

# Complementary porridge based on pearl millet and cow pea

the effect of traditional processing techniques and cooking methods on pasting properties and *in vitro* starch digestibility

Signe Christerson

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Complementary porridge based on pearl millet and cow peathe effect of traditional processing techniques and cooking methods on pasting properties and *in vitro* starch digestibility

Signe Christerson

Supervisor:	Santanu Basu, Swedish University of Agricultural Sciences, Department of Molecular Science				
Assistant supervisor:	Sunera Zulficar Nurmomade, Swedish University of Agricultural Sciences, Department of Molecular Science				
Examiner:	Galia Zamaratskaia, Swedish University of Agricultural Sciences, Department of Molecular Science				

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#### Swedish University of Agricultural Sciences

Faculty of Natural resources and Agricultural Science Department of Molecular Sciences

#### Abstract

In Mozambique, malnutrition in children under 5 years is a serious concern. The condition is thought to be owing to nutritionally inferior diets and improper feeding practices on a mainly cereal-based diet, where porridges from millet- and legume-flours constitute the major staple food. Prior to cooking the porridges, traditional processing techniques are often applied to the cereal grains and grain legumes, such as soaking, germination and fermentation. These processing techniques have been suggested in literature to improve the nutritional quality, by reducing the amount of antinutritional compounds naturally present in both millets and grain legumes. Some of these processing methods have also been reported to modify the starch composition which result in changes in functional properties, e.g. pasting properties, and improve starch digestibility.

The objective of this master thesis was to formulate complementary porridges based on cereal grain; pearl millet, and grain legume; cow pea, to evaluate these for changes in cooking quality (i.e., pasting properties) and *in vitro* starch digestibility due to the effects of cooking method and traditional processing techniques (e.g. germination, fermentation, soaking). Two aims were formulated to meet the objective. The first was to determine the impact of ingredient formulation on product consistency in terms of pasting characteristics. The second was to map the starch digestibility of the formulated porridges.

The pasting properties of formulated complementary porridges were analysed with a rapid visco analyzer (RVA) using two costume temperature running profiles, i.e. "slow cooking" and "rapid cooking". In direct connection to analysing the pasting properties of porridges, the *in vitro* starch digestibility was analysed using a "Digestible starch assay kit" from Megazyme.

The impact of ingredient formulation on product consistency showed that porridges constituting of soaked and germinated pearl millet generally displayed substantially lower viscosities, compared to porridges constituting of only soaked pearl millet. The results also showed that fermentation of cow pea only marginally affected porridge pasting properties. Further, the cooking method, i.e. slow cooking or rapid cooking, did not substantially affect pasting properties of porridges. Lastly, the *in vitro* starch digestibility was neither impacted by ingredient formulation, nor by cooking methods used.

In future research, when formulating complementary porridges based on pearl millet and cow pea for malnourished children, one would optimally utilize the effects of germination as it offers an increase energy- and nutrient density. One would also utilize the effects of fermentation, as literature suggest that the process improves seed nutrient bioavailability.

*Keywords:* cow pea; pearl millet; complementary porridge formulation; traditional processing techniques; germination; fermentation; soaking; pasting properties; starch digestibility; energy density

#### Sammanfattning

I Moçambique är undernäring hos barn under 5 år ett allvarligt problem. Problemet tros bero på näringsmässigt sämre dieter och felaktiga utfodringsmetoder på en huvudsakligen spannmålsbaserad diet, där gröt tillagad av hirs- och baljväxtmjöl utgör den viktigaste basfödan. Innan gröten tillagas tillämpas ofta traditionella bearbetningstekniker på ingredienserna, dessa tekniker innefattar blötläggning, groning och jäsning. Dessa tekniker har föreslagits förbättra näringskvaliten i ingredienserna genom att minska nivåerna av naurligt förekommande antinutrienter. Teknikerna har även rapporterats modifiera ingrediensers stärkelsesammansättning, vilket i sin tur kan leda till förändringar i de funktionella egenskaperma, så som tillagningsegenskaper och stärkelse smältbarhet.

