-based foods, and it plays a crucial role in maintaining health. DF offers various health benefits, such as promoting the growth of beneficial gut bacteria, producing short-chain fatty acids, reducing insulin and glucose responses, stimulating gut transit, and increasing faecal bulk (Qi et al., 2018; Rakha et al., 2011). DF encompasses various components, such as resistant starch, cellulose, hemicellulose, pectins, gums, mucilages, fructan, and fructo-oligosaccharides, as well as lignin (Andersson et al., 2009; Rakha et al., 2010; Theander et al., 1995). Total DF in pearl millet varies from 7 to 15% (Jukanti et al., 2016), whilst cowpea contains approximately 14 to 23% of total DF (Veena et al., 1995). Aside from the nutritional role, DF also influences the pasting properties of plant foods due to the presence of extractable and non-extractable fibre. For example, it absorbs more water than starch granules when placed under higher temperatures (Santos et al., 2008). During processing, endogenous enzymes in seeds are activated which break down complex cell wall polysaccharides into simpler and more fermentable forms (Nkhata et al., 2018). The content and extractability of these fibre components in plant seeds can vary widely.

Resistant starch

Resistant starch (RS) is a component of DF that undergoes fermentation in the colon, which is vital for maintaining one's health, and an important part of the human diet. When starchy foods are cooked, then cooled and stored, they develop type III RS. During the cooling process, gelatinised starch undergoes recrystallisation. This primarily involves the linear fraction of the starch, known as amylose, although amylopectin can also retrograde, but it requires more time. The recrystallised amylose is resistant to the action of human α-amylase (Champ et al., 2003; Cummings & Stephen, 2007). The intervention study by Balamurugan et al. (2019) found that stunted children possessed an impaired capacity to ferment certain types of RS. Thus, lower levels of RS are beneficial when preparing food for undernourished children.

Digestible starch

Digestable starch (DS) varies significantly among cereals, legumes, and tubers. The amount of DS is influenced by the physicochemical and microstructural characteristics of the starch, the composition of the plant seed, as well as the applied processing methods and conditions (Kingman & Englyst, 1994; Liu et al., 2006). Generally, starch in cereal flour is more rapidly digestible compared to that in legume flour.

Based on in vitro starch digestion studies, there are three distinct starch fractions: Rapidly digested starch (RDS) which represents the amount of starch that breaks down within 20 minutes, slowly digested starch (SDS) which breaks down within 120 minutes, and total digestible starch (TDS) which comprises the amount of starch that breaks down within 240 minutes (Englyst et al., 1992).

For malnourished children, it is important to provide foods that are easily digestible, nutrient-dense, and low in viscosity (Makame et al., 2020; Weaver et al., 1995). Processing methods such as soaking, germination, fermentation, and cooking can enhance starch digestibility (Elkhalifa et al., 2004; Nnanna & Phillips, 1990; Sharma & Kapoor, 1996).

Minerals and vitamins

Pearl millet is a rich source of minerals such as potassium, phosphorus, magnesium, iron, zinc, copper, and manganese (Satyavathi et al., 2021). Cowpea, on the other hand, is high in calcium, magnesium, potassium, iron, zinc, and phosphorus. However, it is important to note that the levels of phosphorus, potassium, and manganese in cowpea may vary widely depending on the environmental conditions (Abebe & Alemayehu, 2022). Moreover, millets also provide vitamins and they are particularly rich in B vitamins. Pearl millet has been shown to contain the highest amount of carotene when compared to other millet types (Anandharamakrishnan et al., 2022). Cowpea is also a good source of B vitamins and carotenoids, which may enhance antioxidant activity (Abebe & Alemayehu, 2022).

Additionally, the presence of phytates in seeds may compromise the bio-accessibility of essential minerals by forming a phytate-mineral complex. The absorption of minerals, notably iron and zinc, is significantly reduced in the presence of phytates, which are known to inhibit their uptake (Sandberg, 2002). However, soaking, germination, fermentation, and cooking can effectively reduce phytates levels (Badau et al., 2005; Gabaza et al., 2017; Torre et al., 1991). Consequently, these methods can enhance mineral and vitamin bioavailability, particularly iron, for populations that rely on a cereal and legume-based diet.

2.6.4 Anti-nutritional and phenolic compounds of pearl millet and cowpea

Anti-nutritional compounds

Certain cereals and legumes contain anti-nutritional compounds (ANCs) that can affect and reduce the absorption of macronutrients and micronutrients (Gemede & Ratta, 2014; Kaur et al., 2021; Samtiya et al., 2020). Cereals and legumes contain a broad spectrum of ANCs, including saponins, tannins, goitrogens, trypsin inhibitors, lectins, hemagglutinin, gossypol, proteases inhibitors, amylase inhibitors, and phytates (Kaur et al., 2021; Samtiya et al., 2020). This study placed a specific emphasis on phytates because it is the most abundant ANC in most cereals and legumes, with the potential to inhibit mineral absorption.

Phytate, also known as inositol hexakisphosphate (IP6), is the salt form of phytic acid and is mainly found in legumes, cereals, nuts, and oil seeds (Gemede & Ratta, 2014; Silva & Bracarense, 2016). The phytic acid content in pearl millet ranges between 172 and 327 mg/100 g, with some reports indicating levels as high as 990 mg/100 g (Jukanti et al., 2016). In cowpea, phytate levels vary between 360 and 631 mg/100 g (Avanza et al., 2013; Melini & Melini, 2021). The consumption of food containing phytates may lead to mineral deficiencies, particularly in diets where plant-based foods dominate (Oghbaei & Prakash, 2016).

In Mozambique, children consume the same food as adults, primarily plant-based foods which are associated with a poor bioavailability of protein and micronutrients such as zinc, calcium, and iron. The application of traditional processes is recommended to reduce the ANCs in the seeds and, in turn, improve the bioavailability of minerals (Castro-Alba et al., 2019; Eyzaguirre et al., 2006; Gabaza et al., 2017; Ibrahim et al., 2002; Punia, 2000). On the other hand, recent studies have also highlighted potential health benefits of consuming seeds that contain ANCs (Melini & Melini, 2021; Oghbaei & Prakash, 2016; Silva & Bracarense, 2016).

Phenolic compounds

Phenolic compounds are products of secondary metabolism in plants. They comprise various compounds, including flavonoids, tannins, coumarins, alkylresorcinols, and phenolic acids (Li et al., 2009). These compounds act as defence mechanisms against external factors and contribute to the colour and flavour of plants. In food products, these compounds influence the oxidative status and contribute to the overall aroma, taste, and appearance (Li et al., 2008).

Phenolic acids are divided into two groups: hydroxycinnamic acids and hydroxybenzoic acids. The most abundant hydroxycinnamic acid found in cereal grains is ferulic acid, followed by p-coumaric and caffeic acids (Wang et al., 2014). Earlier findings identified ferulic and p-coumaric as majorly bound phenolic acids in millets (Chandrasekara & Shahidi, 2010; Subba Rao & Muralikrishna, 2001). In graminaceous plants, ferulic acid is ester-linked to various cell wall constituents, such as arabinoxylan (Chandrasekara & Shahidi, 2010; Li et al., 2009). Phenolic compounds can form complexes with protein, starch, and minerals, inhibiting their absorption (Abdelrahaman et al., 2007). Therefore, processing the seeds is important to enhance the extractability and availability of these components. The transformation of polyphenols is also promoted during the germination of seeds through various pathways (Hassan et al., 2020). Oxidative stress is created as a result of PEM, therefore, providing children with foods rich in nutrients and antioxidants may help mitigate oxidative damage and promote recovery (Aly et al., 2014; Khare et al., 2014).

Health benefits of pearl millet and cowpea

Numerous studies have demonstrated that pearl millet confers a range of health benefits, largely due to its bioactive compounds and essential minerals. Its consumption has been linked to the reduced risk of chronic conditions such as cancer and cardiovascular diseases, reflecting an ability to lower cholesterol levels, reduce fat absorption, and delay gastric emptying (Gupta et al., 2012; Jukanti et al., 2016; Kaur et al., 2019; Nambiar et al., 2011; Rai et al., 2008; Saleh et al., 2013; Taylor et al., 2014). Similarly, cowpea is rich in nutrients and contains several health-promoting compounds. This legume has recently received attention for its beneficial properties, including anti-diabetic, anti-cancer, anti-inflammatory, and anti-hypertensive properties, in preventing chronic diseases (Jayathilake et al., 2018; Kumar & Bhalothia, 2020; Phillips et al., 2003; Singh et al., 2003).

When combined in food formulations, pearl millet and cowpea offer complementary nutritional profiles, generating a blend of health-promoting compounds. Such combinations are particularly valuable for addressing PEM in children, especially in low-income countries, such as Mozambique where dietary diversity is often limited.

2.6.5 Pasting and rheological properties of pearl millet and cowpea flour

Pasting properties

Cereals and legume flours contain starch, protein, dietary fibre, lipids, and other minor components. Whilst starch is the major component that controls the pasting properties of flours, these properties are also influenced by other components within the flour. Pasting refers to the changes that occur in starch when it is mixed with water and heated. During this process, the starch granules undergo gelatinisation, transforming from a crystalline structure into a disordered form. The granules absorb water and swell. Sufficient swelling leads to the leaching out of amylose into the liquid phase and finally a rupture of the granules (Balet et al., 2019; Schirmer et al., 2015).

The peak viscosity represents the highest level of viscosity during heating. Factors that typically influence the peak viscosity include the size and shape of the starch granules, the type and degree of crystallinity within the granules, the ionic charge of the granules, molecular size and degree of branching, and other chemical components present in the flour (Henshaw et al., 1996).

Generally, peak viscosity is higher in cereal flours than in legume flours. Legumes usually do not exhibit a distinct peak viscosity, but rather a sharp rise in viscosity over time (Hoover & Sosulski, 1985). The processing methods of seeds significantly affect their pasting properties and the combination of different types of flour can also play a role (Adeyanju et al., 2025). Measuring the pasting properties of processed flour can provide insights into its functional characteristics and help to predict food viscosity, which is a critical factor for children's diets (Thaoge et al., 2003). The ideal apparent viscosity for children's porridge should be between 1000-3000 cP to provide adequate consistency (Mosha & Svanberg, 1990; Thaoge et al., 2003).

Rheological properties

The rheological properties of food play a crucial role in quality control, product development, and sensory evaluation (Liepsch, 2016; Wang & Ren, 2020). Most food products exhibit a combination of viscous and elastic properties (Ramli et al., 2022). The viscoelastic properties of both dispersed and continuous phases, along with their interactions, significantly influence the rheological behaviour of food.

In porridge, rheological properties affect both acceptability and stability. Porridges can be characterised as a three-dimensional network consisting of flour particles and swollen starch granules, embedded within a gel-like phase that affects their rheological properties (Ojijo & Shimoni, 2004). Additionally, the three-dimensional paste structure can be affected by various factors, including the type of flour, particle size, cooking duration, solids content, processing methods, and temperature. The presence of specific solutes, such as sugars, can also significantly influence the rheological behaviour (Wang & Ren, 2020; Yoo & Yoo, 2005). Furthermore, additional components, such as proteins, lipids, and salts, along with their interactions, play a role in modifying the rheological properties of food products (Adeyanju et al., 2025).

2.6.6 Sensory Evaluation

Sensory evaluation plays a key role in determining product acceptability. According to Stone & Sidel (2009) it is a science that assesses, analyses, and interprets how individuals respond to products using their senses: sight, sound, smell, taste, and touch. Consumer testing is used to gather insights into how consumers perceive a newly developed or modified product compared to an existing one. The sensory evaluation of a food can be affected by human response, influenced by psychological factors and previous experiences.

To measure consumers' opinions, various scales are commonly employed. The 9-point hedonic scale is an effective method for assessing liking or preference, as it functions as an approximate equal interval scale that is well-suited for untrained consumer panels. The scale consists of nine points indicating varying degrees of liking or disliking for a specific attribute (Stone & Sidel, 2009). Another method is the facial hedonic scale, which is often used for children or people with limited literacy skills (Guinard, 2000).

For effective evaluations of product acceptability, a sample size of 75 to 150 participants is recommended to ensure significant differences within the population (Singh-Ackbarali & Maharaj, 2014). In addition, questionnaires or interviews can be conducted in multiple forms: through in-person questioning, self- administered paper forms, websites, or telephone interviews. Each method is suitable for different situations. In food consumer tests, the questions regarding the general opinion of the product must be adapted to ensure that the respondents can easily understand them.

3. Aims and objectives

The overall aim of this thesis was to develop a composite porridge with a high nutritional value based on pearl millet and cowpea for undernourished children in Mozambique. Different traditional pre-treatments were employed to enhance the nutritional value and reduce anti-nutritional factors in pearl millet and cowpea.

The specific objectives of the thesis were to:

- Investigate the effect of pre-treatments on the physicochemical and morphological properties of the flour samples (Papers I and II);
- Analyse the chemical composition, digestible starch, rheological behaviour and enzymatic breakdown of the starch in composite porridge samples (Papers III and IV);
- Evaluate the sensory attributes and overall liking for the composite porridges among mothers in Mozambique (Paper V).

4. Materials and methods

The materials and methods used in this thesis are explained below, with more comprehensive descriptions available in Papers I-V.

Sample preparation and pre-treatments were conducted in the laboratory in Uppsala, as described in Papers I and II. The formulations for the composite porridges are outlined in Paper III. The six selected composite porridges that underwent further investigation are described in Paper IV. Lastly, Paper V provides details on the sensory analysis of porridges conducted in Mozambique. Information regarding the porridge formulations used in Papers III, IV, and V can be found in Table 1.

4.1 Material

Pearl millet (*Pennisetum glaucum*) variety Changara and cowpea (*Vigna unguiculata*) variety 10 were acquired from the Mozambican Agricultural Research Institute in Montepuez, Cabo Delgado. These varieties are commercially and locally available in the northern region of Mozambique. They are known for their high yields, which contribute to food and nutritional security in Mozambique.

4.1.1 Sample preparation and pre-treatments

The samples were sorted and cleaned to remove damaged grains, stones, and other extraneous materials. The batches were then divided for pre-treatment processes (washing, soaking, germination, and fermentation). Methods described by Adebiyi et al. (2016); Griffith & Castell-Perez, (1998); Ibrahim et al. (2002), and Ocheme & Chinma, (2008) inspired these pre-treatment methods with minor modifications. A simplified version is described below.

In the washing pre-treatment, the seeds were rinsed in tap water for 10 sec, followed by drying. In the soaking pre-treatment, the seeds were steeped in tap water for 10 min and subsequently dried. In the germination pre-treatment, the seeds were soaked in tap water for 24 h and spread on germinator trays at a temperature of 30 ± 1°C, allowing them to germinate for 48 h and dried. In the fermentation pre-treatment, the seeds were soaked in tap water allowed to ferment by the action of endogenous microflora at 30 ± 1°C for 72 h, and then dried.

The washed samples were milled with the entire hull. The soaked, germinated, and fermented samples were partially dehulled following traditional Mozambican methods and subsequently milled to pass through a sieve with a mesh size of 0.5 mm.

4.1.2 Porridge preparation in the Lab

The formulations shown in Table 1 were prepared in a laboratory in Uppsala using a Rapid Visco Analyser (RVA) to mimic traditional cooking in Mozambique. A three-gram sample of flour was mixed with 25 mL of deionised water in an aluminium canister, resulting in a total solid content of 10.7%. The porridges were freeze-dried using the main drying program at 0 °C and 1.5 mbar for 48 h, followed by secondary drying for 5 h at 30 °C and 0.01 mbar. The freeze-dried porridges were then stored at -80 °C for further analyses, which are detailed in Paper III. In Paper IV, a total solid content of 11.5% was used to prepare the six selected composite porridges for subsequent chemical and rheological analyses.

Table 1. Composite porridge formulations.

Formulations Colour code		Pearl Millet Treatments %				Cowpea Treatments %				Papers III	Papers IV	Papers V
	SMSP	Soaked	60			Soaked	40			III		
	SMFP	Soaked	60			Fermented	40			III		
	SGMSP	Soaked	55	Germinated	5	Soaked	40			III		
	SGMFP	Soaked	55	Germinated	5	Fermented	40			III		
	SMSGP	Soaked	60			Soaked	35	Germinated	5	III		
	SMFGP	Soaked	60			Fermented	35	Germinated	5	III		
	FMSP	Fermented	60			Soaked	40			III	IV	
	FMFP	Fermented	60			Fermented	40			III	IV	V
	FGMSP	Fermented	55	Germinated	5	Soaked	40			III	IV	
	FGMFP	Fermented	55	Germinated	5	Fermented	40			III	IV	V
	FMSGP	Fermented	60			Soaked	35	Germinated	5	III	IV	
	FMFGP	Fermented	60			Fermented	35	Germinated	5	III	IV	V

4.1.3 Porridge preparation in Mozambique communities

Cooked composite porridges were prepared with a total solid content of 13%, using 208 g of total flour mixed with approximately 1400 ml of tap water. The flour-water slurries were cooked in a traditional manner with coal as detailed in Paper V. The cooking process was carried out within 15 min and sugar was added just before the sensory evaluation.

4.2 Methods

An overview of different analyses applied to the flours and composite porridges is briefly described in this section; for a more detailed description, see Papers I–V.

4.2.1 Determination of chemical composition

Proximate composition

Crude protein content was measured through the Kjeldahl method (N x 6.25). Fat content was determined using a Hydrotec 8000 and Soxtec 8000 extraction unit. Ash was determined by incineration in an oven at 600 °C (Paper I).

Dietary fibre components

The dietary fibre components of the flours analysed in Paper II and the composite porridges in Paper III were quantified by the Uppsala method (Theander et al., 1995) and subsequently modified for separate measurement of extractable and unextractable dietary fibre (Andersson et al., 1999). The fructan content was analysed using assay kit K-FRUC (Megazyme, Bray, Ireland).

Total starch and amylose content

The starch contents of the flours analysed in Paper I and the composite porridges in Paper III were determined using thermostable α-amylase and amyloglucosidase (Åman et al., 1994). The amount of released glucose was quantified using kit K-GLUC (Megazyme, Bray, Ireland). Additionally, amylose content was measured using a colorimetric method based on iodine complex formation stabilised with trichloroacetic acid (Chrastil, 1987). Amylose content was assessed in both the flour samples in Paper I, and the porridge samples in Paper III.

Resistant starch, total digestible starch, and in vitro digestible starch

The RS and TDS contents analysed in the freeze-dried composite porridges were measured using the kit K-RAPRS (Megazyme, Bray, Ireland), detailed in Paper III. The amount of digestible starch in freshly prepared composite porridges was measured using the in vitro digestible starch enzymatic kit K-DSTRS (Megazyme, Bray, Ireland) (Paper IV).

Minerals

In Paper II, the mineral content of the flours, such as calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), sulphur (S), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) were determined using ICP-MS.

Phenolic compounds

The analysis of phenolic compounds was performed with a slight modification of the Li et al. (2009) method. Free and bound phenolic acids extraction of the flours is detailed in Paper II and composite porridges in Paper III. The samples were analysed with HPLC.

Phytic acid

The phytic acid contents of the composite porridges were extracted and then determined using HPIC (Carlsson et al., 2001). The inositol phosphates were detected using UV–vis HPLC, and the correction factor used for IP5 come from the standard curve of IP6 0.1–0.6 µmol/mL as reported by Skoglund et al. (1997). A more detailed description can be found in Paper III.

Molar mass distribution

Porridge was prepared in a RVA, and the sample was immediately transferred to an Eppendorf tube containing acetic acid. Centrifugation was carried out and the supernatant was then filtered. The molecular weight distribution of the extractable polysaccharides was determined using a HPSEC-MALS system. Chromatograms were detected by a MALS detector, a viscometer detector and a RI detector. Further details on this can be found in Paper IV.

4.2.2 Physical and microstructural properties

Morphological properties

Pearl millet and cowpea flour were analysed using SEM (Hitachi TM-1000, Tokyo, Japan) with 2500X magnification (30 µm scale) (Paper I).

Particle size distribution

The particle size distribution of the flours was determined by sieve fractionation using an AS 200 shaker (Retsch, Haan, Germany) with a set of five graded sieves (425, 250, 150, 75, and 50 μm) and a collection pan (Paper I).

Colour measurements

Porridge colour was analysed using a Konica Minolta spectrophotometer CM-600d (Konica Minolta, inc., Japan). The colour parameters were expressed as L*, a*, and b* values. The L* values stand for whiteness to darkness. The chromatic portion was analysed by a* (+) redness and a* (−) greenness, and b* (+) yellowness and b* (−) blueness (Paper V).

4.2.3 Rheological Properties

The rheological properties of the six selected composite porridges (Paper IV) were determined at 40 °C using a DHR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40 mm parallel plate geometry. Apparent viscosity was recorded at a shear rate range of 0.01 to 100 s^{-1}. Strain sweep tests (0.1 to 100% strain) were conducted to determine the LVR. For frequency sweep tests, strain was kept at 1.0% within the LVR and angular frequency from 1.0 to 100 rad/s was used for viscoelastic profiling of the porridge samples prepared in the RVA.

Pasting properties

RVA determined the pasting properties of the flours (Paper I) and composite porridges (Papers III-V). The samples were heated from 50 °C to a maximum temperature of 95 °C which they were held for 2.5 min, before cooling to 50 °C. Pasting temperature, peak viscosity, peak time, breakdown, trough, setback, and final viscosity were each recorded.

4.2.4 Sensory evaluation

Sensory evaluation was performed in accordance with the rules of the Ethics Committee of the Medicine Faculty and Central Hospital of Maputo in Mozambique, approved under the number CIBS FM&HCM/15/2023 on 29th July 2024. A 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) as well as a facial hedonic scale was used to assess the sensory attributes of the composite porridges among mothers in three different locations (Copuito, Pulupu, and Nanguasse) in Mozambique. A more detailed description can be found in Paper V.