Syftet med denna masteruppsats var att formulera kompletterande grötar på pärlhirs och koärt, för att utvärdera dennes tillagningskvalitet genom att studera stärkelsens egenskaper och *in vitro* stärkelse smältbarhet. Detta efter att ingredienserna pärlhirs och koärt blivit utsatta för bearbetningstekniker (t.ex. groning, fermentering, blötläggning). Två mål formulerades för att uppnå syftet. Det första målet var att bestämma effekten av ingrediensformulering på produktens konsistens när det gäller stärkelsens egenskaper. Det andra målet var att kartlägga stärkelsens smältbarhet av de formulerade grötarna.

De komplementära grötarnas tillagningsegenskaper analyserades med hjälp av en "rapid visco analyzer" (RVA), där två programprofiler skapades, d.v.s. "långsam tillagning" och "snabb tillagning". I direkt anslutning till utvärderingen av stärkelsens egenskaper, så analyserades även grötarnas smältbarhet genom att använda ett färdigt kit, "Digestible starch assay kit", från Megazyme.

Inverkan av ingrediensformulering på produktens konsistens visade att gröt som består av groddad pärlhirs i allmänhet uppvisade avsevärt lägre viskositeter, jämför med gröt som bestod av blötlagd pärlhirs. Resultaten visade också att jäsning av koärter endast marginellt påverkade viskositeten . Vidare visade tillagningsmetoden, dvs långsam tillagning eller snabb tillagning, inte väsentligt påverka pastaegenskaperna hos gröt. Slutligen påverkades in vitro-stärkelsens smältbarheten varken utav ingredienssammansättningen eller av använda tillagningsmetoder.

I framtida forskning när man formulerar komplementära grötar baserade på pärlhirs och koärter för undernärda barn skulle man optimalt utnyttja effekterna av groning eftersom det ger en ökad energi- och näringstäthet. Man skulle också utnyttja effekterna av jäsning, eftersom litteraturen tyder på att processen förbättrar frönäringsbiotillgängligheten.

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## Abbreviations

RDS	Rapid digestible starch
cP or cPs	Centipoise
СР	Cow Pea
PM	Pearl millet
RC-	Rapid cooking
RVA	Rapid visco analyzer
SC-	Slow cooking
SDS	Slowly digestible starch
-SS	Soaked pearl millet and soaked cow pea
-SF	Soaked pearl millet and fermented cow pea
-SGF	Soaked pearl millet, germinated pearl millet and soaked cow
	pea
-SGS	Soaked pearl millet, germinated pearl millet and soaked cow
	pea
TDS	Total digestible starch
wb	water basis
w/v	Weight per volume

### 1. Introduction

According to the most recent Demographic and Health Survey (DHS), six percent of children under-five years are acutely malnourished or wasted ("have low weight-to height") and 43 percent of children under-five are stunted ("have low height-for-age") in Mozambique (Ministerio da Saude (MISAU) 2013; USAID 2021) The conditions are primarily caused by deficiencies in energy, protein, and micronutrients, due to nutritionally inferior diets and improper feeding practices on a mainly cereal-based diet (Griffith et al. 1998).

Recent research efforts are therefore directed towards the formulation of complementary porridge with high nutritional quality, which are based on local available staples, and suitable for children under 5 years living in Mozambique.

Traditional grains originating from Africa include sorghum, teff, rice, cereals, and several varieties of millets. Among the grains, Pearl millet *(Pennisetum glaucum, syn. P. americanum, P. typhoideum)* is an important source of essential nutrients and contains higher energy compared to other cereal grains e.g., rice and wheat. Pearl millet is further a high producing crop which provides an income to small-scale farmers, and thus contributes to livelihoods and the availability of foods (Hassan et al. 2021). Moreover, it is considered to have one of the most ideal protein quality or amino acid scores, which is considerably enhanced when combined with a grain legume, as the latter complements its profile of essential amino acids (Serna-Saldivar et al. 1991; Almeida-Dominguez et al. 1993).