4.2.5 Statistical analysis

All analyses in Papers I, II and III were conducted on replicate batches used for the pre-treatments. In Papers I and II, analyses were performed on the flour samples whilst in Paper III analyses were performed on the composite porridge samples. In Paper III, the phytic acid analyses was performed on only one batch. Similarly, in Papers IV and V, all analyses were carried out on a single batch.

Statistical analyses were performed using Minitab software (version 19.2.). ANOVA was conducted using the general linear model procedure, and Tukey's pairwise comparisons test was applied to identify significant differences between group means at a 95% confidence level.

Additionally, multivariate analysis was performed using the software SIMCA 17 (Sartorius Stedim Data Analytics AB). Principal component analysis (PCA) score and loadings plots were used to visualise differences between samples and to explore correlations between variables. For further detailed descriptions of the statistical methods refer to Papers I-V.

5. Results and discussion

The research presented in this thesis investigates the effect of different pre-treatment techniques, soaking, germination, and fermentation, which are applied to the pearl millet and cowpea seeds before they are processed into flour. These pre-treated flours were then used to develop different formulations, which were subsequently cooked to create porridges. The resulting flour and porridge samples were analysed and discussed in different research papers.

Papers I and II investigated the effect of the pre-treatment methods on the physicochemical, microstructural, and pasting properties of the flours. Paper III focused on the chemical composition of twelve different composite porridges, as well as the influence of cooking on specific phenolic compounds. Paper IV investigated digestible starch, the enzymatic breakdown of the starch, and the rheological properties in six selected composite porridges. Lastly, Paper V used sensory evaluations with Mozambican mothers to assess their liking of three selected composite porridges for children.

5.1 Effect of pre-treatments on nutritional composition of the flours and composite porridges

The proximate composition of pearl millet and cowpea flour was analysed, and the results are presented in Table 2. The data shows that cowpea flour contains nearly twice the crude protein content of pearl millet, ranging from 23.6% to 27.9% of DM, compared to 12.1% to 12.9% of DM in pearl millet. In cowpea, germination and fermentation significantly increased the protein content, whereas no statistically significant changes were observed among pre-treated pearl millet samples. Legumes are known to have a higher protein content than cereals. Moreover, cowpea is particularly rich in lysine, an essential amino acid often limited in cereal grains (Prinyawiwatkul et al., 1996). Therefore, combining cowpea with pearl millet in porridge formulations has the potential to improve protein quality.

Pearl millet contains nearly three times more crude fat, ranging from 5.9% to 6.6% of DM compared to 2.3% to 2.5% of DM in cowpea, with no statistically significant changes observed between pre-treatments for both crops. The crude protein and fat contents in cowpea align with previously reported by Abebe & Alemayehu (2022), and likewise the composition in pearl millet is consistent with earlier findings by Jukanti et al. (2016). The slight variations in proximate composition across different studies may be due to different varieties and growing conditions.

Crude protein and fat of composite flours were calculated based on individual flour results (Table 2). The addition of cowpea flour, whether soaked or fermented, increased the protein content in the composite flours, resulting in a formulation with a higher protein content compared to pearl millet flour. According to Abebe & Alemayehu (2022), cowpea provides essential amino acids necessary for a cereal-based diet. As a result, the protein quality of composite porridge is better suited to feed children in low-income countries to prevent PEM. As the different studies highlight, the combination of cereals and legumes and applying traditional pre-treatments techniques such as germination and fermentation, to prepare children's porridge are effective strategies for reducing their viscosity and increasing the energy density (Griffith et al., 1998; Nkhata et al., 2018; Ocheme & Chinma, 2008; Thaoge et al., 2003). A high energy density diet is ideal for addressing malnutrition in children (Donnen et al., 1996; Treche, 1999).

Starch is a major component in both cereals and legumes and plays a critical role in the human diet regarding energy gain. As shown in Table 2, the total starch content was significantly higher in pearl millet, ranging from 67.2-71.0 % of DM, compared to cowpea which ranging between 44.6-51.9 % of DM. The amylose content (% of starch) in pearl millet samples ranged from 32.1 to 33.6 %, which is considered relatively high compared to many other cereal starches (Emmambux & Taylor, 2013). Germinated samples resulted in a slight decrease in amylose content (% of starch) compared to soaked samples. Interestingly, fermentation led to an increase in both total starch and amylose content (% of starch). This increase may be due to the breakdown of the cell wall and protein matrix during fermentation, which likely released starch granules that were previously inaccessible. As result, more starch becomes available for measurement. In contrast, washed cowpea showed lower total starch content, possible due to intact protein matrix protecting the surface of the starch granules and thereby limiting their enzymatic degradation. This observation is supported by Zhu et al. (2010) who reported that the protein matrix protects starch granules from enzymatic attack.

Table 2. Nutrient composition of pre-treated pearl millet and cowpea (Paper I) and composite porridges (Paper III).

Sample Codes	Crude Fat (%)	Crude Protein (%)	TDF[1] (%)	Total Starch (%)	Amylose (% of starch)	Ash (%)
			Paper I			
WPM	6.0[a]	12.8[d]	7.0[bc]	71.0[a]	32.1[ab]	1.3[c]
SPM	6.1[a]	12.7[d]	6.0[c]	69.9[ab]	33.1[ab]	1.3[c]
GPM	5.9[a]	12.1[d]	7.7[bc]	67.2[b]	31.5[b]	1.0[cd]
FPM	6.6[a]	12.9[d]	6.8[bc]	70.6[a]	33.6[ab]	0.9[d]
WCP	2.4[b]	23.6[c]	13.5[a]	44.6[e]	33.6[ab]	3.4[a]
SCP	2.3[b]	24.7[c]	7.9[bc]	48.1[d]	32.5[ab]	3.5[a]
GCP	2.5[b]	27.9[a]	8.3[bc]	47.4[de]	32.5[ab]	3.4[a]
FCP	2.3[b]	26.3[b]	8.4[b]	51.9[c]	36.9[a]	2.9[b]
			Paper III			
SMSP	4.6	17.5	7.6	58.7	43.2	
SMFP	4.6	18.1	8.4	59.5	43.1	
SGMSP	4.6	17.5	7.2	58.2	41.6	
SGMFP	4.6	18.1	7.4	62.4	39.6	
SMSGP	4.6	17.7	8.0	58.5	43.0	
SMFGP	4.6	18.2	7.7	62.0	42.1	
FMSP	4.9	17.6	7.3	58.4	43.2	
FMFP	4.9	18.3	7.2	60.3	40.5	
FGMSP	4.8	17.6	7.3	58.6	40.3	
FGMFP	4.8	18.2	7.3	58.0	40.0	
FMSGP	4.9	18.3	7.1	59.6	42.4	
FMFGP	4.9	18.3	7.2	62.0	40.1	

Results are presented as the mean of replicate batches on % of dry weight basis, except for amylose that is expressed in % of starch. Values within columns followed by different letters are significantly different at $p < 0.05$. Tukey's pairwise comparison was made on average values at a 95% confidence level.

For Paper I two factors (treatments and materials) and an interaction were used for comparison, and for Paper III four factors (SFM, GM, SFP and GP) were used. SFM is soaked, fermented pearl millet, SFP is soaked, fermented cowpea, GM is germinated pearl millet and GP is germinated cowpea.

For sample codes, see the list of abbreviations, pages 17-18 or Table 1.

1) Total dietary fibre is the sum of extractable and unextractable fractions, except oligosaccharides (DP 3–9).

5.2 Effect of pre-treatments on pasting and morphological properties

The pasting properties of pre-treated raw materials are discussed in Paper I and the results for composite porridges are presented in Paper III. Figure 2 illustrates significant differences in the pasting properties of flours and composite porridges.

The results show that washed and soaked pearl millet exhibited the highest peak viscosity, approximately 1500 cP, whereas washed and soaked cowpea flour did not exhibit a peak viscosity since it was continuously rising (Figure 2, top). According to Hoover & Sosulski (1985), legumes usually do not exhibit a peak viscosity; instead, they exhibit a gradual increase in viscosity over time. This behaviour may be attributed to the interaction of various components in cowpea flour that possibly interact with starch to varying degrees. According to Henshaw et al. (1996), the presence of protein and fibre may influence pasting properties in cowpea. Other factors that typically influence the pasting properties include starch granule size and shape, the amylose to amylopectin ratio, and degree of branching.

On the other hand, FCP flour showed the highest peak viscosity at around 1500 cP. According to Pranoto et al. (2013) and Zhu et al. (2010), the growth of proteolytic bacteria during fermentation breaks down protein, thereby freeing starch from the protein matrix, which significantly affects the pasting properties. From the SEM images in Figure 3, top panel FPM, and bottom panel FCP, we can observe a visible loose matrix compared to the washed samples.

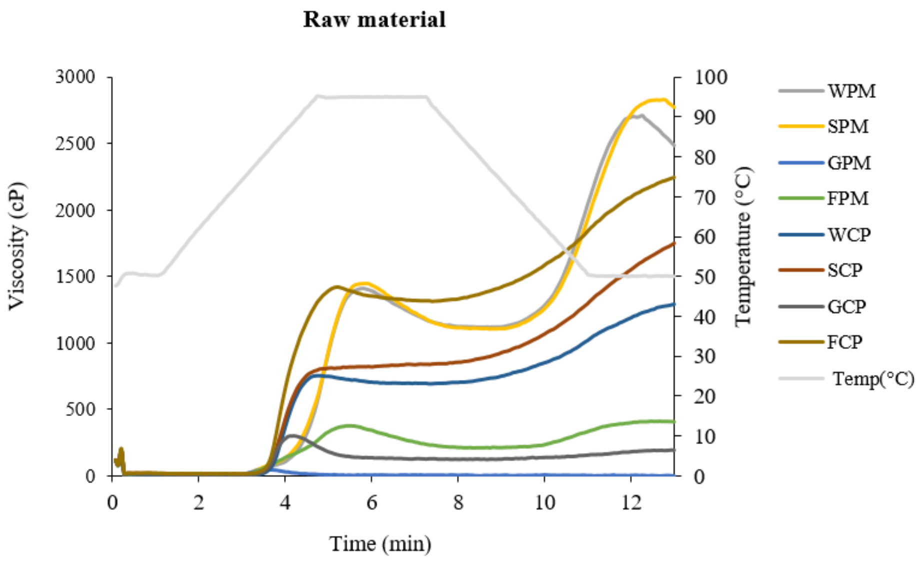
The addition of cowpea flour in the porridges, as explained in Paper III, demonstrated that the viscosity significantly decreased (Figure 2, bottom). The reduction in viscosity of composite porridge can be attributed to the presence of dietary fibre and protein content in cowpea flour. Fibre competes for water, limiting the availability of water for starch granules to swell and gelatinise fully (Henshaw et al., 1996; Rumler et al., 2024). Additionally, starch-protein interactions may also influence the hydration rate of starch granules (Henshaw et al., 1996). These findings suggest that incorporating cowpea flour altered the pasting properties of the composite porridges.

Furthermore, the incorporation of germinated pearl millet significantly lowered the viscosity of composite porridge. All composite porridges containing germinated pearl millet exhibited the lower peak and final viscosity such as SGMSP –dark yellow, SGMFP –dark violet, FGMSP –light yellow, and FGMFP – light violet as shown at the bottom of Figure 2. This reduction is likely due to the activation of various endogenous enzymes during germination, which degrades starch and consequently alters the pasting properties (Alexander, 1983; Griffith & Castell-Perez, 1998; Nkhata et al., 2018). According to Alexander (1983) and Thaoge et al. (2003), an addition of 5% of germinated flour gave an acceptable viscosity for children at a high solid content of 30%. Preliminary experiments performed in our laboratory in Uppsala with concentrations of 2.5%, 5%, and 10%, led us to conclude that an addition of 5% germinated flour is the most effective way to achieve the desired consistency of porridges as consumed in Mozambique.

As illustrated in Figure 2-top panel, germinated pearl millet flour had the lowest viscosity, followed by germinated cowpea flour. The difference in viscosity between pearl millet and cowpea indicates a higher level of enzymatic activity in pearl millet compared to cowpea. According to Lineback & Ponpipom (1977), pearl millet has a very active α-amylase system. This can be observed from the SEM images in Figure 3, top panel GPM, where numerous holes and broken starch granules were visible. However, in the bottom panel GCP, no holes were visible on the starch granules after germination, likely because low enzymatic activity and the protein content protected the starch granules from enzymatic attack. Germination may be a viable strategy in developing countries to reduce viscosity whilst increasing the total solid content of porridges, ultimately increasing energy density (Makame et al., 2020; Moriconi et al., 2023; Oladiran & Emmambux, 2022).

Viscosity is a key contributor in determining both the quantity of food that a child can consume and their overall energy intake. Oladiran & Emmambux (2022) indicated that the recommended apparent viscosity for children's food should be within the range of 1000 to 3000 cP. This viscosity range is essential as it facilitates easier swallowing for small children. Similarly, studies by Mosha & Svanberg (1983) and (1990), showed that gruel with viscosities between 1000 to 3000 cP, measured at a shear rate of 54 rpm with a viscometer, were preferred for feeding small children. Moreover, porridges are best served warm, as cold porridge has a tendency to thicken, making it more difficult for children to consume (Mouquet & Trèche, 2001).

In our exploration of the twelve composite porridges outlined in Paper III, we identified six porridges made with fermented pearl millet that were selected for further investigation in Paper IV. These samples were chosen based on their favourable nutritional profiles, lower levels of final viscosity, phytic acid, and resistant starch whilst also exhibiting a higher total digestible starch content as shown in the principal component analyses (PCA), Figure 4. A detailed description of the PCA can be found in Paper III. This selection was intended to find the appropriate formulations for sensory trial in Mozambique (Paper V).



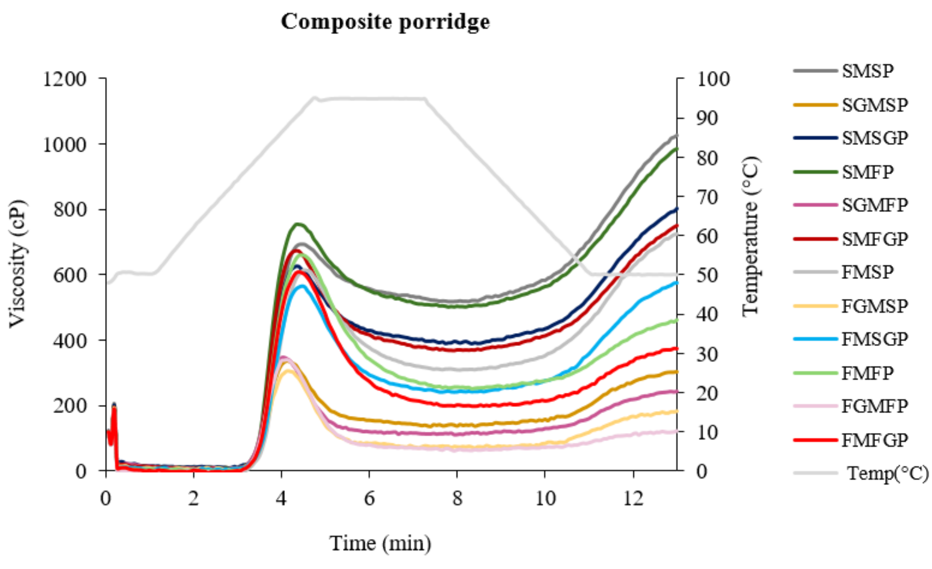


Figure 2. Pasting properties of pre-treated raw material (top panel), composite porridges (bottom panel). For sample and colour coding, see Table 1 and the list of abbreviations, pages 17-18. Composite porridges made with soaked pearl millet (dark lines) and with fermented pearl millet (light lines).

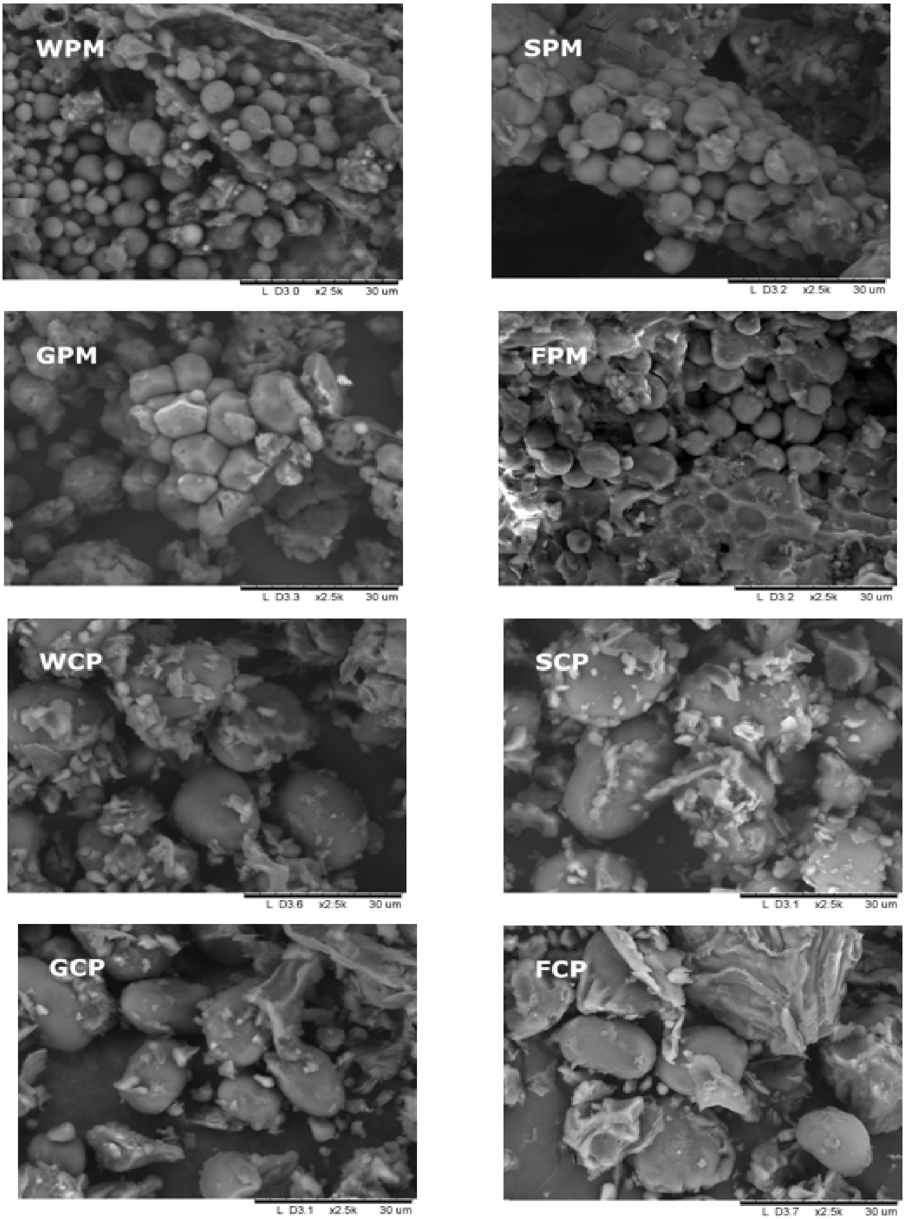


Figure 3. SEM images of starch granules of pearl millet (top panel) and cowpea (bottom panel). For sample codes, see the list of abbreviations, pages 17-18 or Table 1.

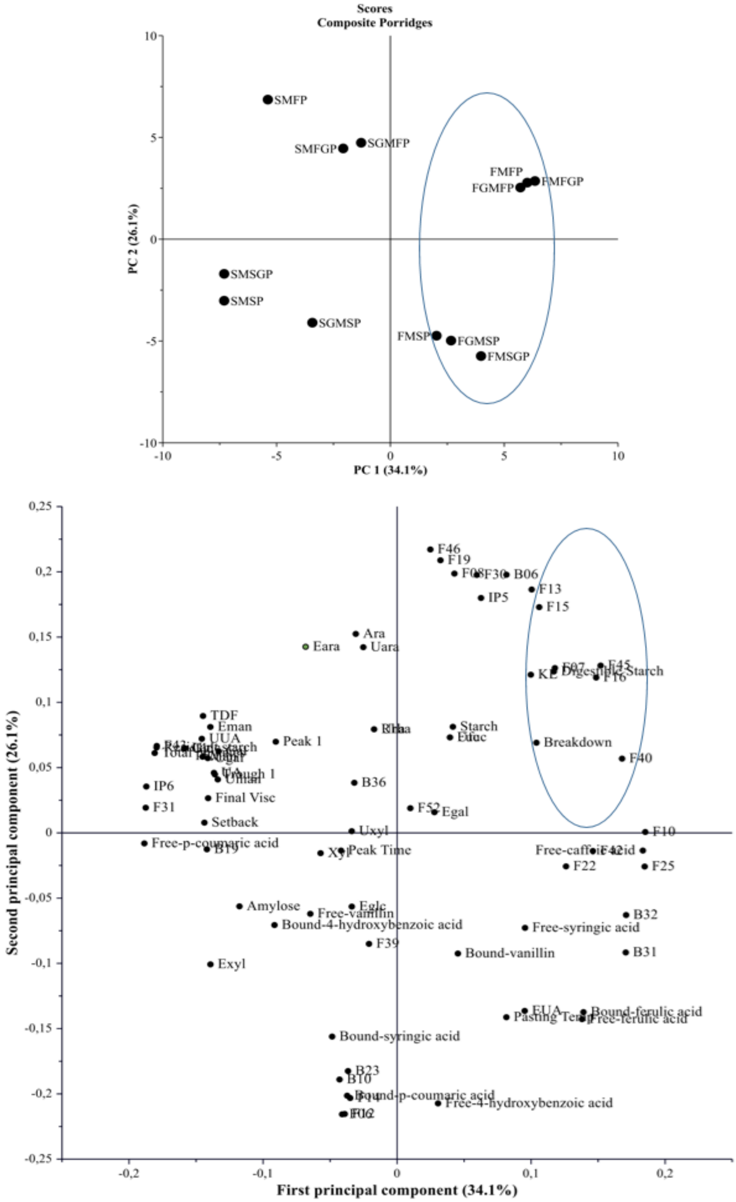


Figure 4. PCA plots of composite porridges. Scores plot (top panel) and loadings plot of variables (bottom panel). For sample codes in score plot, see the list of abbreviations, pages 17-18 or Table 1.

5.3 Effect of pre-treatments on phytic acid, resistant starch, and digestible starch of composite porridges

Pearl millet and cowpea contain high amounts of macronutrients and micronutrients, offer numerous health benefits to humans. However, they also contain anti-nutritional factors which may compromise nutrient bioavailability and lead to micronutrient malnutrition (Kaur et al., 2021; Samtiya et al., 2020). Specifically, phytate is considered a strong anti-nutritional compound; it can bind to and further hinder the activity of necessary food-degrading enzymes such as proteases, amylases, and trypsin in the small intestine of humans (Samtiya et al., 2020; Sandberg & Scheers, 2016).

In this study, the total phytate concentration, including IP6 and IP5, in twelve composite porridges (Paper III) were quantified. The total phytate levels in composite porridges containing fermented pearl millet (Figure 5, light lines) were lower in comparison to the composite porridges containing soaked pearl millet (Figure 5, dark lines). Similarly, Onweluzo & Nwabugwu (2009) and Rasane et al. (2014) reported a significant decrease in phytate content when millet and pigeon pea flour were fermented. Fermentation has been shown to significantly decrease the concentration of phytic acid. During fermentation and germination, enzymatic activities occur, including the activation of endogenous phytase which degrades phytate (Adebiyi et al., 2018; Kaur et al., 2021; Nkhata et al., 2018). In principle, the greater the amount of phytic acid, the lower the rate of starch hydrolysis. Thus, applying fermentation and germination to the seeds could be beneficial for reducing phytic acid and increasing the digestible starch content (Singh et al., 2015). According to Annor et al. (2017), the decrease in phytic acid content during fermentation may also account for improved starch digestibility as phytic acid had a significant negative correlation with in vitro starch digestibility.

In Paper III, we also investigated total digestible starch (TDS) and resistant starch (RS) of freeze-dried composite porridges. The results indicated that the levels of the RS content in composite porridges containing fermented pearl millet significantly decreased ($p < 0.05$), as illustrated in Figure 6, whilst the levels of the TDS content increased, further explained in Paper III. This may be because fermentation led to changes in the cell wall polysaccharides. When cell walls are partially degraded, enzymes have better access to attack. In addition, long amylose chains favour RS type III formation and they may be degraded by fermentation when they leak out from the starch granules. Similarly, Elkhalifa et al. (2004) found that in vitro starch digestibility in sorghum flour markedly increased as a result of fermentation, whilst RS decreased. Sharma & Kapoor (1996) also found a great effect of fermentation and germination in increasing starch digestibility. Further investigations were carried out on six selected composite porridges made with fermented pearl millet to determine in vitro digestible starch amount in freshly prepared composite porridges, as presented in Paper IV.

The content of slow, rapid, and total digestible starch was measured for the six freshly prepared composite porridges made with fermented pearl millet, as shown in Figure 7. All six composite porridges exhibited very high amount of rapidly digestible starch (RDS), with almost no slowly digestible starch (SDS). RDS is beneficial for children; it serves as the primary source of dietary energy for growing children, which can promote weight gain, especially among undernourished children in developing countries such as Mozambique, as reported by Michaelsen et al. (2009) and Weaver et al. (1995).

The application of fermentation and germination method to staple foods has become an important area of research as it offers many health benefits for humans. These processes not only help to reduce anti-nutritional factors and enhances digestible starch, but also influence the composition of dietary fibre and phenolic compounds. The changes brought about by these pre-treatments will be explored in the following section.

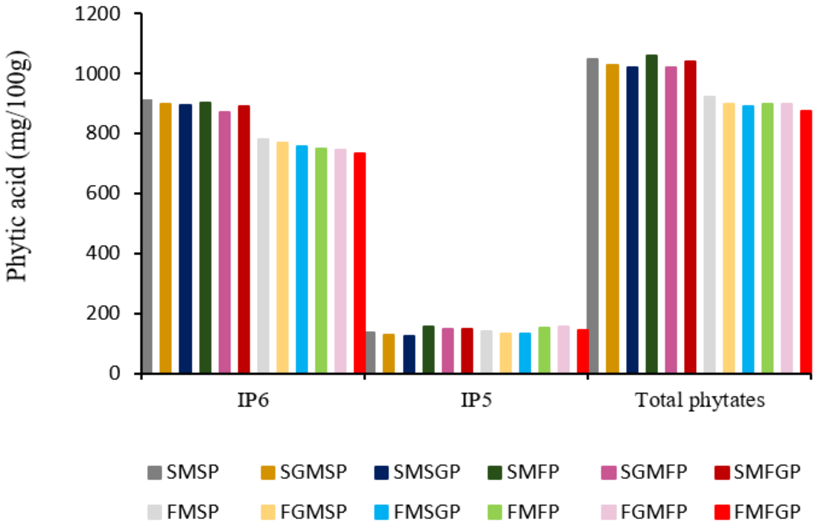


Figure 5. Phytic acid content in composite porridges. For colour coding, see Table 1.

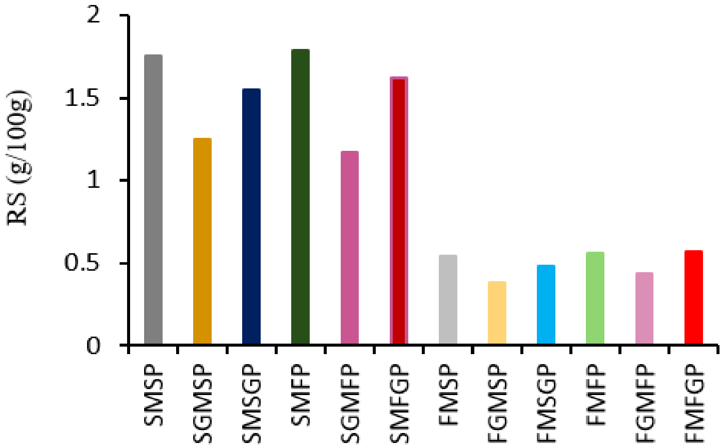


Figure 6. Resistant starch content of freeze-dried composite porridges. For colour coding, see Table 1.

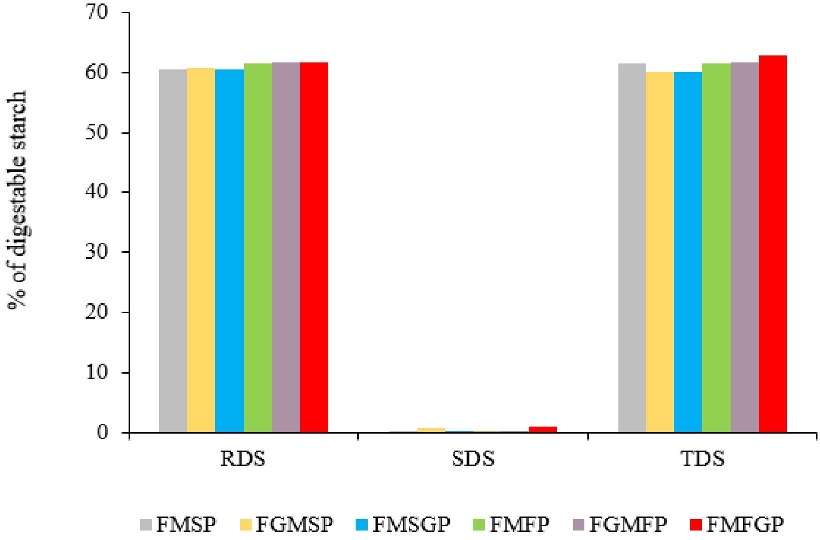


Figure 7. Amount of digestible starch of freshly prepared composite porridges in g/ 100g of flour. For colour coding, see Table 1.

5.4 Effect of pre-treatments on dietary fibre, phenolic compounds, and minerals

The total dietary fibre (TDF) components of pre-treated raw materials and composite porridges made from pearl millet and cowpea are presented in Table 3. Paper II examines the TDF in raw materials, whilst Paper III explores the TDF in the cooked composite porridges. In the raw materials, TDF content varied significantly depending on the pre-treatment. Among the cowpea samples, washed cowpea flour exhibited the highest TDF content (13.5%), which was significantly higher than the other pre-treated cowpea flours. This is likely due to the presence of the hull, as the washed samples were milled with the hull intact. In contrast, no significant differences in TDF content were observed among the pearl millet samples. These findings align with the previous studies by Griffith et al. (1998) and Veena et al. (1995), who reported that removing the hull from cowpea leads to substantial differences in fibre content, whereas no notable variations were observed in pearl millet.

In Paper III, which investigates the twelve formulations of composite porridges, no significant differences in TDF components were observed, apart from slight significant differences in glucose sugar residues. The addition of soaked, germinated or fermented cowpea flour in composite porridges did not result in significant variation in DF components (Table 3). However, porridges made with fermented pearl millet exhibited lower levels of glucose sugar residues compared to those made with soaked pearl millet. The higher glucose sugar residues levels in porridges made with soaked pearl millet may be attributed to the formation of RS type III. Moreover, an increase in glucose sugar residues was observed in our experiments when both soaked pearl millet flour and soaked cowpea flour were cooked. Previous studies have also shown that cooking can increase RS, thereby increasing the TDF content (Mahajan et al., 2021). According to Cummings & Stephen (2007), gelatinised starch, when cooled, undergoes recrystallisation, which makes it resistant to human α-amylase activity.

DF plays a crucial role in promoting positive health outcomes. However, for children with moderate malnutrition, TDF intake should be as low as possible (Aggett et al., 2003; Michaelsen et al., 2009). Unrefined cereals and legumes, such as those commonly used in homemade food in Mozambique, are naturally rich in insoluble and soluble dietary fibre, which may compromise child growth. In this study, composite porridges formulated from pre-treated pearl millet and cowpea exhibited a favourable composition of dietary fibre components.

Both seeds contributed to dietary fibre components in composite porridge. The inclusion of cowpea also contributed to the presence of uronic acid residues in the composite porridges. Indeed, legumes are generally known to contain high proportions of uronic acid residues (Longe, 1980) as pectin substances are a major compound in cowpea. The cell wall of the cotyledons contains a range of polysaccharides, including pectic substances of about 55% (Guillon & Champ, 2002).

In Paper II, we explored DF components in-depth and the results suggested that processing methods such as germination and fermentation enhanced the extractable fractions of the DF. These findings align with earlier studies on different varieties of millets by Malleshi et al. (1986) and soybean seeds by Kaczmarska et al. (2017). This enhancement can be attributed to either the breakdown of cell wall polysaccharides by endogenous or microbial enzymes that are activated during germination and fermentation, which facilitate the extractability of DF components.

Macro and micronutrients as well as bioactive compounds are abundant in plant-based food. Among these, phenolic compounds contribute to the antioxidants capacity of plant foods and are associated with many health benefits (Kaur et al., 2019; Kumar & Bhalothia, 2020). The profile of phenolic compounds in raw materials and composite porridges are presented in Papers II and III, respectively. In Paper II, which focused on raw materials (pre-treated flours), significant differences were observed in both free and bound phenolic compounds depending on the type of pre-treatments applied. Germination and fermentation were found to activate enzymes, which breaks down cell wall components and facilitate the release of phenolic compounds. These processes also led to the formation of new phenolic compounds and a transformation of the existed ones, likely due to enzymatic action and *de novo* synthesis. Additional details are included in Paper II.

As illustrated in Figure 8 (top panel), fermentation significantly increased the concentration of free ferulic acid in pearl millet, both before and after fermented pearl millet was cooked, compared to the other pre-treated pearl millet samples. Consequently, the high levels of free ferulic acid in fermented pearl millet may have contributed to the elevated levels of free ferulic acid in composite porridges made from fermented pearl millet, as illustrated in Figure 9 (top panel) and further detailed in Paper III.

On the other hand, germination led to a significant increase in bound ferulic acid levels in pearl millet (Figure 8, bottom panel). This increase is likely attributed to enzymatic hydrolysis of cell wall components during germination, facilitating the release of ester-linked ferulic acid. These observations align with previous findings in germinated and fermented millets, which also reported elevated levels of bound ferulic acid (Balli et al., 2020; Chandrasekara & Shahidi, 2010).

Further, free ferulic acid was not detected in cowpea samples prior to cooking (Figure 8, top panel). However, quantifiable levels of free ferulic acid were observed following the cooking of pre-treated cowpea samples. This suggests that heat treatment promoted the release of free ferulic acid in cowpea.

Additionally, p-coumaric acid was also detected in both free and bound fractions of pearl millet and cowpea (Figure 8). However, germination and fermentation significantly decreased the levels of p-coumaric acid in both seeds. These findings are consistent with previous studies in which similar pre-treatments led to decreased concentrations of free p-coumaric acid in cowpea (Dueñas et al., 2005) and finger millet (Subba Rao & Muralikrishna, 2001). However, upon cooking the pre-treated samples, the levels of free p-coumaric acid increased, particularly in soaked pearl millet (SPM). This increased concentration likely contributed to the elevated levels of free p-coumaric acid observed in composite porridges formulated with soaked pearl millet, Figure 9 (top panel) and discussed in Paper III.

In addition to known phenolic compounds, several unknown peaks were detected in both raw and cooked samples, as detailed in Papers II and III, respectively. In Paper II, Peak F33, observed in the soluble extracts of pearl millet, was tentatively identified as a flavonoid based on its spectral characteristics (Supplementary Figure S2, Paper II). The concentration of this compound decreased significantly with germination and fermentation as illustrated in Figure 10 (top panel), and decreased further after cooking. This reduction may be attributed to degradation of phenolics through heat treatment making it less extractable. In line with these results, Towo et al. (2003) reported that boiling finger millet for 15 min resulted in a 40% reduction in total extractable phenolics.

The bound fraction of pearl millet, Peak B33, as shown in Figure 10 (top panel), was tentatively identified as a diferulic acid and displayed an increase upon germination and a decrease after cooking (spectra in supplementary Figure S3, Paper II). On the other hand, the bound fraction of cowpea, Peak B35, as shown in Figure 10 (bottom panel), was also tentatively identified as a diferulic acid, and disappeared with germination (spectra in supplementary Figure S5, Paper II). This compound is structurally similar to ferulic acid. Diferulic acids are potent antioxidants and are ester-linked to cell wall polysaccharides such as arabinoxylan (Ralph et al., 1994). They are only bioavailable after being released from cell structures by digestive enzymes or microorganisms in the intestinal lumen (Andreasen et al., 2001). Certain studies have indicated that malnourished children often exhibit more oxidative stress and compromised antioxidant defence mechanisms (Aly et al., 2014; Khare et al., 2014). Thus, dietary interventions enriched with antioxidant compounds may be beneficial in supporting immune recovery and overall health among malnourished children.

Alongside to enhanced phenolic compounds, germination and fermentation also resulted in an increase in key minerals, such as calcium, copper, and magnesium, in raw materials. Adebiyi et al. (2017) reported a significant increase in calcium content when pearl millet was germinated. Devi et al. (2015) reported similar results for cowpea. The increase in calcium may be attributed to the salts present in the tap water used for soaking, germination, and fermentation or a loss of dry matter. Cowpea is an excellent source of calcium, magnesium, potassium, and phosphorus, making it suitable to combine with pearl millet porridge.

Table 3. Total dietary fibre[1], sugar residues, and Klason lignin (content %) in raw materials (Paper II) and composite porridges (Paper III).

Sample Codes	**TDF**	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	**UA**	**Klason l.**
				Paper II				
WPM	7.1^{bc}	1.5^{bc}	1.8^{a}	0.3^{bc}	0.3^{d}	2.1^{b}	0.4^{e}	0.7^{ab}
SPM	6.0^{c}	1.3^{c}	1.4^{a}	0.2^{c}	0.3^{d}	1.7^{b}	0.4^{e}	0.6^{ab}
GPM	7.9^{bc}	1.7^{abc}	1.9^{a}	0.3^{bc}	0.4^{cd}	2.1^{b}	0.5^{d}	0.7^{ab}
FPM	6.8^{bc}	1.5^{bc}	1.7^{a}	0.2^{bc}	0.3^{d}	2.0^{b}	0.4^{de}	0.7^{ab}
WCP	13.5^{a}	2.1^{ab}	1.6^{a}	0.4^{a}	0.7^{a}	5.7^{a}	1.7^{a}	1.1^{a}
SCP	8.0^{bc}	1.9^{abc}	0.6^{b}	0.3^{abc}	0.5^{bc}	2.7^{b}	1.2^{c}	0.5^{b}
GCP	8.6^{b}	2.3^{a}	0.6^{b}	0.4^{ab}	0.6^{b}	2.6^{b}	1.3^{b}	0.5^{b}
FCP	8.5^{b}	2.2^{ab}	0.6^{b}	0.4^{a}	0.6^{b}	2.9^{b}	1.2^{b}	0.4^{b}
				Paper III				
SMSP	7.6	1.5	1.1	0.2	0.4	3.0	0.8	0.5
SMFP	8.4	1.6	1.1	0.3	0.4	3.4	0.8	0.5
SGMSP	7.2	1.5	1.1	0.2	0.4	2.6	0.8	0.4
SGMFP	7.4	1.6	1.1	0.3	0.4	2.6	0.8	0.5
SMSGP	8.0	1.6	1.2	0.3	0.4	3.3	0.8	0.5
SMFGP	7.7	1.6	1.1	0.3	0.4	2.9	0.8	0.5
FMSP	7.3	1.6	1.2	0.3	0.4	2.5	0.8	0.5
FMFP	7.2	1.6	1.1	0.2	0.4	2.4	0.8	0.5
FGMSP	7.3	1.6	1.2	0.3	0.4	2.5	0.8	0.5
FGMFP	7.3	1.7	1.2	0.2	0.4	2.4	0.8	0.5
FMSGP	7.1	1.5	1.1	0.3	0.4	2.4	0.8	0.4
FMFGP	7.2	1.6	1.1	0.2	0.4	2.3	0.8	0.6

Results are presented as a mean of replicate batches on % of dry weight basis. Values within columns followed by different letters are significantly different at $p < 0.05$. Tukey's pairwise comparison was made on average values at a 95% confidence level.

For Paper II two factors (treatments and materials) and an interaction were used for comparison, and for Paper III, four factors (SFM, GM, SFP and GP) were used. SFM is soaked, fermented pearl millet, SFP is soaked, fermented cowpea, GM is germinated pearl millet and GP is germinated cowpea.

For sample codes, see the list of abbreviations, pages 17-18 or Table 1.

1) Total dietary fibre is the sum of fructan, sugar residues, and Klason lignin, except oligosaccharides (DP 3-9).

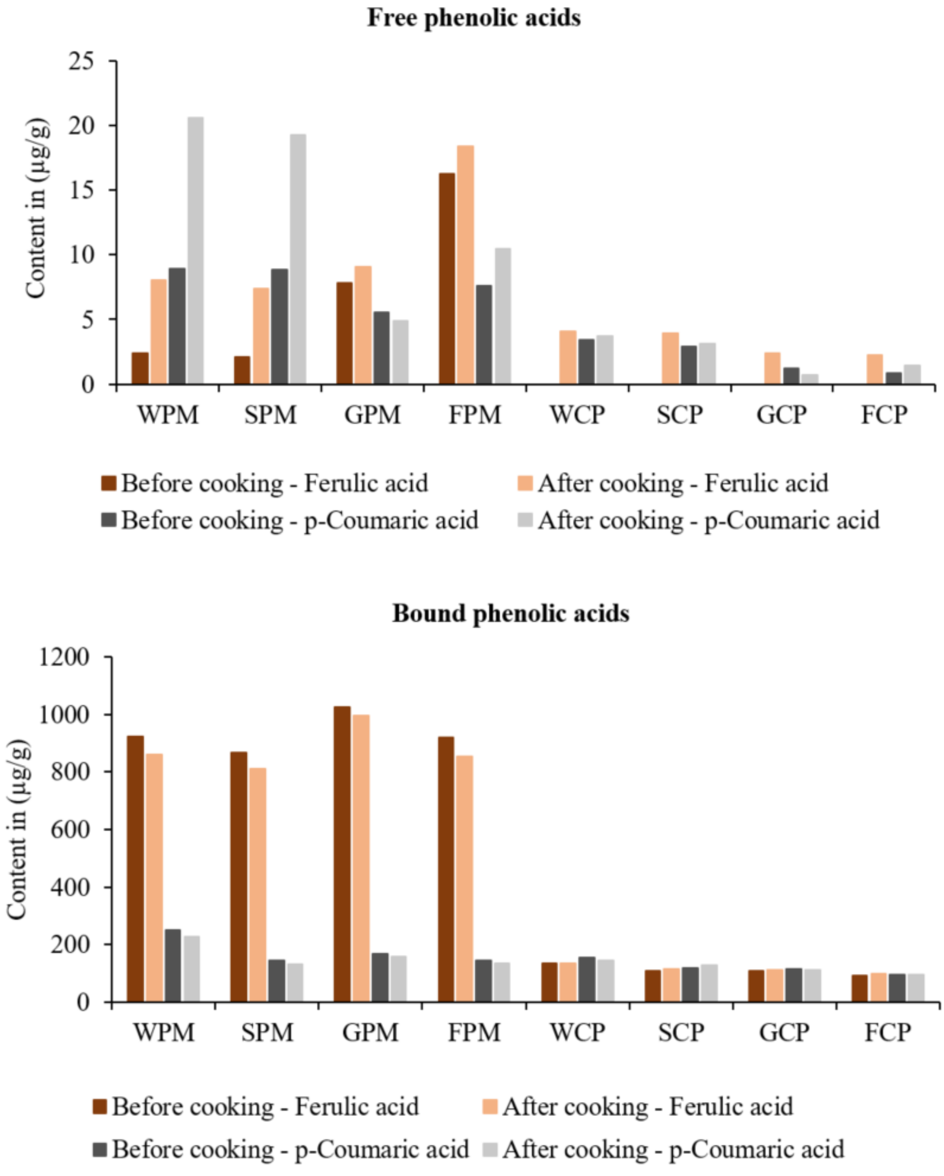
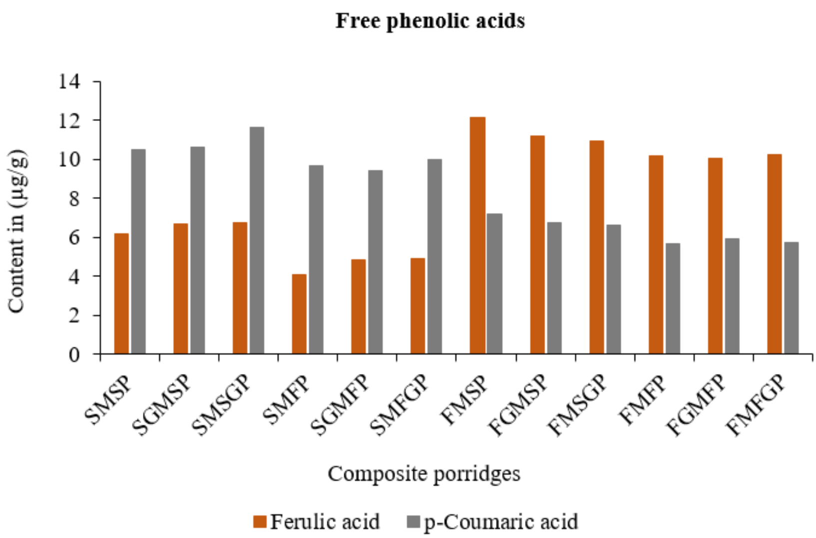


Figure 8. Content of free and bound ferulic and p-coumaric acids in pre-treated raw materials before and after cooking. Free phenolic acids (top panel) and bound phenolic acids (bottom panel). For sample codes, see the list of abbreviations, pages 17-18.