Grain legume, Cow pea (*Vigna unguiculata*), is a promising and practical protein source, which is locally available and acceptable. The potential of these staples to be used when formulating complementary porridges can thus have a powerful overall effect on diminishing the prevalence of malnutrition in children.

Unfortunately pearl millet and cow pea also contain large quantities of antinutritional compounds, which inhibit the starch hydrolysis and mineral bioavailability, thus impairing the growth among children.

In this regard, traditional processing techniques (e.g. soaking, germination, and fermentation), have been identified as inexpensive and effective technologies to improve the nutrient bioavailability, by reducing the amount of antinutritional compounds. These processing techniques have also been reported to lead to modifications in starch composition which alters the grains functional properties. For instance, germination has been reported to increase the digestibility of starch

and reduce a foods viscosity upon cooking, with food viscosity being identified as the critical issue and often limiting factor prohibiting adequate nutritional intake (Griffith et al. 1998).

#### 1.1 Aim and objective

The objectives of this study were to formulate complementary porridges on pearl millet and cow pea based on traditional processing techniques (soaking, germination, fermentation) to evaluate its cooking behaviour in terms of pasting properties and starch digestibility.

The aims of the thesis were 1) to determine the impact of ingredient formulation on product consistency in terms of pasting characteristics and 2) to map the starch digestibility of the formulated porridges

The thesis was a part of a bigger research project *Complementary porridge with high nutritional quality for children in Mozambique*, funded by Swedish International Development Corporation Agency, Sida.

### 2. Background

#### 2.1 Traditional porridge in Mozambique

Traditional thick or thin porridges prepared from milled cereal grain staples (e.g., pearl millet, finger millet, sorghum, teff, African rice, maize etc), constitutes the major source of nutrients and complementary food for many children in sub-Saharian Africa, including Mozambique (Calvin & Wanjala 2018). Traditional technologies available for processing of cereal grains include, threshing, cleaning, washing, dehulling, soaking, germination, wet and dry milling and fermentation while roasting of cereals is rarely practiced (Makuru, 1992). Although, the preparation techniques of traditional porridges are known to vary from one locality to another (Mtebe et al. 1993). This has resulted in different types of porridges, varying in consistency and ingredients used.

Prior to the introduction of maize across the continent, traditional porridges were made from millet varieties and sorghum (Muyambo 2020). Maize has since then overshadowed many indigenous African cereal grains. As a result, pearl millet lags behind other major cereal grains in its genetic development, due to limited research and industrial support (National Research Council 1996).

However, over more recent years, the untapped potential of pearl millet has gained the attention of researchers. The reason for this is that pearl millet is considered a climate resilient crop with a highly favourable nutritional profile (Jukanti et al. 2016).

Like other grain cereals, pearl millets are incomplete in proteins, as they are deficient in essential amino acid *lysine*. To provide a complete protein profile in the diet, pearl millets can be accompanied by a grain legume. Legumes and millets are highly complementary, and when combined, create a powerful nutritional profile with high levels of proteins, complete protein (all essential amino acids), high protein digestibility and high levels and wide range of major micronutrients (Anitha et al. 2020).

Cow pea is an example of such as a grain legume. Similar to the pearl millets, cow peas has a potential to contribute to the major challenges in food and nutrition security, as well as in agricultural sustainability (Gomes et al. 2021).

Therefore, the crops; pearl millet and cow pea, are interesting and completory ingredients when designing complementary porridges.

#### 2.1.1 Pearl millet

Pearl millet, (*Pennisetum glaucum*), are robust annual grass, which produce panicles that generate 500-2000 seeds per panicle (Gustavsson & Jacobsson 2020).