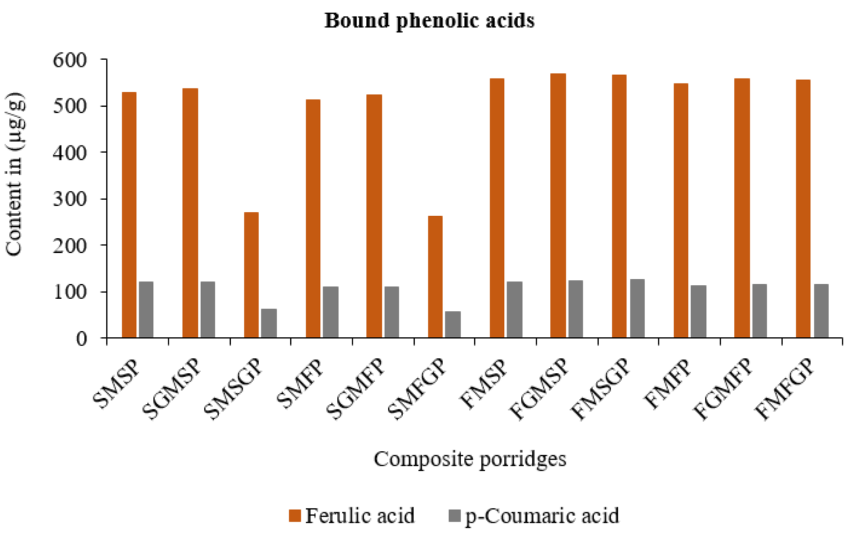


Figure 9. Content of free and bound ferulic and p-coumaric acids in composite porridges. Free phenolic acids (top panel) and bound phenolic acids (bottom panel). For sample codes, see the list of abbreviations, pages 17-18.

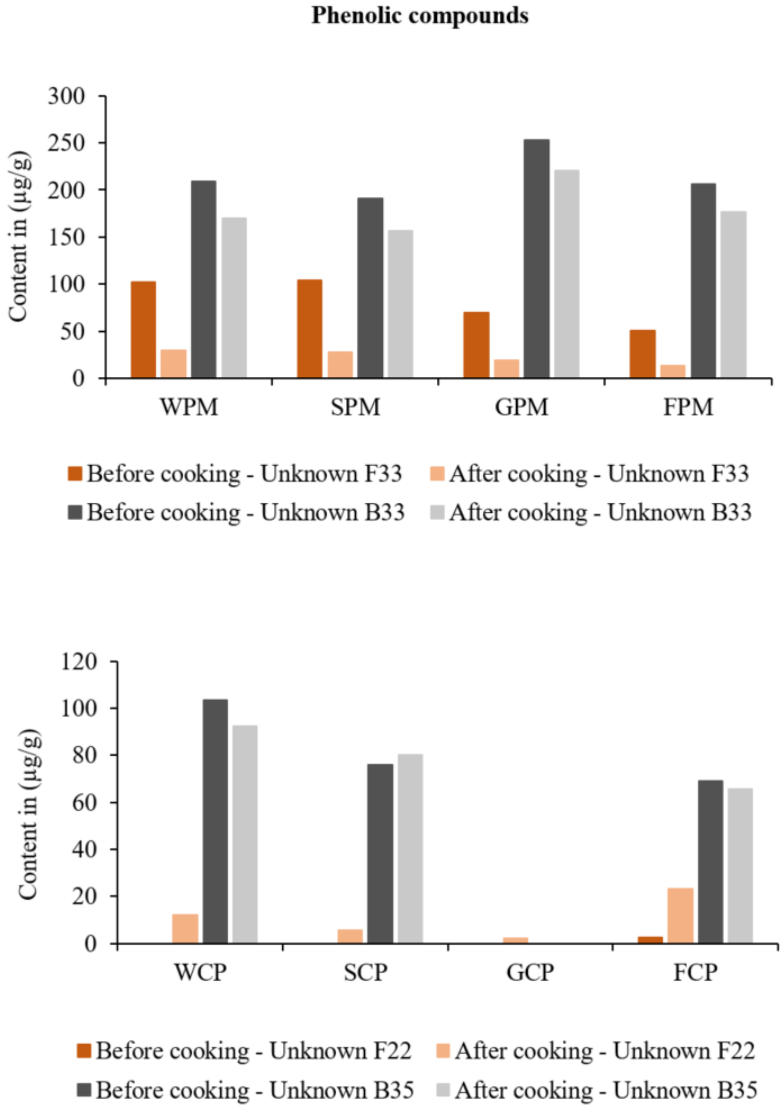


Figure 10. Content in micrograms of gallic acid equivalents per gram (µg GAE/g) of free and bound phenolics before and after cooking in pre-treated raw materials. Pearl millet (top panel) and cowpea (bottom panel). For sample codes, see the list of abbreviations, pages 17-18.

5.5 Rheological behaviour and enzymatic breakdown of the starch in composite porridges

In terms of rheology, the six selected composite porridges made with fermented pearl millet exhibited non-Newtonian shear-thinning behaviour as well as characteristics of viscoelastic material (Paper IV). The flow behaviour is crucial for assessing a product's quality and ensuring that it meets the desired sensory characteristics, particularly for porridges intended for consumption by children. The apparent viscosity of all samples decreased with an increasing shear rate, as illustrated in Figure 11 (top panel). Similar findings have been reported for fermented finger millet porridge by Ojijo & Shimoni (2004) as well as more recent observations for cooked cowpea flour by Kumari & Sit (2024). Shear-thinning behaviour is typical for starch-based food systems (Moriconi et al., 2023; Tsai & Lai, 2021). The ideal flow behaviour of the porridge for feeding children includes moderate viscosity, shear-thinning, and viscoelastic properties. This rheological profile is important for ease swallowing (Cichero, 2017).

Among the six tested samples (Paper IV), composite porridges containing germinated pearl millet consistently showed a lower apparent viscosity compared to the other formulations (Figure 11, light violet and light yellow lines) measured with rheometer. This trend was also observed in their pasting properties measured with RVA, where final viscosity was generally lower in the porridges containing germinated pearl millet (Paper IV). This reduction in viscosity may be due to increased enzymatic activity in germinated pearl millet flour, which facilitates hydrolysis of the starch granules. This can be seen from the scanning electron microscopy images in Paper I or in Figure 3 top panel GPM, where numerous holes and broken starch granules were visible in pearl millet flour, suggesting extremely active α-amylase enzyme. Nkhata et al. (2018) reflected that during traditional germination and fermentation, various endogenous enzymes are activated, and these enzymes break down complex macronutrients into their simple forms which alters the rheological properties.

The six tested samples were further investigated using size exclusion chromatography to analyse the soluble starch fractions in the extractable fractions present in the cooked composite porridges (Paper IV). The chromatographic profiles, illustrated in Figure 12, revealed three distinct starch fractions with varying molecular weight populations. The first population was observed with a molecular weight between 2.4×10^6 and 102×10^6 g/mol. The second population had a molecular weight between 0.8×10^6 and $2.4 \text{ x } 10^6$ g/mol. Lastly, the third population eluted between 0.4×10^6 and 0.8×10^6 g/mol. The starch fractions from all samples ranged in order of decreasing molecular weight from Peak 1 to Peak 3, similarly to the polydispersity values which were also found to decrease (Paper IV).

Polydispersity (Mw/Mn) is an important parameter for describing the variation in molecular weight of the starch; a higher polydispersity value indicates a wider molecular weight distribution, suggesting the presence of more complex polymer components. The polydispersity values of FGMFP (fermented and germinated pearl millet with fermented cowpea) and FGMSP (fermented and germinated pearl millet with soaked cowpea) were higher compared to the other formulations. This suggests a wider distribution of polymer chains lengths in the above-mentioned samples.

Notably, the porridges FGMFP and FGMSP, represented by light violet and light yellow lines, respectively, exhibited higher concentrations of starch fractions compared to the other formulations. This suggests that the addition of germinated pearl millet in the samples enhanced the enzymatic breakdown of starch into smaller fractions and more soluble saccharides. Sharma & Sharma (2022) and Yang et al. (2021) reported that germination of grains activates enzymes that accelerate starch degradation, thereby improving digestibility.

This characteristic is beneficial when preparing porridge for children, as smaller starch fragments are often associated with rapidly digestable starch, which ensures a swift energy gain, a crucial factor for children suffering from PEM. In this study, all six selected composite porridges that were analysed in Paper IV exhibited RDS values.

Based on the multivariate analysis results in Paper IV, we selected three composite porridges made from fermented pearl millet and fermented cowpea, either together or combined with germinated pearl millet or cowpea, such as FMFP, FGMFP, and FMFGP, which displayed higher values of RDS. Further investigation was conducted to identify which composite porridge was the most accepted in Mozambican communities, as detailed in the following section and more comprehensively in Paper V.

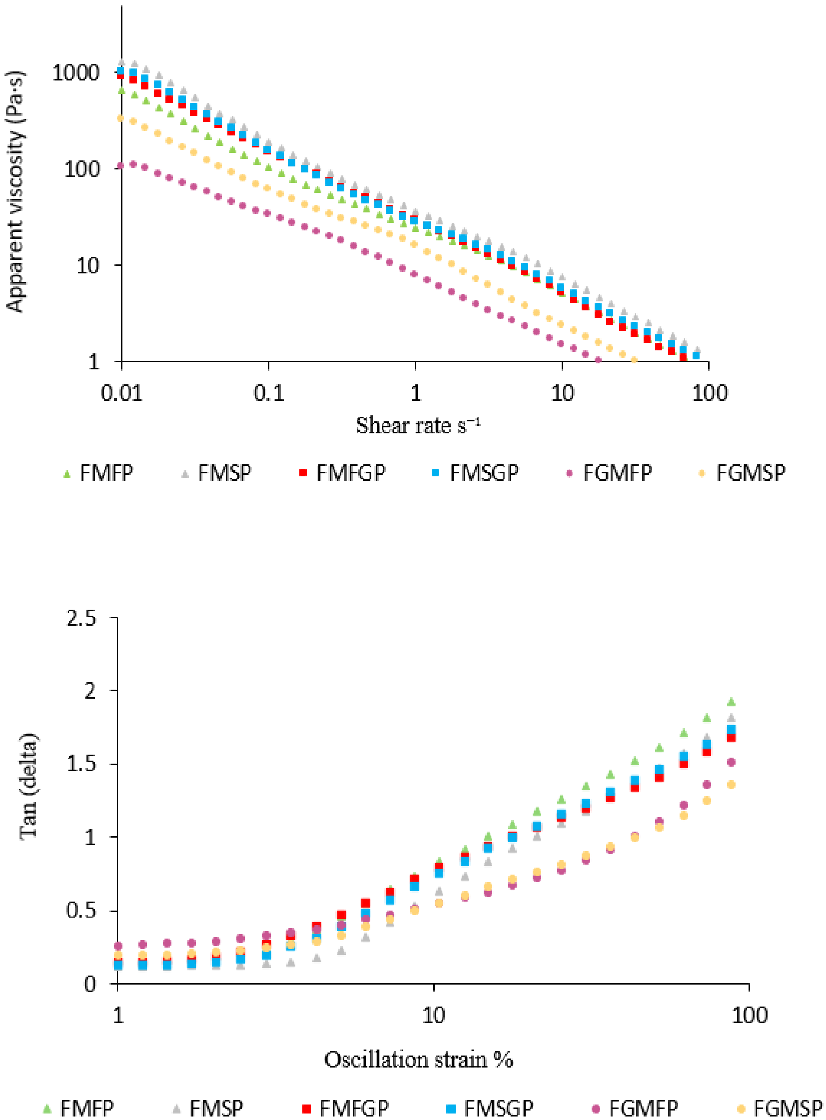


Figure 11. Flow sweep (top panel) and strain sweep (botton panel) in composite porridges. For sample codes, see the list of abbreviations, pages 17-18.

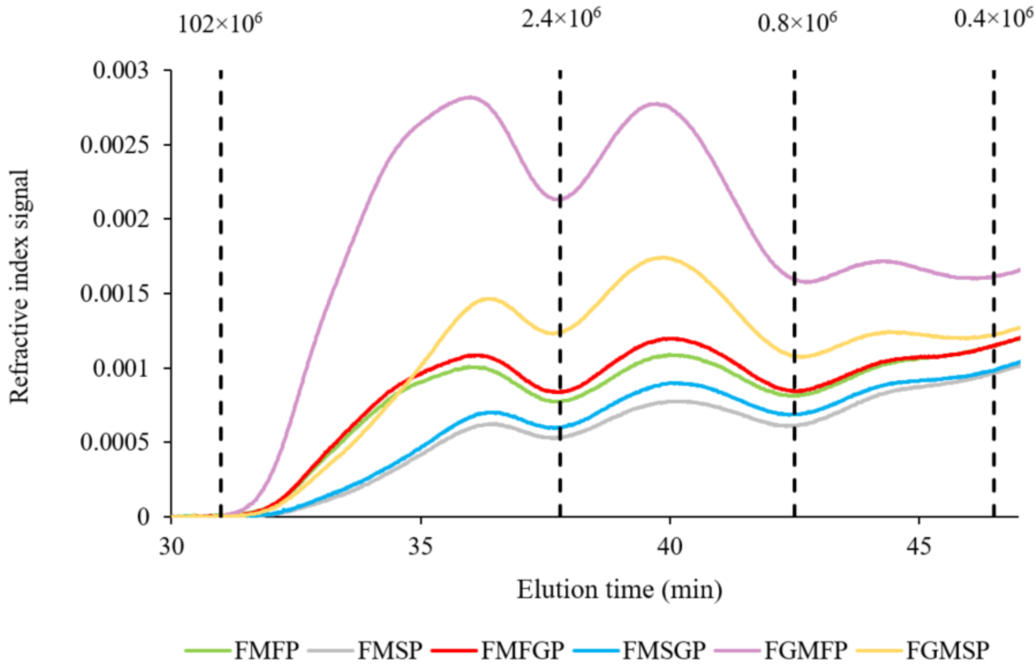


Figure 12. Size exclusion chromatogram of the extractable fractions in composite porridge samples. For sample codes, see the list of abbreviations, pages 17-18.

5.6 Sensory evaluation

Sensory evaluation revealed significant variations among Mozambican mothers for different sensory attributes of composite porridges, as detailed in Paper V. This was particularly the case for overall acceptability, as shown in Figure 13.

Three composite porridges made from fermented pearl millet and fermented cowpea, either together or combined with germinated pearl millet or cowpea such as FMFP, FGMFP, and FMFGP, were evaluated alongside the traditional soaked pearl millet (SM) porridge in three locations (Copuito, Pulupu and Nanguasse) in northern Mozambique (Figure 13). Although SM was very well received by the participant mothers, likely due to their familiarity with pearl millet porridge and cultural acceptance, it exhibited the highest viscosity among porridges. This high viscosity in porridges is not recommended for children under five as it limits gastric capacity and is typically low in energy density. Mosha & Svanberg (1990) and Thaoge et al. (2003) suggested the ideal apparent viscosity for children's porridge is between 1000-3000 cP to provide adequate consistency.

The composite porridge FMFGP, containing fermented pearl millet with fermented and germinated cowpea, was liked by Mozambican mothers and no significant differences in acceptability were observed with SM. Both porridges SM and FMFGP were the most liked across all sensory attributes, appearance, colour, aroma, and taste, in all three locations and this is reported in Paper V.

Among the tested composite porridges, FGMFP containing germinated pearl millet had the lowest final viscosity measured by the STD1 method in RVA and was approximately 600 cP. In comparison, FMFGP, containing germinated cowpea, measured approximately 1800 cP, and FMFP, without germinated samples, measured approximately 2200 cP. The traditional SM porridge exhibited a much higher final viscosity of approximately 4000 cP (see Paper V). The incorporation of germinated flour significantly reduced the viscosity of the porridges. Although FGMFP had the lowest final viscosity, it carried the advantage of allowing for a greater addition of more flour, thus increasing energy density.

Overall, all porridges evaluated by Mozambican mothers were generally well appreciated, likely because of their familiarity with these staple crops which are widely available and affordable in the northern region of Mozambique. The addition of cowpea in the porridges enhances protein quality, although it slightly decreases the sensory appeal. This study demonstrates that composite porridges made from fermented and germinated flours are culturally acceptable and can be consumed by children and have the potential to provide adequate energy density. These porridges could be effectively introduced as a nutritional intervention to help combat child malnutrition in Mozambique.

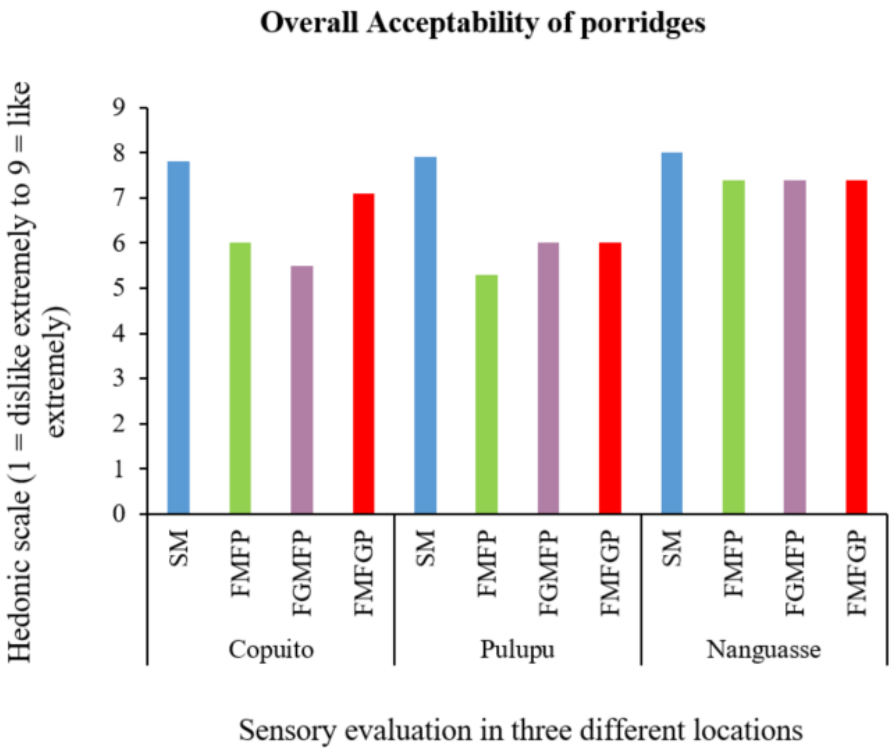


Figure 13. Sensory evaluation of composite porridges in three locations in Mozambique. For sample codes, see the list of abbreviations, pages 17-18.

6. Conclusions

The following conclusions can be drawn from this thesis:

- Traditional pre-treatments significantly altered the microstructure of pearl millet and cowpea. Scanning electron microscopic analysis revealed a structural breakdown in pre-treated raw materials. Germinated pearl millet showed numerous holes and broken starch granules and fermented pearl millet showed slightly irregular loose matrix.
- Germination and fermentation increased the extractability of dietary fibre as well as released free and bound phenolic compounds, specifically ferulic acid, and promoted the formation of new phenolic compounds.
- Composite porridges prepared with germinated pearl millet exhibited a lower final viscosity, which is beneficial for children as it facilitates easier swallowing and allowing for an increase in energy density.
- Composite porridges containing germinated pearl millet exhibited a higher concentration of three extractable starch fractions with different molecular weight and showed higher polydispersity values in the largest starch fractions.
- Composite porridges containing fermented pearl millet showed shear-thinning behaviour, lower levels of phytic acid, resistant starch and, exhibited rapidly digestible starch, which is beneficial for children suffering from chronic malnutrition.
- The best received formulation by Mozambican mothers and described as good porridge for feed their children was the composite porridge made from fermented pearl millet with fermented and germinated cowpea (FMFGP).
- Composite porridges prepared with fermented pearl millet and fermented cowpea, either together or combined with germinated pearl millet or cowpea demonstrated a strong potential for community level adoption and can be used as a nutritional intervention to address child malnutrition in Mozambique.

7. Future work

This thesis explores the effects of traditional pre-treatments on pearl millet and cowpea seeds by using these pre-treated flours to develop composite porridges. The physicochemical characterisation of seed flour and porridges helped to elucidate the modifications induced by pre-treatments, as well as cooking. Sensory studies with mothers helped to identify a suitable porridge formulation from pearl millet and cowpea for children in Mozambique. However, several important questions remain unanswered and thus certain aspects require further investigation:

- Quantification of the amino acid profile and assessment of protein digestibility in the composite porridges.
- Evaluation of the bioavailability of key minerals and vitamins in the composite porridges.
- Characterisation of unknown phenolic compounds present in pre-treated pearl millet and cowpea flour as well as composite porridges.
- Characterisation of microorganisms involved in the traditional fermentation process.
- Assessment of the shelf life of the pre-treated pearl millet and cowpea flours under different storage conditions.
- Exploration into the relationship between rheological properties of composite porridges and their impact on satiety and food intake among children.
- Implementation of composite porridges in community-based nutrition programmes in childhood centres to evaluate the nutritional impact of these porridges in real life settings.

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Popular science summary

Uncovering the nutritional potential of pearl millet and cowpea can transform children's diets, promoting healthier and stronger growth.

Pearl millet and cowpea are two nutritious, affordable, and available grains in northern Mozambique. These grains contain essential nutrients and play an important role in improving children's diets. They are also well-adapted to adverse climate conditions. Pearl millet is considered a promising crop. In recognition of its importance, the Food and Agricultural Organisation (FAO) designated 2023 as the "International Year of the Millets" to raise awareness about the agronomic, nutritional, and socioeconomic benefits of this crop. When pearl millet is combined with cowpea in children's foods, they provide good protein quality, essential nutrients, and bioactive compounds that offer several health benefits. These grains do also contain anti-nutritional factors; however, these compounds can be reduced through traditional processing techniques.

This thesis analysed the effects of traditional pre-treatment methods such as soaking, germination, and fermentation when applied to pearl millet and cowpea seeds, focusing on nutritional and anti-nutritional factors. The resulting flours were then combined into different formulations to identify the most suitable option for children in Mozambique. The results were promising. They showed that composite porridges made with fermented pearl millet were found to have higher levels of rapidly digestible starch, which provides quick energy to children, a crucial factor for those suffering from chronic malnutrition. These porridges also had lower levels of phytic acid, an anti-nutritional compound that can block the body's ability to absorb minerals.

On the other hand, porridges made with germinated pearl millet and cowpea had a lower viscosity, eliminating the need for dilution with water. This helps to increase the energy density of the porridge. Adding cowpea flour also helped to balance the protein profile of the composite porridges, reinforcing their potential as a nutritious and culturally appropriate composite food. Traditional pre-treatments also released compounds known for their antioxidant and anti-inflammatory properties, which are potentially relevant in the prevention of chronic diseases.

In Mozambique, child malnutrition is not always associated with food scarcity, but rather with inadequate feeding practices and limited knowledge

on how to prepare and combine locally available grains. This project highlight the importance of traditional processing techniques and the use of local grains in developing culturally accepted composite porridges with high nutritional value.

In this context, this study contributes to the United Nations Sustainable Development Goals (SDGs), particularly SDG2 (Zero Hunger) and SDG3 (Good Health and Well-being), by reinforcing local and sustainable strategies to combat undernutrition in Mozambique. By incorporating endogenous crops such as pearl millet and cowpea, this approach not only promotes the improvement of children's nutritional status in Mozambique but also supports global recognition of the nutritional and functional importance of these underutilised crops.

Populärvetenskaplig sammanfattning

Upptäck potentialen hos pärlhirs och ögonböna i att förändra barns kost, och främja en hälsosammare och starkare tillväxt.

Pärlhirs och ögonböna är två näringsrika, prisvärda och lättillgängliga grödor i norra Moçambique. De innehåller viktiga näringsämnen och kan spela en betydande roll för att förbättra barns kost. De är dessutom anpassade till ogynnsamma klimatförhållanden. Hirs anses vara en lovande gröda, och den uppmärksammades av FN:s livsmedels- och jordbruksorganisation (FAO) som utsåg år 2023 till ”Internationella hirsåret” för att öka medvetenheten om dess agronomiska, näringsmässiga och socioekonomiska fördelar. När hirs kombineras med ögonböna i barnmat berikas den med ett protein av god kvalitet, samt andra viktiga näringsämnen och bioaktiva ämnen som bidrar till flera hälsofördelar. Även om råvarorna innehåller antinutritionella faktorer kan dessa minskas genom traditionella bearbetningstekniker.

Denna studie analyserade effekterna av traditionella förbehandlingar som blötläggning, groddning och fermentering tillämpade på hirskärnor och ögonbönor, med fokus på både näringsmässiga och antinutritionella faktorer. De resulterande mjölerna kombinerades därefter i olika sammansättningar för att identifiera det mest lämpliga alternativet för barn i Moçambique. Resultaten var lovande och visade att sammansatta grötar med fermenterad hirs innehöll mer snabbt nedbrytbar stärkelse (RDS), vilket ger barn snabb energi - en avgörande faktor för barn som lider av kronisk undernäring. Dessa grötar hade också lägre halter av fytinsyra, ett antinutritionell ämne som kan blockera kroppens upptag av mineraler.

Grötar tillverkade mjöl från groddade råvaror hade en lägre viskositet, vilket eliminerar behovet av utspädning med vatten. Detta bidrar till att öka grötens energitäthet. Tillsats av ögonbönsmjöl bidrog också till att balansera proteinprofilen i gröten, vilket stärker dess potential som ett näringsrikt och kulturellt accepterat komplementärt livsmedel. Traditionella förbehandlingar främjade även frisättningen av föreningar kända för sina antioxidativa och antiinflammatoriska egenskaper, vilket kan vara relevant för förebyggandet av kroniska sjukdomar.

I Moçambique är barnundernäring inte alltid kopplad till livsmedelsbrist, utan snarare till otillräckliga matvanor och begränsad kunskap om hur lokalt tillgängliga sädesslag bäst kombineras och bearbetas. Detta projekt lyfter fram vikten av traditionella bearbetningstekniker och användningen av lokala grödor vid utvecklingen av sammansatta grötar med högt näringsvärde och kulturell förankring.

I detta sammanhang bidrar studien till målen inom Agenda 2030, särskilt mål 2 (Ingen hunger) och mål 3 (God hälsa och välbefinnande), genom att stärka lokala och hållbara strategier för att bekämpa undernäring i Moçambique. Genom att integrera inhemska grödor såsom hirs och ögonböna främjar detta tillvägagångssätt inte bara förbättrad näringsstatus bland barn i Moçambique, utan också globalt erkännande av den näringsmässiga och funktionella betydelsen hos dessa underutnyttjade grödor.

Resumo de divulgação científica popular

Conheça o potencial nutricional da mexoeira e do feijão nhemba na transformação da alimentação infantil, promovendo um crescimento mais saudável e fortalecido.

A mexoeira e o feijão-nhemba constituem grãos de elevado valor nutricional, acessíveis e disponíveis na região norte de Moçambique. Esses grãos destacam-se pelo seu teor de nutrientes essenciais e desempenham um papel importante na melhoria da alimentação infantil. São culturas adaptadas a adversas condições climáticas. A mexoeira é uma cultura alimentar promissora, a Organização das Nações Unidas para a Alimentação e Agricultura (FAO) designou o ano de 2023 como o “Ano Internacional dos Milhetos”, com o intuito de promover a conscientização acerca dos benefícios agronômicos, nutricionais e socioeconómicos dessa cultura. Quando a mexoeira é combinada com o feijão-nhemba na formulação de alimentos para crianças, contribuem com proteínas de elevada qualidade, nutrientes essenciais e compostos bioativos com potenciais benefícios à saúde. Embora apresentem fatores antinutricionais, estes podem ser substancialmente reduzidos mediante a aplicação de técnicas tradicionais adequadas de processamento.

Esta pesquisa analisou os efeitos de pré-tratamentos tradicionais, como imersão, germinação e fermentação aplicados às sementes de mexoeira e feijão-nhemba, com foco em fatores nutricionais e antinutricionais. As farinhas resultantes desses processos foram posteriormente combinadas em diferentes formulações, com o objetivo de identificar a mais adequada à alimentação infantil em Moçambique. Os resultados foram promissores, demonstraram que as papas compostas contendo mexoeira fermentada apresentaram alto teor de amido de digestão rápida, característica nutricionalmente desejável na alimentação infantil, por proporcionar rápida disponibilidade energética. Estas papas, também apresentaram menores níveis de acido fitíco, um composto antinutricional que impede a absorção de minerais pelo organismo.

Por outro lado, as formulações com farinhas germinadas mostraram uma redução da viscosidade, sem necessidade de diluição com água, favorecendo, assim, o aumento da densidade energética da preparação. A inclusão da farinha de feijão-nhemba contribuiu para o equilíbrio do perfil proteico das papas compostas, reforçando seu potencial como alimento complementar nutritivo e culturalmente apropriado. Os pré-tratamentos também favoreceram a liberação de compostos reconhecidos por suas propriedades antioxidantes e anti-inflamatórias, com potencial relevante na prevenção de doenças crônicas.

No contexto moçambicano, a desnutrição infantil não está, necessariamente, associada à escassez de alimentos, mas sim a práticas alimentares inadequadas e à limitada compreensão sobre o processamento e a combinação adequada de grãos disponíveis localmente. Esta pesquisa ressalta a relevância de técnicas tradicionais de processamento e o uso de grãos localmente disponíveis na formulação de papas compostas de alto valor nutricional e culturalmente aceites.

Nesse contexto, este estudo contribui para a Agenda dos objectivos de Desenvolvimento Sustentável (ODS), em especial o ODS 2 (Fome Zero) e o ODS 3 (Saúde e Bem-Estar), reforçando estratégias locais e sustentáveis de combate à desnutrição em contextos vulneráveis. Ao incorporar culturas endógenas como a mexoeira e o feijão-nhemba, promove-se não apenas a melhoria do estado nutricional infantil em Moçambique, mas também o reconhecimento global da importância nutricional e funcional dessas espécies alimentares subutilizadas.

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Effect of pre-treatment on physicochemical, microstructural and pasting properties of pearl millet and cowpea

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ABSTRACT

The effects of soaking, germination and fermentation on physicochemical, microstructural and pasting properties of improved and drought-tolerant Mozambican varieties of pearl millet and cowpea were investigated.

Total starch content in both germinated pearl millet (GPM, 67.2 % of DM) and germinated cowpea (GCP, 47.4 % of DM) was found to be affected by germination, which consequently affected the amylose content of GPM and GCP. Morphological and pasting properties of pearl millet starch granules were altered by germination, with numerous holes and broken starch granules developing, leading to a drastic reduction in final viscosity (4 mPa.s). Cowpea starch granule structure was not markedly affected by pre-treatment, but peak (310 mPa.s) and final viscosity (196 mPa.s) were decreased by germination. Cowpea flour had smaller particle size distribution than pearl millet, but no significant differences in the flour were observed after pre-treatment (soaking, germination, fermentation). Therefore, these simple, low-cost and culturally acceptable treatments can be used to alter technical functionality and improve the nutritional benefits of flour, e.g. different pre-treatments of pearl millet and cowpea could be used to develop food products with high energy density and acceptable sensory profile such as porridge for undernourished children in low and middle-income countries.

1. Introduction

Pearl millet and cowpea are well-known seeds cultivated in many countries worldwide and contribute significantly to African food security. In Mozambique, these seeds play an essential role in the diet due to their high nutrient content and potential value as an affordable source of protein. The protein in pearl millet has a balanced amino acid profile and a high methionine content, which makes it an excellent complement to legumes. Overall, pearl millet seeds are nutritionally comparable or even superior to cereals such as wheat and rice (Rai, Gowda, Reddy, & Sehgal, 2008). Cowpea is a plant protein source that could be used to enrich infant cereal food in low and middle-income countries in Africa or Asia. Its high lysine content makes it an excellent complement to cereal seeds to enhance protein quality in the human diet. Cowpea is therefore one of the most important legumes in Africa (Prinyawiwatkul, McWatters, Beuchat, Phillips, & Uebersak, 1996). In recent years, pearl millet and cowpea have attracted attention due to their capacity to grow in harsh climate conditions and potential nutritional benefits. Therefore, they are an excellent choice for incorporation into children's diets to alleviate protein-energy malnutrition in climate-vulnerable countries such as Mozambique (Goudar et al., 2023; Kapravelou, Martínez, Martino, Porres, & Fernández-Fígares, 2020; Almeida-Dominguez, Serna-Saldivar, Gomez, & Rooney, 1993).

The use of simple, affordable and culturally acceptable pre-treatments, such as soaking, germination and fermentation, are common at a household level in many low-middle-income countries like Mozambique before milling to soften the seeds and to enhance their nutritional value. These pre-treatments reduce the levels of anti-nutritional factors and improve digestibility and sensory characteristics (Nkhata, Ayua, Kamau, & Shingiro, 2018; Oladiran & Emmambux, 2022). Pearl millet and cowpea seeds are usually soaked for different food preparations (Henshaw, McWatters, Oguntunde, & Phillips, 1996; Ocheme & Chinma, 2008). This soaking process activates endogenous enzymes, such as alpha- and beta-amylase, and some water-soluble components leach out into the soak water. Germination pre-treatment increases the activity of hydrolytic enzymes, which break down

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starch, protein and cell-wall polysaccharides. Germination of seeds effectively reduces the viscosity of thick porridges without dilution with water, and thus maintains high energy density (Alexander, 1983; Mosha & Svanberg, 1990; Ocheme & Chinma, 2008). In fermentation pre-treatment, microorganisms and enzymes produced by the natural microflora alter the composition of seed components, thus improving the nutritional value and altering the microstructure, leading to improved digestibility and texture (Nout & Ngoddy, 1997). The study is of particular interest because there is currently a lack of detailed information on the effects of these pre-treatments on the physicochemical, microstructural and pasting properties of Mozambican varieties of pearl millet and cowpea. In Mozambique, the changara variety of pearl millet and the variety 10 of cowpea are among the highly recommended, improved and locally available varieties due to their adaptability to volatile climatic conditions like drought. These varieties are high-yielding which contributes to food and nutritional security (Intsormil, 2012; Huynh et al., 2013). A better understanding of the chemical and physical modifications made to these seeds by pre-treatments such as soaking, germination and fermentation is therefore essential to successfully develop food products with high nutritional value, better texture and sensory acceptability for undernourished children in Mozambique.

This initial study aimed to determine the effects of seed pre-treatment (soaking, germination and fermentation) on physicochemical, microstructural and pasting parameters, proximate composition, starch and amylose content in pearl millet and cowpea. The overall goal was to gain better insights into the physicochemical attributes of different flour samples, produced by simple processing steps, intended for use in developing nutritional porridge products for children in Mozambique.

2. Materials and methods

2.1. Sample preparation and pre-treatments

Pearl millet (*Pennisetum glaucum*) variety Changara and cowpea (*Vigna unguiculata L.*) variety 10 were acquired from the Mozambican Agricultural Research Institute in Montepuez, Cabo Delgado. The samples were divided into two batches (800 g each), and sorted and cleaned, with damaged grains, stones and all other extraneous material removed and discarded. Each batch was then divided into four 200-g portions for four treatments (washing, soaking, germination and fermentation).

In the washing pre-treatment, the seeds were washed quickly for 10 s in tap water to remove dust, followed by drying in an oven with fan (Memmert, Germany) at 40 °C for 24 h. In the soaking pre-treatment, the seeds were steeped in tap water at room temperature (21 ± 2 °C) for 10 min and then dried. In the germination pre-treatment, the seeds were soaked in tap water at room temperature for 24 h, in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, and then spread on germinator trays at 30 ± 1 °C in an oven without fan. Seeds were sprayed with water twice a day and removed from the oven after 48 h of germination. The sprouted seeds were washed for 10 s in tap water and dried in an oven with fan, after which dried rootlets were removed by rubbing between the hands. In the fermentation pre-treatment, the seeds were soaked in tap water at room temperature (21 ± 2 °C), in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, and allowed to ferment by the action of endogenous microflora at 30 ± 1 °C for 72 h in an oven without fan. The seeds were then washed for 10 s in tap water, dried in an oven with fan.

The seeds were pre-milled using a Tecator machine (Cemotec, Sweden) to break them into small pieces. The washed samples were further milled with the entire hull. In the soaking, germination and fermentation pre-treatments, the seeds were partially dehulled following the Mozambican tradition, which consists of spreading the pre-milled seeds on a tray and removing the hulls using compressed air, and then milling the remaining seed components. All samples were milled using a laboratory cyclone sample mill (Retsch, Haan, Germany) to pass through a sieve with mesh 0.5 mm.

These pre-treatment methods were inspired by methods described by Adebiyi, Obadina, Mulaba-Bafubiandi, Adebo, and Kayitesi (2016), Griffith and Castell-Perez (1998), Ibrahim, Habiba, Shatta, and Embaby (2002) and Ocheme and Chinma (2008), with minor modifications relating to duration of drying for the materials and length for fermentation for cowpea, in order to standardise the treatments and make them easily replicable in practice in Mozambican communities.

The milled samples were stored in polyethylene zipper plastic bag at room temperature (21 ± 2 °C) before analysis. Samples from the two original batches of each seed type were used to prepare replicates for all treatments. Each treatment was analysed at least in duplicate and results are reported as mean values of repeats.

2.2. Proximate composition analysis

Dry matter (DM) content was determined by drying for 16 h at 105 °C according to AACC method 44-15A (2000). Total dietary fibre was quantified according to the Uppsala method (Theander, Aman, Westerlund, Andersson, & Petersson, 1995), further modified for separate analysis of extractable and unextractable dietary fibre components (Andersson, Merker, Nilsson, Sørensen, & Åman, 1999). Crude protein content was measured by the Kjeldahl method, according to the Nordic Committee on Food Analysis (1976), using a 2520 digestor, Kjeltec 8400 analyser unit and 8460 sampler unit (all from Foss, Denmark). Protein content was calculated from nitrogen content (N x 6.25). Fat content was determined according to EU Commission Directive 152/2009 EC (2009) using a Hydrotec 8000 and Soxtec 8000 extraction unit (both from Foss, Denmark). Ash was determined by incineration in an oven at 600 °C for 3 h.

2.3. Total starch and amylose content

The starch content in flours was determined by selective hydrolysis with thermostable α-amylase and amyloglucosidase (Åman, Westerlund & Theander, 1994). The amount of glucose released was quantified using kit K-GLUC (GOPOD: glucose oxidase/peroxidase) from Megazyme (Bray, Ireland). Amylose was measured using a colorimetric method based on iodine complex formation stabilised with trichloroacetic acid (Chrastil, 1987). The amylose content in flour samples was determined based on a standard curve developed using waxy barley and barley starch (Lyckeby, Kristianstad, Sweden) with varying amylose level (0%, 16%, 32% and 48%). The absorbance value was read against water at 620 nm. The results are reported as mean percentage of total starch content.

2.4. Morphological properties

Pearl millet and cowpea flour were analysed using scanning electron microscopy (SEM) (Hitachi TM-1000, Tokyo, Japan) with 2500X magnification (30 μm scale). Samples were mounted on aluminium stubs and spray-coated with a thin film of carbon using a carbon coater (Cressington Scientific Instruments, Sputter coater-108 auto, Watford, UK). After coating, the samples were transferred to the SEM specimen chamber and subjected to an electron beam under vacuum.

2.5. Particle size distribution

Particle size distribution of the flours was determined by sieve fractionation using an AS 200 shaker (Retsch, Haan, Germany). Each sample (50 g) was sifted with a set of five graded sieves (425, 250, 150, 75 and 50 μm) and a collection pan. Fractionation was carried out at 1.5 mm amplitude for 10 min with 10 s sieving intervals to ensure complete fractionation.

2.6. Pasting properties

Pasting properties were determined by a rapid visco analyser (RVA) (Newport Scientific, Australia), using a 3 g sample of flour dispersed in 25 mL deionised water in an aluminium canister. The samples were heated from 50 °C to maximum temperature 95 °C and held at 95 °C for 2.5 min, before cooling to 50 °C, using the general standard method (Std1). Pasting temperature, peak viscosity, peak time, breakdown, strength, setback and final viscosity were recorded.

2.7. Statistical analysis

All analyses were carried out on the two replicate batches used for the pre-treatments. Statistical analyses of data were performed using the statistical software Minitab version 19.2. Analysis of variance (ANOVA) was performed using the general linear model procedure, with pre-treatment, seed types and their interaction as factors. Tukey's comparison test was used to identify significant differences between group means, with significance level set at 95% confidence level.

Multivariate analysis of the data obtained in the study was applied separately for pearl millet and cowpea, to assess the effect of the pre-treatments. Principal component analysis (PCA) score and loadings plots were used to visualise differences between batches, and differences and relationships between variables. These analyses were performed using the software SIMCA 17 (Sartorius Stedim Data Analytics AB).