The crop is cultivated in many semi-arid areas in Africa, including Mozambique, and has been found to be resilient to climate change. This is due to its inherent ability to withstand drought and high temperatures and its tolerance to saline-, acidic- and low fertility soils. The crop requires a short growing season and has been found to have fewer pest and disease problems compared to other cereals (Jukanti et al. 2016). In addition, it produces high yields even under challenging conditions, subsequently making it a cheap source of nutrition when compared to other major cereal crops less suitable to harsh climates (Inyang & Zakari 2008). It has also been found to have the ability to be cultivated either as a monocrop or in intercropping and mixed cropping (with e.g. sorghum or cow pea). The latter two practises provide financial security to smallholder farmers and secure the availability of food, as good yields of maize or sorghum cannot be counted on under similar climatic conditions (Serna-Saldivar et al. 1991; National Research Council 1996; Jukanti et al. 2016).

The crop is mainly used for human consumption in versatile manners (e.g. thick or thin porridges, fermented or unfermented dough and local beers), but also as a forage crop or for stover as a source of dry fodder (Abdelrahaman et al. 2007; Jukanti et al. 2016). It significantly contribute to human and animal diets owing to their high levels of energy, dietary fibre, lipids, proteins with a balanced amino acid profile, many essential minerals (e.g. calcium, iron, zinc, magnesium), some vitamins and antioxidants (Jukanti et al. 2016; Hassan et al. 2021).

However, the abundance of anti-nutritional factors (e.g. some polyphenols and phytic acid) interfere with the mineral bioavailability and inhibit proteolytic- and amylolytic enzymes, which reduce the digestibility of proteins and starch (Jukanti et al. 2016).

#### 2.1.2 Cow pea

Cow pea (Vigna unguiculata) is an annual herbaceous legume, indigenous to sub-Saharan Africa (Boukar et al. 2013). Similar to pearl millet, cow peas are also grown in semi-arid areas, as it tolerates drought and performs well in a wide variety of soils. It's resistance to drought and ability to improve soil fertility, whilst preventing soil erosion, makes it an important economic crop in many developing regions. The crops has root nodules that can fix atmospheric nitrogen, which allows it to be effectively intercropped with other cereals as it tolerates shade (Jackai et al. 2018).

Humans consume the young pods, immature pods and seeds and the mature dried seeds. The crop is also extensively grown as forage for livestock, as green manure and as cover crop (Jackai et al. 2018).

Cow pea seeds are an important source of nutrients. The legume provides high quality, inexpensive protein and energy to diets otherwise generally based on cereal grains and starchy foods (Som & Hazra 1993; Ibrahim et al. 2002). The crops is also a good source of soluble and insoluble fibre, resistant starch, bioactive peptides and several important micronutrients including folate, copper, thiamine, and iron (Benítez et al. 2013). They are also high in important bioactive compounds polyphenols (e.g., phenolic acids and flavonoids) which act as antioxidants in the body to prevent cell damage and protect against disease (Awika & Duodu 2017).

However, the nutritional quality of cow peas is reduced by the presence of antinutritional compounds which may have adverse effect on human or animal nutrition. Some antinutritional compounds include protease inhibitors, phytic acids, tannins, lectins, and flatus-producing oligosaccharides, which inhibit the digestibility of both proteins and starch by inhibiting proteolytic enzymes and amylolytic enzymes as well as reduces the bioavailability of some essential minerals (Ibrahim et al. 2002; Avanza et al. 2013; Jukanti et al. 2016).

#### 2.2 Traditional processing techniques

To enhance the nutritional quality and reduce the level of antinutritional factors naturally present in pearl millet and cow pea, different processing techniques including germination, fermentation, soaking and cooking have been suggested in scientific literature.

#### 2.2.1 Germination

Germination is defined as the process by which the seed embryo begins growth. A seed is considered to have germinated when the embryonic root emerges from the seed coat (Heslop-Harrison 2022). During germination, various endogenous enzymes are activated, and the starch, fat, and protein are hydrolysed into sugars, fatty acids, free amino acids and biopeptides (Baranzelli et al. 2018).