3. Results and discussion

3.1. Proximate composition

Cowpea samples contained nearly twice as much crude protein (23.6–27.9% of DM) as pearl millet samples (12.1–12.9% of DM) (Table 1). The protein in cowpea is rich in lysine, but low in methionine, so combining cowpea with pearl millet, which is rich in methionine, can result in a porridge with high protein quality suitable for undernourished children (Prinyawiwatkul et al., 1996). Germinated cowpea had a significantly higher protein content than soaked cowpea (Table 1), which is in agreement with previous findings (Ghavidel & Prakash, 2007). During germination, carbohydrates stored in the endosperm are consumed by the plant embryo to provide energy for cellular processes, which leads to DM losses due to carbon dioxide and water incorporation in growing shoots and roots. This process may have contributed to the observed increase in protein content in cowpea. In contrast, Jirapa, Normah, Zamaliah, Asmah, and Mohamad (2001) found no significant differences in protein content in cowpea after germination, although their germination treatment had a significant effect on in vitro protein digestibility. Pre-treatment by fermentation significantly increased the protein content in cowpea (Table 1), again possibly as a result of loss of dry matter (mainly carbohydrates), which fermentative microorganisms consume as a source of energy. In contrast, Giami (1993) observed only a non-significant increase in crude protein content of cowpea after 72 h of fermentation.

The crude protein content in pearl millet samples did not show any significant variation between the different pre-treatments (Table 1). Thus, germination and fermentation of pearl millet did not increase the protein content, whereas some previous studies have found that these pre-treatments can improve protein digestibility due to breakdown of complex proteins into more soluble peptides (Annor, Tyl, Marcone, Ragaee, & Marti, 2017; Elkhalifa & Bernhardt, 2010). Adebiyi, Obadina, Adebo, and Kayitesi (2017) and Sade (2009) observed an apparent increase in the protein content of germinated and fermented pearl millet seed.

In the present study, the total fat content was significantly higher in pearl millet samples (5.9–6.6% of DM) than in cowpea samples (2.3–2.5% of DM) (Table 1). However, pre-treatment by soaking, germination or fermentation did not affect the fat content in either pearl millet or cowpea, which is in agreement with previous findings by Sade (2009) for germinated and fermented pearl millet. For cowpea, however, Jirapa et al. (2001) observed a significant decrease in total fat content after 48 h of germination. The total fat content in pearl millet is much higher than that in major cereal crops like rice, wheat and maize, improving the energy value (Rai et al., 2008).

Table 1
Total dietary fibre, crude protein, crude fat, ash and starch content (% of dry matter (DM)) and amylose content (% of starch) in pearl millet and cowpea subjected to different pre-treatments[1].

Pre-treatment	Total dietary fibre [2]	Crude protein (N x 6.25)	Crude fat	Ash	Total starch	Amylose (% of starch)
Pearl millet						
Washed	7.0 ± 1.1[bc]	12.8 ± 0.1[d]	6.0 ± 0.4[a]	1.3 ± 0.1[c]	71.0 ± 0.5[a]	32.1 ± 1.4[ab]
Soaked	6.0 ± 0.3[c]	12.7 ± 0.02[d]	6.1 ± 0.2[a]	1.3 ± 0.0[c]	69.9 ± 0.2[ab]	33.1 ± 0.6[ab]
Germinated	7.7 ± 0.3[bc]	12.1 ± 0.2[d]	5.9 ± 0.2[a]	1.0 ± 0.1[cd]	67.2 ± 0.7[b]	31.5 ± 0.4[b]
Fermented	6.8 ± 0.4[bc]	12.9 ± 0.3[d]	6.6 ± 0.3[a]	0.9 ± 0.0[d]	70.6 ± 0.1[a]	33.6 ± 1.0[ab]
Cowpea						
Washed	13.5 ± 1.1[a]	23.6 ± 0.0[c]	2.4 ± 0.4[b]	3.4 ± 0.0[a]	44.6 ± 0.6[e]	33.6 ± 0.7[ab]
Soaked	7.9 ± 0.4[bc]	24.7 ± 0.2 [c]	2.3 ± 0.2[b]	3.5 ± 0.0[a]	48.1 ± 0.1[d]	36.2 ± 0.0[ab]
Germinated	8.3 ± 0.0[bc]	27.9 ± 0.6[a]	2.5 ± 0.3[b]	3.4 ± 0.2[a]	47.4 ± 1.8[de]	32.5 ± 3.1[ab]
Fermented	8.4 ± 0.2[b]	26.3 ± 0.4 [b]	2.3 ± 0.1[b]	2.9 ± 0.1[b]	51.9 ± 0.2[c]	36.9 ± 0.8[a]

Values within columns followed by different letters are significantly different at $p < 0.05$ in Tukey's pair-wise comparison of pre-treatments and seed types.
1) Mean value ± standard deviation of two replicates on % of dry weight basis, except for amylose.
2) Sum of extractable and unextractable fractions, except oligosaccharides (DP 3–9).

Ash content in pearl millet samples (0.9–1.3% of DM) was significantly lower than that in cowpea samples (2.9–3.5% of DM) (Table 1). In both cases, fermentation of samples significantly decreased the ash content compared with soaked samples, possibly due to leaching of soluble inorganic salts during fermentation. Similar results have been reported by Adebiyi et al. (2017) and Sade (2009) for fermented pearl millet, and by Onweluzo and Nwabugwu (2009) for fermented pigeon pea flour. In the present study, there was a slight decrease in ash content of germinated samples of pearl millet and cowpea compared with soaked samples, but the differences were not statistically significant.

Total dietary fibre content in pearl millet and cowpea was calculated as the sum of extractable and unextractable dietary fibre. Pre-treatment and seed type both had statistically significant effects on total dietary fibre (TDF) content, with cowpea samples having significantly higher TDF levels (7.9–13.5% of DM) than pearl millet (6.0–7.7% of DM) (Table 1). Washed cowpea seeds had a significantly higher TDF content than soaked, germinated or fermented cowpea. This might be due to presence of the hulls, since washed cowpea was milled as whole grains while seeds used for other pre-treatment were dehulled, indicating that dehulling significantly affected the TDF content. No significant differences were observed in TDF content in pearl millet. Similarly, Griffith and Castell-Perez (1998) found that grain decortication significantly reduced the TDF content of cowpea, but not pearl millet.

In comparison with the soaking pre-treatment, germination and fermentation tended to increase the TDF content in both cowpea and pearl millet (Table 1). Veena, Urooj, and Puttaraj (1995) and Benítez et al. (2013) observed similar changes after germination and fermentation of cowpea, while Sharma, Saxena, and Riar (2016) observed similar changes after germination of barnyard millet. This increase in TDF content may be because of enzymatic breakdown of cell-wall polysaccharides during seed germination or fermentation.

3.2. Total starch content and amylose

Starch is an essential component of cereals and legumes used in the human diet. Total starch content was significantly higher in pearl millet (67.2–71.0% of DM) than in cowpea (44.6–51.9 % of DM) (Table 1). Total starch content in both germinated pearl millet and cowpea was slightly lower than in the soaked samples, possibly due to breakdown of starch granules by α-amylase, the principal enzyme activated during germination. It is known that the germination process is responsible for activating enzymatic activity in sprouted seeds, resulting in breakdown of carbohydrates into simpler forms. Similarly, in previous studies, germination of seeds has generally been found to decrease starch content (Elkhalifa & Bernhardt, 2010; Griffith & Castell-Perez, 1998; Veena, Urooj, Puttaraj, 1995; Yang et al., 2021).

Total starch content in fermented pearl millet (70.6% of DM) showed no statistically significant differences compared with soaked pearl millet (69.9% of DM) (Table 1). In contrast, Khetarpaul and Chauhan (1990) observed a decrease in starch content in pearl millet during fermentation in a study where pure cultures were used for fermentation. On the other hand, total starch content in fermented cowpea (51.9% of DM) was significantly higher than in soaked cowpea (48.1% of DM) (Table 1). The higher protein content in fermented cowpea might have protected the starch granules surface for enzymatic action due to reduced starch swelling. A study by Zhu, Liu, Sang, Gu, and Shi (2010), reported after the protein matrix is disrupted, the starch granules become less rigid and susceptible to enzymatic attack. However, Veena et al. (1995) found no significant differences in total starch content in cowpea after fermentation.

Amylose comprised 33.1% of the total starch content in soaked pearl millet and 36.2% of total starch in soaked cowpea (Table 1). A study by Badi, Hoseney, and Finney (1976) reported much lower amylose content in pearl millet starch (17% of total starch). The amylose content in pearl millet in the present study ranged from 32.1 to 33.6 %, which is a high level compared with some cereal starches (Emmambux & Taylor, 2013). Different varieties, seed origins, crop growing conditions and estimation methods may explain these differences. A study by Faki, Desikachar, Paramahans, and Tharanathan (1983) and Wani et al. (2016) reported the amylose content in cowpea starches ranged from 25.8 to 33% of total starch, which is slightly lower than in the present study. Legume starch has a relatively high amylose/amylopectin ratio compared with cereals (Hoover & Sosulski, 1985).

The pre-treatments tested in this study showed no significant effects on amylose content of cowpea and pearl millet. However, the amylose content in germinated pearl millet and cowpea samples decreased slightly compared with soaked samples (Table 1). Yang et al. (2021) reported similar results for germinated proso millet. The slight decrease in amylose after germination may have been caused by an increase in α-amylase activity and enzyme hydrolysis of macromolecules such as amylose and amylopectin during germination, leading to production of small molecules such as dextrin, maltose and oligosaccharide (Li, Oh, Lee, Baik, & Chung, 2017). According to Frias, Fornal, Ring, and Vidal-Valverde (1998), the digestibility of starch may improve with decreasing amylose in germinated samples.

3.3. Scanning electron microscopy

The SEM images showed different morphological properties of pearl millet and cowpea starch granules (Figs. 1 and 2). Pearl millet starch granules ranged in diameter from 6 to 12 μm and were round in shape, irregular and polygonal with several faces. Cowpea starch granules ranged in diameter from 12 to 24 μm and were oval or spherical in shape. Similar findings have been reported by Badi et al. (1976) and Faki et al. (1983) for pearl millet and cowpea starch, respectively.

From the SEM images, it was evident that washed and soaked pearl millet starch granules had similar morphology, with intact starch granules visible in both cases. Thus short-duration soaking (10 min) did not affect the morphology of the starch granules. On the other hand, the germination pre-treatment clearly affected pearl millet starch granule morphology, resulting in numerous holes and broken starch granules. In addition, the layers became apparent after germination, due to attack by amylases (Fig. 1, panel GPM). Some previous studies have also observed pores in starch granule in cereal grains after 48 h of germination, as a result of enzymatic degradation (Adebiyi et al., 2016; Li, Oh, Lee, Baik & Chung, 2017). It has been suggested that amylases penetrate the granules during germination and hydrolyse from the hilum region towards the outside (Krishna & Thayumanavan, 1998). However, the SEM images of germinated cowpea showed no holes in the granules (Fig. 2, panel GCP), probably due to low enzyme activity and higher protein content protecting the starch granules from damage (Zhu et al., 2010). Similar findings have been reported previously for starch granules in cowpea (Faki et al., 1983). Hoover and Sosulski (1985) postulated that the degree of attack by α-amylase also depends on factors such as granule size, amylose/amylopectin ratio, degree of crystallinity and degree of polymerisation.

A slightly irregular, loose matrix was apparent in fermented pearl millet (Fig. 1, panel FPM), probably due to enzymatic attack, whereas this type of irregular, loose matrix feature was less visible in fermented cowpea (Fig. 2, panel FCP). During fermentation, water uptake by the seed makes the starch granules smoother and loosely embedded in the protein matrix, microstructural differences that were more clearly visible in pearl millet than in cowpea. According to Lineback and Ponpipom (1977), pearl millet has a very active α-amylase system.

3.4. Particle size distribution

Fig. 3 shows particle size distribution (PSD) retained in each sieve, with values expressed as percentage of the total. Pearl millet flour retained significantly larger particle sizes (≥150 μm mesh size) than cowpea. The most significant percentage of fine particles was observed in cowpea flour, with most particles retained in the sieve with <150 μm mesh size. Similarly, Griffith and Castell-Perez (1998) observed very fine particle size in cowpea flour, with high percentages in a <74 μm sieve and the collection pan.

Large particle size distribution influences the resistance of starch granules to expand and rupture. According to Kerr, Ward, McWatters, and Resurreccion (2000), milling conditions and particle size influence the functional properties of the flour, as do type and amount of material extracted by water. In the present study, there were differences in particle size distribution depending on seed type, seed hardness and pre-treatment. Pre-treatments such as soaking, germination and fermentation are often used in Mozambique to soften the seed and facilitate the milling process. Milling can be done manually or using a commercial roller mill, which gives different particle sizes. Griffith and Castell-Perez (1998) found that germination of pearl millet and cowpea influenced the particle size distribution, with germinated flours producing a higher percentage of particles that passed through the sieve 210 μm mesh size. In the present study, no significant differences were observed between pre-treatments in pearl millet and cowpea. However, germinated and fermented pearl millet flour produced a higher percentage of particles that passed through in the sieve 150 and 75 μm mesh size and cowpea flour produced a higher percentage of particles that passed through in the sieve 50 and < 50 μm mesh size (Fig. 3). As suggested by Griffith and Castell-Perez (1998), all samples were

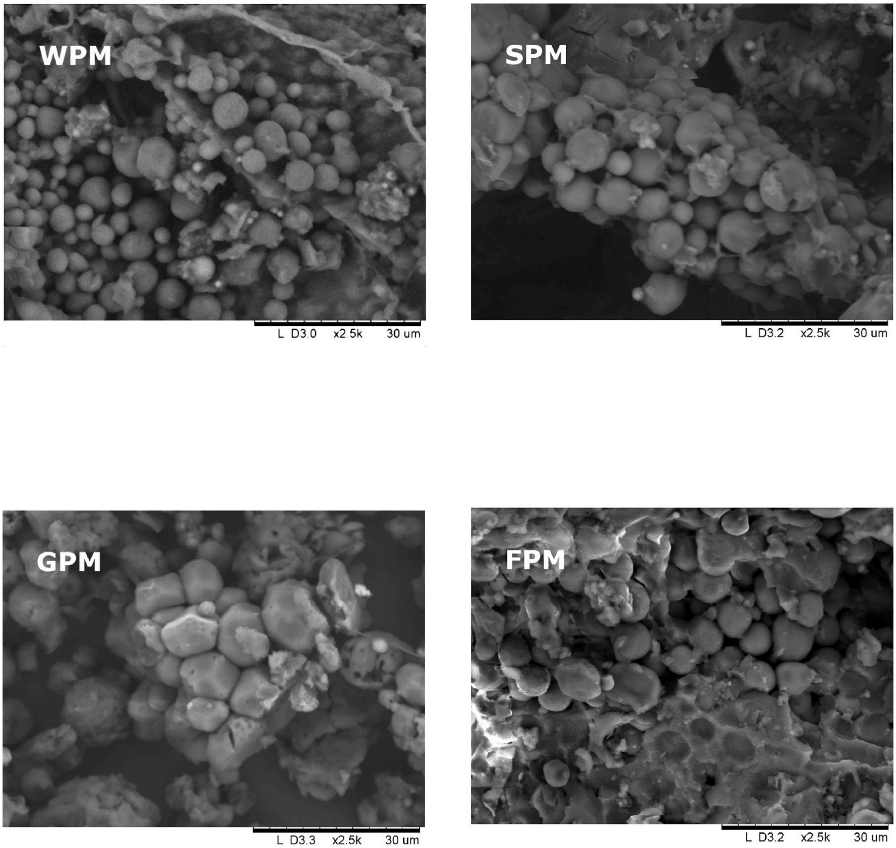

Fig. 1. SEM images of starch granules in pearl millet after different pre-treatments: WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet.

classified as fine because they passed through the sieve 425 μm mesh size, which is reported to be a good particle size range to prepare complementary porridge with desirable viscosity values for undernourished children. Viscosity is an important characteristic in children's foods, as children and babies have less developed oral motor skills.

3.5. Pasting properties

The pasting properties of pearl millet and cowpea flours were assessed based on pasting graphs (Fig. 4), which provided information on the viscosity of the flour in water. The pasting characteristics of starches are affected by amylose and amylopectin content and by the arrangement of these in the starch granule. In pearl millet, the highest viscosity (2773 mPa.s) was observed in soaked samples, followed by washed samples (2488 mPa.s) (Fig. 4A). The highest peak viscosity (1387 mPa.s) in cowpea was observed in fermented samples (Fig. 4B). The high peak viscosity of the pearl millet samples indicated high swelling power of the starch granules and their resistance to shear. However, soaked cowpea showed no peak in viscosity, but rather a gradual rise over time. Hoover and Sosulski (1985) reported a similar pattern of peak viscosity for legume starches, which were characterised by absence of peak viscosity due to a gradual rise during a holding period at 95 °C. Peak viscosity reflects the ability of starch granules to swell freely before they start to break down. The absence of peak viscosity in soaked cowpea (Fig. 4B) might be because of presence of other molecules such as proteins and dietary fibre in large amounts, which might have affected the starch swelling process and the development of peak viscosity. For instance, higher protein and fibre content, fibre-starch and starch-protein interactions, and the structural arrangements of amylose and amylopectin molecular configurations influence the hydration rate of starch (Henshaw et al., 1996). The lowest peak viscosity was observed in the germinated pearl millet (53 mPa.s) and germinated cowpea (310 mPa.s) (Fig. 4), possibly because of enzymatic breakdown of the starch macromolecule to simple sugars during germination to support sprout growth. Breakdown of pearl millet and cowpea starch was observed when the samples were subjected to constant high temperature (95 °C) and stirring conditions (Fig. 4). However, the breakdown was more accentuated for pearl millet (Fig. 4A) than cowpea (Fig. 4B), indicating fragility of the swollen starch granules in pearl millet at shear and temperature. Interestingly, the results showed that germinated (55 mPa.s) and fermented (158 mPa.s) pearl millet underwent lower breakdown compared with the washed (300 mPa.s) and soaked (346 mPa.s) pearl millet. This is probably because the starch granules were already broken and could not swell to

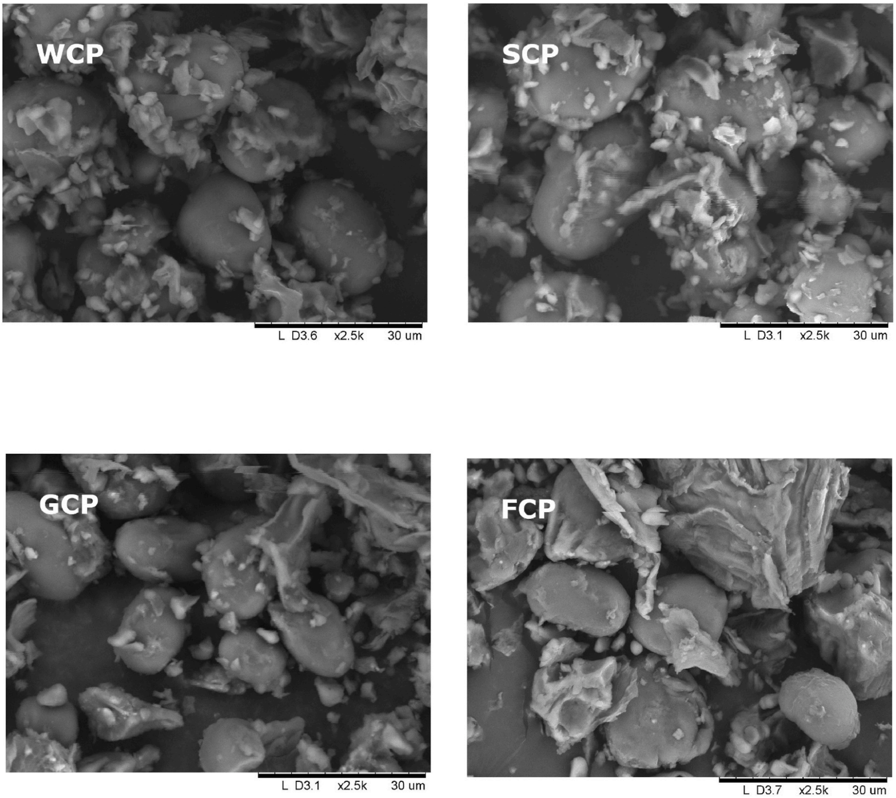

Fig. 2. SEM images of starch granules in cowpea after different pre-treatments: WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea.

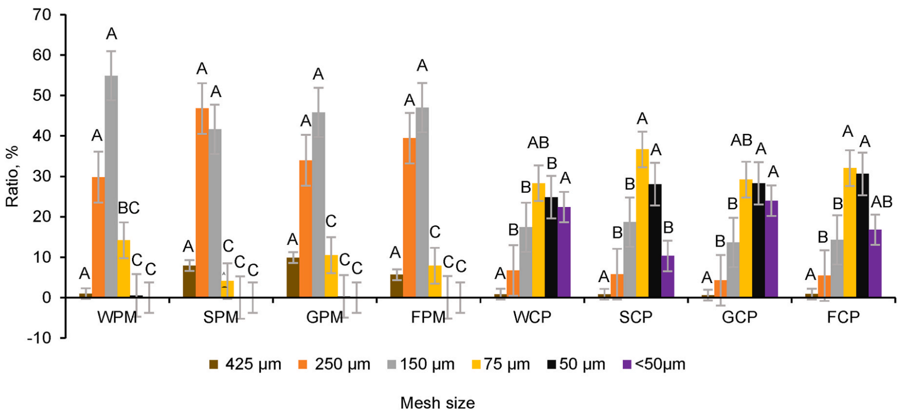

Fig. 3. Particle size distribution of pearl millet and cowpea flour: WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet, WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea. Error bars represent standard error. Bars marked with different letters are significantly different ($p < 0.05$) within each mesh size.

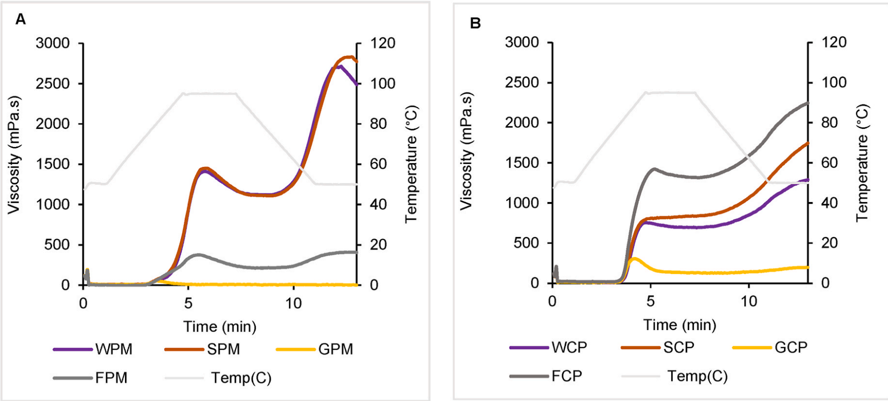

Fig. 4. Pasting properties of (A) pearl millet (WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl mille) and (B) cowpea (WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea).

the same extent as those in washed and soaked pearl millet. Yadav, Chhikara, Anand, Sharma, and Singh (2014) also reported shear-thinning behaviour of pearl millet samples. The behaviour of starch granules in cowpea was completely different, with very low breakdown of washed (65 mPa.s) and soaked (18 mPa.s) cowpea compared with germinated (187 mPa.s) and fermented (192 mPa.s) cowpea. Indicating that washed and soaked cowpea were more resistant to shear thinning than germinated and fermented cowpea. Germinated and fermented cowpea showed higher breakdown, probably because of the higher activity of amylolytic and proteolytic enzymes activated during the germination and fermentation process.

Setback indicates how starch behaves after heating, cooking and cooling, and it is an essential parameter in aggregation of starch molecules. The decrease in temperature in the flour paste during cooling allows more scope for hydrogen bonding and entanglement between the starch chains. Cooling of starch pastes also leads to renewed crystal formation of amylose-lipid complexes and recrystallisation of starch. Soaking pearl millet gave the highest setback of all pre-treatments (Fig. 4A). Germinated and fermented pearl millet had the lowest setback, probably due to breakdown of amylose by enzymes minimising the scope for entanglement between starch chains. For cowpea, the highest setback was observed in fermented samples (Fig. 4B). This was possibly because of disruption of the cell wall matrix by enzymatic action during fermentation, allowing the starch to swell freely, as observed by Zhu et al. (2010). This information must be considered in efforts to formulate complementary porridge with good appearance, paste stability and the right consistency for children under five years old.

Final viscosity in the pearl millet samples ranged widely, from 4 to 2773 mPa s (Fig. 4A), with soaked pearl millet showing higher final viscosity followed by washed, fermented and germinated pearl millet. Final viscosity in the cowpea samples also ranged widely, from 196 to 2117 mPa s (Fig. 4B), with fermented cowpea showing higher final viscosity followed by washed, soaked and germinated cowpea. This higher final viscosity in fermented cowpea might be because of the high protein content (Table 1), which possibly protected the starch granules from breakdown by enzymes during fermentation (Zhu et al., 2010). The germination process lowered the final viscosity of both pearl millet and cowpea samples, with the lowest values observed for germinated pearl millet. This difference reflects higher enzymatic attack on the starch granules in germinated pearl millet than in germinated cowpea, as also observed in the SEM images (Fig. 1, panel GPM, and Fig. 2, panel GCP). Previous studies have also found that germination decreases viscosity in cereal and legume flour due to breakdown of starch granules by α-amylase activated during germination (Griffith & Castell-Perez, 1998; Malleshi, Daodu & Chandrasekhar, 1989; Mosha & Svanberg, 1990).

Therefore, decreasing the viscosity by germinating seeds can be a good strategy when developing complementary porridge for undernourished children. Lower viscosity provides more flexibility in adjusting flour concentration and thus increasing energy density (Alexander, 1983; Mosha & Svanberg, 1990).

3.6. *Principal component analysis (PCA)*

Exploratory multivariate analysis was used to further distinguish the effects of pre-treatments on physicochemical and pasting properties of pearl millet and cowpea and to identify any differences between replicate batches (Fig. 5). In general, variables opposite to each other showed a negative relationship, while variables close to each other revealed a positive relationship. Based on the pearl millet PCA score and loading plot (Fig. 5A1 and 5A2), PC1 and PC2 accounted for 39.0% and 30.6% of the total variance, respectively. Any differences between the pre-treatments were mainly associated with higher pasting viscosity in washed and soaked pearl millet and higher total dietary fibre content in germinated pearl millet. Based on the score plots of pearl millet and cowpea (Fig. 5A1 and B1), there were differences between batch 1 and batch 2. These differences between batches were mainly seen for replicate germination and fermentation samples, and can be challenging to control due to complex biological and environmental factors varying to some extent. This is because these pre-treatments further modify the seeds during processing by increasing activity of enzymes, thereby changing the macromolecules present. PCA score and loading plots for cowpea (Fig. 5B1 and B2), PC1 and PC2 accounted for 40.9% and 30.2% of the total variance, respectively. They indicated that differences between pre-treatments were mainly related to higher total dietary fibre and ash content in washed cowpea, due to the presence of the hull, higher pasting properties in soaked and fermented cowpea, and germination contributing to increasing the protein content. Total dietary fibre content in both loading plots was inversely related to parameters such as starch, % amylose and some pasting parameters.

4. Conclusions

Soaking, germination, and fermentation can be low-cost processing methods to modify the physicochemical properties and alter the food microstructural arrangements of different macromolecules present in pearl millet and cowpea seeds. This study showed that these pre-

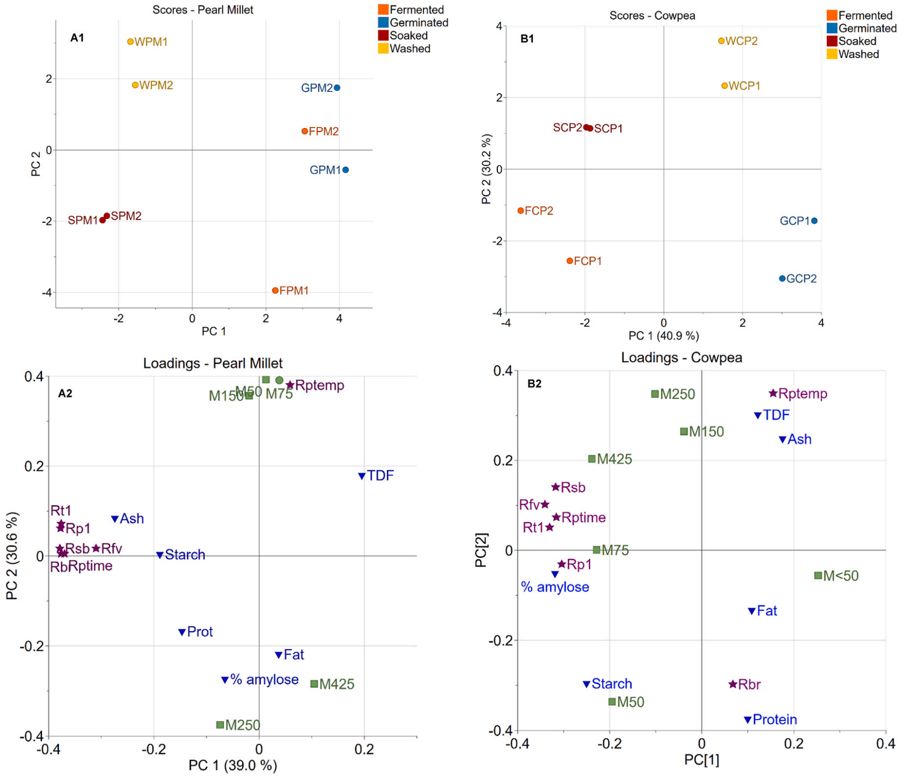

Fig. 5. Principal component analysis (PCA) plots for pearl millet and cowpea. (A1) Score plot for pearl millet (WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet), 1 = batch 1, 2 = batch 2. (A2) Loading plot for pearl millet (TDF = total dietary fibre, M = mesh size, R = rapid visco analyser: p1 = peak 1, ptemp = pasting temperature, sb = setback, fv = final viscosity, br = breakdown, ptime = peak time, t1 = through 1. (B1) Score plot for cowpea (WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea). (B2) Loading plot for cowpea.

treatments can also enhance the nutritional profile. Activation of enzymes during germination and fermentation was likely responsible for the changes in pasting and microstructural properties. Germination decreased total starch content in both pearl millet and cowpea, resulting in less entanglement between starch chains, as verified by the pasting properties. Numerous holes and broken starch granules in pearl millet were observed, drastically reducing the final viscosity of the pearl millet flour, while in cowpea germination did not affect the microstructure but led to reduced final viscosity. This important new knowledge on the effect of pre-treatments on the physicochemical and microstructural properties of Mozambican varieties of pearl millet and cowpea flour milled from pre-treated seeds can be applied to develop complementary porridge for malnourished children.

CRediT authorship contribution statement

Sunera Nurmomade: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Santanu Basu:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Irene de Carvalho:** Writing – review & editing, Supervision, Conceptualization. **Maria Eduardo:** Writing – review & editing, Supervision, Conceptualization. **Roger Andersson:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Sensory evaluation of composite porridges based on pearl millet and cowpea for Mozambican children under five years old

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ABSTRACT

Undernutrition remains a major public health problem in Mozambique. Traditional porridges made from cereals like maize, rice, and millet are typically low in protein, nutrient density, and energy content. This study aimed to evaluate caregivers' liking of newly developed composite porridge made with pearl millet and cowpea, formulated to meet the nutritional needs of children by enhancing protein content and nutrient density.

Sensory evaluations were conducted in three distinct locations: Copuito, Pulupu, and Nanguasse in Cabo Delgado Province, Mozambique. Four porridges were tested for appearance, colour, aroma, and taste: (1) **SM** - 100 % soaked pearl millet (control); (2) **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; (3) **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and (4) **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

Results showed that caregivers in all locations liked FMFGP very much, with both SM and FMFGP described as "good porridge". Although all composite porridges were generally liked, FMFP and FGMFP were less liked in Copuito and Pulupu because of their thinner viscosity and the taste of beans. The thin viscosity was due to the addition of germinated pearl millet, which allows for an increase in energy density. Overall, FGMFP and FMFGP have the potential to be successfully introduced and used as a nutritional solution to address children's malnutrition in Mozambique.

1. Introduction

Porridge, a staple meal made from cereals like maize, rice and millet, is commonly given to children in developing countries, including Mozambique. However, this porridge is often too viscous, making it hard for children to swallow and digest, and usually it is low in protein and energy density. When the porridge is diluted with water, there is a reduction in its nutrient content (Makame et al., 2020; Onofiok and Nnanyelugo, 1998). Many caregivers, unfortunately, prepare cereal porridge that lacks energy, protein and other nutrients. To overcome these concerns, optimizing a composite porridge made from cereal and an affordable source of protein like legumes and using pre-treated flour can be an alternative approach. Griffith et al. (1998), Singhavanich et al. (1999) and Thaoge et al. (2003) showed that using pre-treated flour could reduce porridge viscosity while enhancing nutrient and energy density. When porridge reduces the viscosity, it opens the possibility of adding more flour, ultimately leading to high-energy-density porridge and an improved food source for children.

Inadequate intake of nutrients stands as a critical contributor to undernutrition in developing countries, as highlighted by Saaka et al. (2021). Undernutrition significantly impacts public health in Africa, causing stunted growth and impaired development among children under the age of five(United Nations Children's Fund, 2023). In Mozambique, approximately 37 % of children under the age of five suffer from chronic malnutrition, which is commonly due to a lack of protein and micronutrients, with higher rates most widely observed in the northern region, specifically in Cabo Delgado Province (INE/ICF, 2023).

Recent research highlights the significant benefits of incorporating legumes and pre-treated flour into traditional cereal porridges. By combining legumes with pre-treated flour, protein quality is enhanced, in addition to an improvement in protein content. When legumes and

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pre-treated flour are added to cereal porridge, they also lower the viscosity and reduce the dietary components to increase nutrient density and bioavailability (Moriconi et al., 2023; Nout and Ngoddy, 1997; Oladiran and Emmambux, 2022).

A composite porridge made from pearl millet and cowpea can be a potential choice to improve the nutritional status of undernourished children in Mozambique. Pearl millet and cowpea are climate-resilient crops that are cultivated and consumed in many parts of developing countries, including Mozambique. These crops are affordable and available in the northern region of Mozambique, making them good candidates for preparing composite porridges for children to improve their nutritional status.

Twelve different composite porridges with varying proportions of pre-treated pearl millet and cowpea flour were developed and tested in our laboratory. Both crops are excellent sources of energy, and their combination provides an affordable source of protein. Pearl millet, variety Changara contains 12.8 % of crude protein content and cowpea variety 10 contains 23.6 % of crude protein content (Nurmomade et al., 2024). Starch digestibility improved with the germination process in pearl millet (Archana et al., 2001), in cowpeas (Nnanna and Phillips, 1990) and in composite porridges based on cereals and legumes (Gahlawat and Sehgal, 1994). Additionally, using germinated and fermented cereal and legumes as ingredients enhances starch and protein digestibility, increases mineral absorption and reduces resistant starch (Abbas and Ahmad, 2018; Oghbaei and Prakash, 2016).

Traditional processing methods, such as germination and fermentation, can alter the characteristics of these grains in beneficial ways. These pre-treatments are effective in reducing anti-nutritional factors like phytic acid that may limit the bioavailability of minerals and impair protein digestion (Adebo et al., 2022; Chaves-López et al., 2020; Griffith and Castell-Perez, 1998; Hassan et al., 2020). In addition, germination and fermentation improve several sensory attributes of the final product. Specifically, they help reduce bitterness, enhance natural sweetness and improve visual appearance, often making the product lighter (Garrido-Galand et al., 2021; Lemmens et al., 2019; Verni et al., 2022). These pre-treatments also soften the seed and reduce cooking time, contributing to a better mouthfeel and palatability. For example, Togwa, a traditional non-alcoholic drink made from maize and germinated finger millet flour, has a natural sweet taste without the need for adding sugar (Kitabatake et al., 2003). Lemmens et al. (2019) also reported that sprouted seeds improved consumer perceptions of flavour and overall taste, highlighting the positive sensory impact of germination.

Therefore, understanding the caregivers' liking and acceptability of composite porridges made from pearl millet and cowpea is essential for successfully introducing these products into Mozambican communities, particularly to address the nutritional needs of children. This study explores the perceived liking of a newly developed composite porridge formulated using fermented and germinated pearl millet and cowpea among caregivers of children under the age of five, conducted in Cabo Delgado Province, Mozambique. The findings of this study propose sustainable food solutions that reduce reliance on imported or industrial products by using locally available, climate-resilient crops such as pearl millet and cowpea. Moreover, the results can help shift cultural perspectives on food choices and encourage stakeholders and policymakers to promote and disseminate these composite porridges as a practical strategy to reduce undernutrition in Mozambique.

2. Materials and methods

2.1. Production of ingredients

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) variety changara and cowpea (*Vigna unguiculata* (L.) Walp) variety 10 were obtained from the Mozambican Agricultural Research Institute in Montepuez, Cabo Delgado. The samples were sorted and cleaned to remove damaged grains, stones, and extraneous material. The seeds were prepared following the method described by Nurmomade et al. (2024) with slight modifications using natural conditions.

For soaking pre-treatment, the seeds were soaked in tap water for 10 min in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, then sun-dried for 24 h. For germination, the seeds were soaked in tap water for 24 h in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea and then allowed to germinate for 48 h at room temperature (25 ± 2 °C). Then, the seeds were washed and sun-dried for 24 h, and the dried rootlets were removed by rubbing between the hands before milling. For fermentation, the seeds were soaked in tap water at room temperature (25 ± 2 °C), in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, and allowed to ferment for 72 h. Then, the seeds were washed and sun-dried for 24 h.

After sun-drying, all seeds were milled following a traditional process typically followed in Mozambique, which consists of pre-milling seeds with a wooden pestle and mortar until the bran was considered satisfactorily detached then the bran was winnowed from the endosperm grits using a winnowing basket and further milling with a wooden pestle and mortar to obtain the flour. Flour was stored in a polyethylene plastic bag at room temperature (25 ± 2 °C) until cooking the porridge for sensory evaluation.

2.2. Porridge formulation

The formulations used in this study were based on the proportions presented in Table 1. The composite porridges were formulated in ratios of 60 % cereal to 40 % legume, according to Griffith et al. (1998).

2.3. Pasting properties

Pasting properties were determined by a rapid visco analyser (RVA) (Newport Scientific, Australia), using 3.8 g of flour dispersed in 25 mL deionised water in an aluminium canister, mimicking the traditional way of preparing porridge. The samples were heated from 50 °C to a maximum temperature of 95 °C and held at 95 °C for 2.5 min before cooling to 50 °C, using the common Standard 1 method (Std1). Pasting temperature, peak viscosity, peak time, breakdown, trough, setback and final viscosity were recorded.

2.4. Colour measurements

The colour measurements were done to the porridges made with RVA in the laboratory at Uppsala, using a Konica Minolta spectrophotometer CM-600d (Konica Minolta, inc., Japan). The instrument was calibrated using a supplied white calibration plate. The samples were uniformly packed in a transparent Petri plate (Fig. 1). The instrument was placed on the plate with lid on, and three exposures at different places were conducted. The colour parameters were expressed as L*, a* and b* values. The L* values stand for whiteness to darkness. The chromatic portion was analysed by a* (+) redness and a* (−) greenness, b* (+) yellowness and b* (−) blueness.

2.5. Porridge preparation in Mozambique

Cooked porridges were prepared in a 13 % (w/v) concentration (208 g of flour mixed with approximately 1400 ml of tap water). An initial flour paste was made by mixing 208 g of flour and half of the water at room temperature. The paste was added to the other half of the boiled water and mixed well. Flour-water slurries were cooked in a traditional way, using coal to keep boiling the porridge (Fig. 2). Cooking was done within 15 min; sometimes, an additional 5 min was necessary to make sure that the porridge was cooked when the temperature of cooking was affected due to the coal type. Stirring was necessary throughout the cooking of the porridge to avoid lump formation. Cooked porridge was served at the appropriate temperature for consumption, between 40 and 50 °C and sugar was added just before the sensory test (1.5 g of sugar to

Table 1
Experimental design of different formulations.

CODE		Pearl Millet				Cowpea			
		Treatments (%)				Treatments (%)			
1	**SM**	Soaked	100	–		–		–	
2	**FMFP**	Fermented	60	–		Fermented	40	–	
3	**FGMFP**	Fermented	55	Germinated	5	Fermented	40	–	
4	**FMFGP**	Fermented	60	–		Fermented	35	Germinated	5

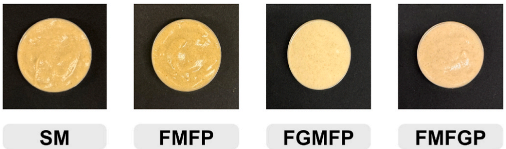

Fig. 1. Porridges on a Petri plate.
SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

Fig. 2. The traditional way to cook porridge using coal.

40 g of porridge).

2.6. Sensory analysis of the porridges

Sensory evaluation was performed in accordance with the rules of the Ethics Committee of the Medicine Faculty and Central Hospital of Maputo, in Mozambique, approved under the number CIBS FM&HCM/15/2023 on July 29, 2024.

A 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) was used to assess the sensory attributes of the composite porridges. Samples were evaluated by adult caregivers in Cabo Delgado Province, in Mozambique. The sensory evaluation was conducted in three different locations in Cabo Delgado. There were 64 caregivers in Copuito, 66 in Pulupu, and 77 in Nanguasse. Inclusion criteria for participation consisted of being over 18 years old, being the mother of children aged five or under, confirming prior experience consuming pearl, to ensure general familiarity with the seed, and wishing to participate in the study without being paid. The participants were recruited and selected with the help of leaders in each location.

The sensory evaluation test took place outdoors, and the participants were sitting on the floor in a shady place according to available conditions in each location (Fig. 3). To avoid allergic reactions and ambiguities, participants were explained about the process and informed in advance about the composition of the porridges. Then, the consent form to sign was given before proceeding with the sensory evaluation.

Each participant received an evaluation form, a pen, a bottle of mineral water, and a plastic spoon for each sample (Fig. 4). The porridge samples (40 g/person) were randomly distributed in a transparent plastic cup. Each treatment sample was designated a random three-digit code, which appeared on the corresponding cup. One sample was tested per time to avoid misunderstanding. Given that most caregivers have limited literacy in Portuguese, all instructions were delivered in the local languages, Emakhuwa and Kimwani, by a trained community nutritionist. Participants were then assisted in rating the hedonic scale and instructed to avoid communication with each other during the sensory evaluation.

The sensory attributes (appearance, colour, aroma, and taste) were used to evaluate the samples. The caregivers analysed the appearance first, following the above order. At the end of the evaluation, participants were selected based on their willingness to provide feedback. In total, 55 participants from the three communities were selected and were asked further questions to clarify their evaluations. The face scale was included in the evaluation form (sensory evaluation form in the annexure) to help caregivers understand the explanation.

Porridges were served at an appropriate temperature for consumption, between 40 and 50 °C. According to Mouquet & Trèche (2001), the typical consumption temperature for porridges is 43 ± 2 °C. The participants were instructed to cleanse their palates by drinking mineral water between the samples.