The process of germination have been shown to improve the nutritive value of cereals and legumes (Nkhata et al. 2018). The process has also been found to reduce the levels of antinutrients (e.g. tannins, phytic acid and oxalate), and maximize the levels of some of the utilizable nutrients such as bioactive components (e.g phenolics, flavonoids and antioxidants) (Saleh et al. 2013; Chen et al. 2017).

Besides improving the nutritional value of seeds, germination has been shown to significantly affects the physiochemical, rheological and *in vitro* starch- and protein- digestibility properties of flours (Cornejo et al. 2019; Yang et al. 2021).

In a study by Cornejo et al. (2019), germinated seeds showed a significant reduction in pasting and viscoelastic properties, a reduction in the total- and resistant starch content as well as a significant lower glycaemic index and higher protein digestibility. Similairly, other researchers have reported that germination significantly decreases the content of resistant starch, while observing an increase in available starch (Benítez et al. 2013).

Germination has further been showed to promote the development of more desirable aromas and flavours, thereby enhancing the organoleptic qualities of foods (Yang et al. 2021).

These mentioned changes resulting from germination could be useful to improve energy density, bioavailability of nutrients and acceptability of complementary porridges (Mtebe et al. 1993). Further, germination has been found useful when designing high-nutrient density porriges, as the process exhibits viscosity-thinning properties reducing dietary bulk (Marero et al. 1988; Nout & Ngoddy 1997).

#### 2.2.2 Fermentation

Fermentation is a natural process through which naturally present fermenting microorganisms (e.g., yeast and bacteria) and endogenous enzymes produced by them, modifies the composition of the substate (Nkhata et al. 2018; Kohajdova & Karovicova 2007). Fermentation activates starch-hydrolysing enzymes e.g, alfa-amylase and maltase, which degrade starch into dextrin and simple sugars (Akpapunam & Achinewhu 1985; Osman 2011).

Fermentation has been found to increase the protein solubility and the availability of limiting amino acids (Inyang & Zakari 2008). The effect of fermentation on protein content has yielded inconsistent results, ranging from an increase to a decrease in protein content upon fermentation (Akpapunam & Achinewhu 1985; Giami, Sunday Y & Okwechime, Ozydima I. 1993). Fermentation has however improved digestibility of plant protein (Inyang & Zakari 2008; Nkhata et al. 2018).

The micronutrient availability is also enhanced because of significant reductions in phytates, tannins and oligosaccharides (Chavan & Kadam 1989). Fermentation has further been reported to lead to an in increase in titratable acidity and reduction of the pH of the porridge to levels below 4.5, which has been shown to prevent proliferation of contaminating acid-intolerant species of bacteria and fungi (Nout & Ngoddy 1997). Fermentation may further provide preservative effects as the antimicrobial activity of lactic acid as inhibiting growth of pathogenic bacteria, the process has further been reported to detoxify aflatoxin (Lòpez et al. 2014). The process also results in improved organoleptic properties (e.g., texture, taste aroma) and improved storage stability (Lòpez et al. 2014).

The effect of fermentation as an isolated treatment on the viscosity of porridge is still uncertain and even controversial (Wanink et al. 1994; Amankwah et al. u.å.). While microbiological exogenous enzymes from the fermentation can have a thinning effect on the viscosity due to the hydrolysis of starches and protein, a lowered pH of the medium towards the isoelectric point may induce a neutralizing effect on the viscosity (Nout & Ngoddy 1997).

The *in vitro* starch digestibility have been reported to markedly increase as result of fermentation, while the total amount of starch has been reported to decrease (Elkhalifa et al. 2004).

#### 2.2.3 Soaking

Soaking involves extended steeping of cereals- or legume- grains in a liquid. This allows the grains to absorb the liquid and become saturated and softened to reduce the cooking time or to aid in seed coat removal. In addition, it involves cleansing of grains and extraction of water-soluble compounds.

Soaking has been reported to influence the nutritional quality and protein solubility of seeds, by reducing the concentration of water-soluble antinutritional factors (el-Adawy et al. 2000). Soaking has further been reported to affect swelling power and water absorption capacity (Ocheme & Chinma 2008; Adegunwa et al. 2012; Agume et al. 2017).