Fig. 3. Open environment (outdoors) for sensory analysis.

Fig. 4. Caregivers' evaluating composite porridges.

2.7. Statistical analyses

Sensory data were analysed using the statistical software Minitab version 19.2. Two-way ANOVA was generated using the general linear model procedure with composite porridge, location and their interaction as factors. Tukey's pairwise comparison test was used to identify significant differences between group means, with a significance level set at a 95 % confidence level.

Pasting properties and colour measurements were analysed using the statistical software Minitab version 19.2, and one-way ANOVA was performed. For porridge differences, Tukey's pairwise comparison test was used.

Principal component analysis (PCA) was performed using the software SIMCA 17 (Sartorius Stedim Data Analytics AB) to visualize the liking of porridges in different locations in Mozambique. The perceived liking of these composite porridges in different locations was analysed in relation to their pasting properties and colour measurements obtained in the laboratory in Uppsala.

A word cloud was generated using the free online application WordClouds.com to assess the caregivers' opinions on composite porridges.

3. Results and discussion

3.1. Pasting properties

The pasting properties of composite porridges are illustrated in Fig. 5. Significant differences in final viscosity were observed between porridges. Final viscosity values indicate the ability of flour to form a viscous paste/gel after cooking and cooling. The final viscosity of composite porridge containing germinated pearl millet (FGMFP) decreased dramatically compared to soaked pearl millet porridge (SM). SM porridge exhibited the highest final viscosity value of approximately 4000 mPa s (Table 2), indicating a greater swelling capacity of the starch granules in soaked pearl millet. SM is a typically traditional cereal porridge known for its high viscosity, which necessitates dilution with water to reduce thickness, ultimately resulting in a porridge with lower energy density. Similar results were reported (Kikafunda et al., 1997; Oladiran and Emmambux, 2022).

In contrast, the final viscosity of FGMFP porridge was recorded at approximately 600 mPa s (Table 2). Utilizing germinated flour to prepare porridges is a good strategy to liquefy thick porridges. This is due to the breakdown of starch granules by α-amylase, which is activated during the germination process. A study by Yenasew and Urga (2023) demonstrated that adding a small amount (5 %) of germinated millet flour as an ingredient reduces viscosity to a semi-liquid consistency. Consequently, the energy density of the porridge can be increased by properly combining ingredients and adjusting the type of flour used to achieve the desired viscosity (Kikafunda et al., 1997). Brandtzaeg et al. (1981) and Oladiran and Emmambux (2022) have reported similar results, clearly indicating that germination has a high potential for increasing energy density.

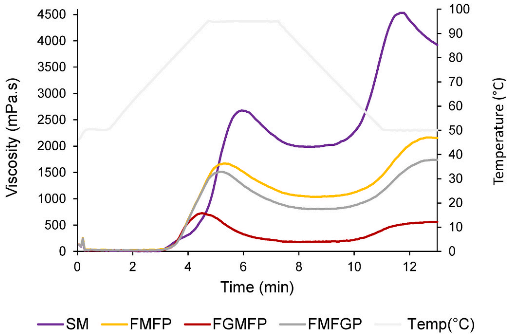

Fig. 5. Pasting properties of composite porridges
SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

When germinated cowpea flour was added to the composite porridge (FMFGP), the reduction in viscosity was less pronounced than with germinated pearl millet flour (Fig. 5). The final viscosity of the FMFGP porridge was approximately 1800 mPa s (Table 2). Nurmomade et al. (2024) noted a lower breakdown of starch granules in germinated cowpea flour compared to germinated pearl millet flour.

Porridge viscosity is a critical parameter in the formulation of composite foods for children, as it directly affects swallowing, nutrient intake, and satiety. According to Thaoge et al. (2003) and Oladiran and Emmambux (2022), the recommended apparent viscosity for children's porridges, measured at 54 rpm, ranges between 1000 and 3000 mPa s to ensure adequate consistency. In this study, the final viscosity of the composite porridge measured using Rapid visco analyser (RVA) was 2163 mPa s for FMFP and 1749 mPa s for FMFGP (Table 2), both of which fell within the recommended viscosity range (Fig. 5).

It is important to note that viscosity measurements can vary significantly depending on the measurement method and instrument, for instance, laboratory or field, or industrial settings (Mouquet and Trèche, 2001). As reported by Ojijo and Shimoni (2004), porridge viscosity is a complex characteristic influenced by several factors, including shear rate, temperature, and cooking duration. Moreover, the sensory perception of viscosity can differ based on cultural preferences and food habits, which may influence caregiver acceptance.

Several researchers working with traditional porridges in developing countries, such as those in Africa, have reported practical methods at the household level to reduce high viscosity while increasing energy density (Makame et al., 2020; Moriconi et al., 2023; Oladiran and Emmambux, 2022). Viscosity is a crucial factor that can significantly influence both the quantity of food a child can consume and their overall energy intake. Porridges are best served to children while warm, as cold porridge tends to thicken (Mouquet and Trèche, 2001).

3.2. Colour measurement of composite porridges

Results of lightness (L*), green/red (a*) and yellow/blue (b*) of composite porridges are presented in Table 3. The values of lightness (L*) ranged from 49.8 to 56.0, statistically significant. The darkest porridge was SM, with the lowest (L*) value, and the lightest porridge

Table 2
Pasting properties of composite porridges.

Composite porridge	Peak Viscosity (mPa.s)	Trough Viscosity (mPa.s)	Breakdown Viscosity (mPa.s)	Final Viscosity (mPa.s)	Setback Viscosity (mPa.s)	Peak Time (min)	Pasting Temp. (°C)
SM	2637^{a}	1968^{a}	670^{a}	3828^{a}	1860^{a}	5.9^{a}	77.5^{b}
FMFP	1685^{b}	1037^{b}	648^{a}	2163^{b}	1126^{b}	5.3^{b}	79.1^{a}
FGMFP	719^{d}	168^{d}	552^{b}	545^{d}	377^{c}	4.5^{d}	79.1^{a}
FMFGP	1507^{c}	790^{c}	718^{a}	1749^{c}	960^{b}	5.2^{c}	80.0^{a}

Pasting properties values are the mean of duplicates. Different letters within columns indicate significant differences $p \leq 0.05$ in Tukey's pair-wise comparison.
SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

Table 3
Colour measurements of composite porridges.

Composite porridge	L*	a*	b*
SM	49.8^{c}±0.1	0.7^{b} ± 0.1	11.4^{a}±0.2
FMFP	54.5^{b} ± 0.1	1.2^{a}±0.03	10.2^{b} ± 0.2
FGMFP	56.0^{a}±0.1	1.1^{a}±0.00	10.2^{b} ± 0.1
FMFGP	54.5^{b} ± 0.2	1.1^{a}±0.04	10.0^{b} ± 0.2

Values shown are mean ± standard deviation of duplicates. Different letters within columns indicate significant differences $p \leq 0.05$ in Tukey's pair-wise comparison of colour measurements.
SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

was FGMFP, containing germinated pearl millet, with the highest L* value. The addition of germinated pearl millet might have influenced the lightness. Similarly, Nefale & Mashau (2018) reported that the lightness increased when finger millet was germinated for 72 h in comparison with ungerminated finger millet.

3.3. Sensory evaluation

The sensory evaluation showed significant variations in the caregivers' liking for the sensory attributes of the composite porridge, as detailed in Table 4. The traditional porridge, described as SM, is made exclusively from 100 % soaked pearl millet and is well-known in Copuito, Pulupu and Nanguasse. Interestingly, there were no significant differences in liking between SM and one of the newly developed composite porridges, FMFGP, which consists of 60 % fermented pearl millet, 35 % fermented cowpea, and 5 % germinated cowpea. Both porridges were the most liked across all sensory attributes, appearance, colour, aroma, and taste in all locations, as represented in Fig. 6, word cloud. A word cloud presents caregivers feedback about their opinions, along with suggestions for improvement. The majority of caregivers expressed familiarity with soaked pearl millet porridge (SM) taste, as it is their traditional porridge, and they also expressed a liking for the taste of FMFGP. As shown in Table 4, no statistically significant difference was observed between these two porridges in terms of taste.

Fig. 6. Word cloud of responses of face-to-face interviews about caregivers opinions on the composite porridge. The largest font size represents words that were mentioned at least 6 times, and the smallest font size represents words that were mentioned at least one time.

Table 4
Caregiver's evaluation for composite porridges in three different locations.

Composite porridges	Location	Appearance	Colour	Aroma	Taste	Overall acceptability
SM	Copuito	8.1^{a}±1.3	7.6^{ab} ± 1.5	7.4^{abc}±1.8	8.1^{a}±1.0	7.8^{ab} ± 1.0
FMFP	Copuito	5.9^{bc}±2.6	5.8^{de} ± 2.5	6.2^{de} ± 2.4	5.9^{de} ± 2.7	6.0^{c}±2.0
FGMFP	Copuito	5.6^{cd} ± 2.6	5.7^{e}±2.7	5.1^{f}±2.7	5.4^{e}±2.9	5.5^{c}±2.3
FMFGP	Copuito	7.5^{a}±1.8	6.9^{bcd} ± 2.1	7.0^{bcd} ± 2.0	7.0^{abcd}±2.4	7.1^{b} ± 1.6
SM	Pulupu	7.6^{a}±1.3	8.0^{a}±1.1	8.0^{ab} ± 1.1	8.1^{a}±1.2	7.9^{ab} ± 0.6
FMFP	Pulupu	4.3^{d} ± 2.9	5.9^{de} ± 2.5	5.7^{ef}±2.5	6.6^{bcde} ± 2.9	5·3^{c}±2.1
FGMFP	Pulupu	4.8^{cd} ± 3.2	6.3^{cde} ± 1.8	6.7^{cde} ± 1.8	6.7^{b} ± 2.2	6.0^{c}±1.6
FMFGP	Pulupu	7.1^{a}±2.1	7.1^{abc}±1.8	7.6^{abc}±1.6	7.5^{abc}±2.1	7.3^{ab} ± 1.5
SM	Nanguasse	8.0^{a}±1.6	7.9^{a}±1.6	8.0^{ab} ± 1.7	8.1^{a}±1.6	8.0^{a}±1.3
FMFP	Nanguasse	7.0^{ab} ± 2.2	7.7^{ab} ± 1.4	7.5^{abc}±1.7	7.6^{abc}±1.9	7.4^{ab} ± 1.5
FGMFP	Nanguasse	7.0^{ab} ± 2.5	7.9^{a}±1.0	7.9^{ab} ± 1.3	6.8^{cd} ± 2.7	7.4^{ab} ± 1.5
FMFGP	Nanguasse	8.1^{a}±1.2	8.1^{a}±1.2	8.1^{a}±1.3	8.0^{ab} ± 1.4	8.1^{a}±1.0

Values shown are the mean of sensory ±standard deviation. Different letters within columns indicate significant differences $p \leq 0.05$ in Tukey's pair-wise comparison of composite porridges and locations.
SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

In contrast, the porridges that were least liked were FMFP, which contained 60 % fermented pearl millet and 40 % fermented cowpea, and FGMFP, which consists of 55 % fermented pearl millet, 5 % germinated pearl millet, and 40 % fermented cowpea. Caregivers observed that these two porridges had a thin viscosity, which influenced their lower liking. However, some caregivers mentioned that they like a thin porridge to feed their children. Although there were several comments concerned with viscosity, all porridges were accepted, as shown in Fig. 6. Common words used in caregivers' answers are presented in a word cloud, Fig. 6. The largest font size represents words that were mentioned 6 times, and the smallest font size represents words that were mentioned only one time. Composite porridge, FGMFP, had the lowest viscosity of around 600 mPa s. In comparison, FMFGP measured about 1800 mPa s and FMFP about 2200 mPa s. The highest final viscosity of porridge was SM, which was approximately 4000 mPa s.

Using germinated and fermented flour as ingredient played a crucial role in reducing the viscosity of the porridges. The enzymes present in germinated flour break down the starch granules, leading to less swelling and lower water-binding capacities (Kaur and Gill, 2020). Nurmomade et al. (2024) reported that germinated pearl millet and cowpea flour do not swell as much compared to soaked flour, resulting in low viscosity. This characteristic allows for adding more flour to the porridge, producing a high-energy-density food (Alexander, 1983; Oladiran and Emmambux, 2022; Griffith et al., 1998).

The composite porridge FGMFP had the lowest viscosity among the tested porridges (Fig. 5). Although it presents an opportunity to increase energy density, caregivers liked other options due to the intense taste and aroma of beans. The higher amount of fermented cowpea (40 %) may have contributed to its more pronounced taste and aroma of beans. Chanadang and Chambers (2019) found similar results, noting that as the quantity of legumes in formulations increases, the beany characteristics become more pronounced.

It is important to note that although the intense aroma and taste of beans were present in all three newly developed composite porridges in this study, caregivers generally liked them. Roland et al. (2017) reported that off-flavour compounds in legumes are primarily derived from the oxidation of unsaturated fatty acids, and many of these characteristics are often linked to the action of the lipoxygenase enzyme.

Regular exposure to these composite porridges made from cereals and legumes may be necessary to enhance their acceptance and potentially shift cultural choices in Mozambique. Repeated exposure to novel extruded fortified blended foods increased children's acceptability of the products in Tanzania (Chanadang and Chambers IV, 2020). Moreover, they suggested that children may increase their perceived liking of some food products after repeated exposure and may increase their liking for some food products more than for others over an extended time period.

All composite porridges in this study were served with sugar, a common practice in Mozambique to enhance the palatability of porridges. Some caregivers mentioned that composite porridge FGMFP was very sweet, even though the same amount of sugar was added to all samples. The sweetness perceived in FGMFP may be attributed to the presence of dextrin, maltose and oligosaccharide in germinated flour. Using germinated flour to prepare porridge can be a good strategy to avoid adding sugar to the composite porridge for children. According to Lineback and Ponpipom (1977) and Yang et al. (2021), during germination, starch is broken down by α-amylase, the principal enzyme activated during germination, resulting in dextrin, maltose and oligosaccharide. A traditional non-alcoholic drink, Togwa, made with maize and germinated finger millet flour, has a sweet taste without adding sugar, as reported by Kitabatake et al. (2003).

3.4. Principal component analysis

The liking of four sensory attributes (appearance, colour, aroma, and taste) was analysed using principal component analysis (PCA) across three different locations in the northern region of Mozambique, along with pasting properties and colour measurements performed in the laboratory in Uppsala. PCA helped to visualize the differences in porridge liking in Copuito, Pulupu and Nanguasse (Fig. 7). The first Principal Component (PC1) accounted for 65.6 % of the variation and effectively differentiated the samples, while the second Principal Component (PC2) explained 20.3 % of the variation.

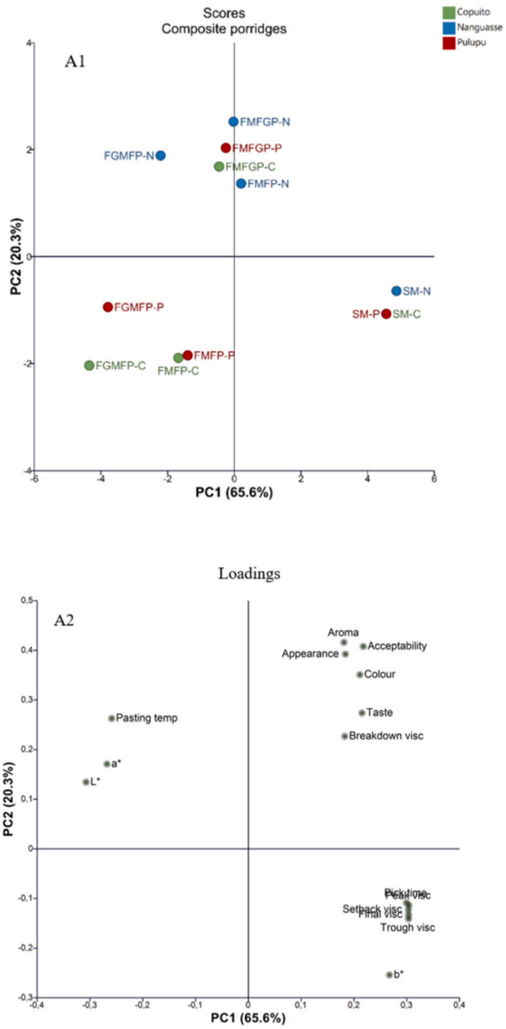

Fig. 7. Principal component analysis (PCA) plots. (A1) Score plot of composite porridges in three locations: C – Copuito (green), N – Nanguasse (blue), and P – Pulupu (red). (A2) Loading plot with sensory attributes, pasting properties and colour measurements.

Composite porridges: SM - 100 % soaked pearl millet (control); **FMFP** - 60 % fermented pearl millet + 40 % fermented cowpea; **FGMFP** - 55 % fermented pearl millet + 5 % germinated pearl millet + 40 % fermented cowpea; and **FMFGP** - 60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea.

Among the four porridges evaluated, the traditional porridge made with soaked pearl millet (SM) was liked across all sensory attributes in all three locations, which was expected due to its familiar appearance, taste and sociocultural relation. The PCA results indicated that SM and FMFGP were grouped on the positive side of PC1, as both were characterised as "good porridge," the caregivers expressed a strong liking for them, and both porridges, SM and FMFGP showed no statistically significant differences in all sensory attributes such as appearance, colour, aroma, and taste.

All composite porridges were liked for their appearance, colour, aroma, and taste in all locations. However, the combinations of FMFP (60 % fermented pearl millet and 40 % fermented cowpea) and FGMFP (55 % fermented pearl millet, 5 % germinated pearl millet, and 40 % fermented cowpea) were the least liked in Copuito and Pulupu and were grouped on the negative side of PC1. It is also important to note that in Nanguasse, all porridges were liked very much since people consume more pearl millet than in the other two locations, which makes it easier for them to like all porridges because it is part of their cultural practices.

4. Conclusion

The results of this study demonstrate that all composite porridges were liked in all locations. The newly composite porridge most liked was FMFGP (60 % fermented pearl millet + 35 % fermented cowpea +5 % germinated cowpea).

The addition of germinated pearl millet affected the appearance of the composite porridge (FGMFP), resulting in a thinner porridge. This porridge has the potential to be enhanced by adding solid content to adjust the viscosity, nutrient and energy density.

The distinct aroma and taste of beans were due to the presence of cowpea in the composite porridges, which caregivers were not familiar with, affecting their liking. Nevertheless, the quantity can be adjusted according to participants liking.

Composite porridges FGMFP and FMFGP have the potential to be successfully introduced in communities and used to improve children's nutritional status to address malnutrition challenges in Mozambique.

Study limitations

This study had certain limitations. The composite porridge formulations were tested with caregivers of children aged five or under, although the intended group for the porridges is children, particularly those weaning and pre-school aged. In Mozambique, mothers usually serve as the primary caregivers and cooks for their children. Additionally, a second limitation was ensuring a consistent cooking temperature, as we used a traditional way to cook porridges in the communities using coal, as shown in Fig. 2.

CRediT authorship contribution statement

Sunera Nurmomade: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Eduardo:** Writing – review & editing, Supervision, Conceptualization. **Santanu Basu:** Writing – review & editing, Supervision, Conceptualization. **Irene de Carvalho:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Roger Andersson:** Writing – review & editing, Validation, Supervision, Conceptualization. **Karin Wendin:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization.

Implications for gastronomy

The findings of this study provide valuable insights for the gastronomy sector, presenting an opportunity to incorporate pearl millet and cowpea into a variety of recipes, encouraging innovation while preserving ancient culinary practices through fermented and germinated seeds. Caregivers responded positively to the newly developed composite porridge made from fermented and germinated pearl millet and cowpea, highlighting the potential of these nutrient-dense, climate-resilient crops to support health and address malnutrition. This effort not only seeks to maintain cultural identity but also aims to enhance the sensory attributes and inspire chefs, food entrepreneurs, and product developers worldwide to explore new applications of fermented and germinated seeds, encouraging the development of sustainable, culturally relevant food products.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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Sensory evaluation form

District: ______________, Community: ______________, Date: ______/09/2024

Name (optional): ______________________________ Age: ________,

Primary school: ______________, Secondary school: ______________,

University level: ______________ other: ______________________,

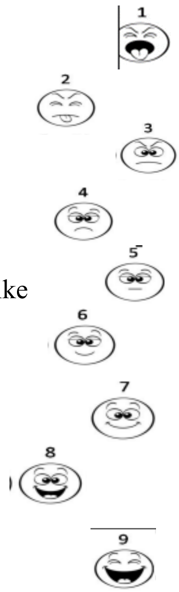
How many kids: ______________,kids less than five or equal five: ______________,

Dear consumer, you have at your disposal 4 coded samples of porridge made from pearl millet and cowpea. Please test and rate the attributes of each sample, using the scale below, how much you liked or disliked it. Between one sample and another, drink water. Mark with a number the position on the scale that best reflects your judgment.

Attributes	Sample 610	Sample 710	Sample 810	Sample 910
Appearance				
Colour				
Aroma				
Taste				
Acceptability				

Hedonic scale:

1. Dislike extremely
2. Dislike very much
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like very much
9. Like extremely



Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2025:63

This thesis investigated the effects of soaking, germination, and fermentation on the physicochemical and morphological properties of pearl millet and cowpea. The aim was to develop a nutrient-dense composite porridge to address child undernutrition in Mozambique. The findings revealed that germination and fermentation promoted enzymatic starch hydrolysis and enhanced the extractability of dietary fibre and phenolic compounds. Porridges prepared with fermented pearl millet exhibited *in vitro* rapidly digestible starch, lower phytic acid, and were well accepted within the communities.

Sunera Zulficar Nurmomade received her graduate education at the Department of Molecular Sciences, SLU, Uppsala. She obtained her M.Sc. degree in Food Technology at Eduardo Mondlane University in Maputo.

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