#### 2.2.4 Cooking

Cooking generally allows for foods to be easier consumed and digested. Moreover it makes the food safe to eat and more appetizing and palatable.

Cooking has been shown to improve the digestibility of poor and intermediately digestible starches and increase the absorption of many nutrients (Dreher et al. 1984; Carmody & Wrangham 2009). Some cooking methods have further been shown to reduce several key nutrients such as some water-and fat-soluble vitamins and minerals (Lee et al. 2017).

Cooking greatly influences the pasting properties of foods as it allows for changes to occur in the food as a result of application of heat in the presence of water. These changes affect the texture, digestibility and the end use of the food product (Ocheme et al. 2018).

The effect of cooking on starch digestibility has been widely studied. In a study by Tamura et al. (2016), starch digestibility was reported to not be affected by cooking degree related to starch gelatinization. Rather it was suggested that it was influenced by the modification/destruction of the grain structure during mechanical processing (Tamura et al. 2016).

#### 2.3 Complementary porridge

When formulating complementary porridges for malnourished children, several factors must be considered.

Complementary porridge is often the main source of nutrients, and it is therefore essential that these porridges are formulated in a way that meets the nutritional needs of the growing child. The porridge energy density must therefore be sufficient to permit adequate caloric intake and meet the energy needs of the young child (Nout & Ngoddy 1997; Griffith et al. 1998). In addition, the total food intake must supply protein and a variety of micronutrients (Nout & Ngoddy 1997). The porridge must also be easy-to-swallow and of a semi-liquid consistency (Nout & Ngoddy 1997; Griffith et al. 1998). Further, ingredients for the porridge must be locally available and acceptable and the level of antinutritional factors occurring naturally in the ingredients must be minimized by adequate processing. Lastly, low-cost processing techniques must be employed, using simple equipment and energy conserving operations (Nout & Ngoddy 1997).

For the complementary porridge blend to meet the nutritional aspects, Griffith & Perez (1998) proposed formulating it in a 60:40 cereal to legumes ratio. As this ratio was reported to yield the highest projected amino acid scores based on infant lysine requirements (Griffith & Castell-Perez 1998).

Besides meeting the child's nutritional requirements, the foremost challenge when producing complementary porridges is to produce a suitable porridge consistency. This as the native starch present in the porridge ingredients allows binding of much water, which in turn results in fluid consistency suitable for children. However, a too fluid like porridge consistency results in increased dietary bulk, which renders it more difficult to consume in one sitting, which in turn limits the amount of nutrients derived (Marero et al. 1988). In contrast, if increasing the concentration of solids the porridge may become too thick, which risks children to choke (Marero et al. 1988). There is therefore a need to reduce the viscosity of porridges, whilst still meeting the nutritional requirement (Nout & Ngoddy 1997).

#### 2.4 Porridge viscosity and satiety

Pasting properties, measured through food viscosity is an important parameter to study when designing complementary porridges for malnourished children, as it determines food consumption. Viscosity changes the flow properties of a liquid food and influences the appearance and the consistency of a product.

The viscosity of food has been linked to satiety by several authors (de Graaf 2012; Campbell et al. 2017).

Research have shown that structural transformations (e.g. jaw and tongue movement, muscle activity, and time) that require longer time, as well as simply increasing oro-sensory time, are associated with increase satiation/satiety. Although, the separate roles of initial food structure, structural transformations, oral processing, and dynamic sensory perception in satiation/satiety has not fully been established (Campbell et al. 2017). Similar conclusion was also made by de Graaf, 2012, who showed that prolonging the oro-sensory exposure time of foods led to earlier meal termination and/or a higher satiety response (de Graaf 2012).

However, upon formulation of complementary porridge for malnourished children one should aim to produce a porridge with a lower satiation response, since it will allow for a higher frequency of food intakes, thereby resulting in a total greater food intake (de Graaf 2012).

Other authors have also identified food viscosity as the critical issue and often frequently the limiting factor prohibiting adequate nutritional intake (Griffith et al. 1998). Porridge viscosity ranging from 1,000-3,000 cps (i.e. meaning that the porridge ranged from a liquid to a semi-liquid state) have been suggested to be as the most suitable for young children (Mosha & Svanberg 1983).

To study the functional properties of cereal- and legume starches, instruments such as the Rapid visco Analyzer (RVA) are often used. This instrument can be used to determine a porridge functional properties such as swelling, gelatinization, pasting and retrogradation (Balet et al. 2019).

The Rapid visco analyzer (RVA) is a heating and cooling viscometer that measures the viscosity of a sample over a given period of time while it is stirred (Gamel et al. 2012). It is important to note that heating and cooling rate, holding temperatures, and solid content will affect the pasting properties (Emmambux & Taylor 2013).

Under a measurement, the process of pasting is observed in a starch-based sample and water suspension subjected to continuous stirring and heat, i.e., the formation of a gel as the starch granules swell and totally dislocate following gelatinization (Balet et al. 2019).

The test is usually divided into five stages (i.e., initial stage, heating stage, holding stage cooling stage (setback) and final holding stage to reflect the complex interactions of the starch and water, affected by temperature and time.

Measured properties are displayed as pasting curves using explanatory parameters including; Peak 1 viscosity which indicates the highest recorded viscosity reached during the heating or pasting; holding strength indicating sheathinning property of starch; breakdown indicating the start of viscosity decrease; final viscosity which is the viscosity at the end of the pasting cycle; setback determined by the difference between the peak viscosity and the final viscosity, peak time the time required for starch sample to reach Peak 1 viscosity and pasting temperature which is the temperature at which the viscosity of the starch increases.

# 2.5 Role of ingredients and cooking methods on pasting properties

The functionality and quality of starch-based products, e.g. porridge, mainly depend on starch properties and starch characteristic (Delcour et al. 2010). The starch pasting property is one of the most important starch physicochemical properties and is affected by multiple factors (Fan et al. 2019). It is highly influenced by the starch source and its swelling power, cultivar/variety and the composition proportion and structure of starch (e.g. total starch as well as amylose and amylopectin content, the ratio of amylose and amylopectin, the proportion of starch granules with distinct size and the distribution of chain length) (Ahmed & Thomas 2017; Fan et al. 2019).

Starch granules consist of two distinct glucose polymer fractions, amylose and amylopectin. A quarter of the starch granules is constituted of amylose molecules, which forms a three-dimensional network when molecules associate upon cooling, and they are responsible for the gelatinization of cooked, cooled starch pastes (Delcour & Hoseney 2013). Sang et al. (2008) suggested that amylose prevents swelling of starches during pasting by forming a barrier around the granules. The remaining three quarters of the starch granules is constituted of amylopectin. Amylopectin is highly branched and therefore less soluble in water compared to the linear amylose. Hence, a higher amylopectin content results in a more viscous starch paste, whereas a higher amylose content results in a stronger gel (Delcour & Hoseney 2013). Further, the amylopectin structure mostly determines the gelatinization temperatures and has been found to be positively correlated with the rate of retrogradation (Emmambux & Taylor 2013).

In pearl millet the starch granule size has been reported to range between 3-15  $\mu$ m, while in cow pea to range between 5.0-37.5  $\mu$ m. The shape of the starch granules in pearl millet is polygonal, dented and round, while in cow pea it is oval or oval and kidney shaped. In both grains the granular starch is of the simple, individual type. In pearl millet the general granule composition ranges for protein 0.8-1.0 %, for fat 0.1-0.5 % and for ash 0.03-0.1%. For cow pea, the general composition ranges for protein 0.4-0.5%, for fat 0.2% and for ash 0.1% (Emmambux & Taylor 2013). Amylose content in pearl millet and cow pea have been reported by many different authors. The amylose content in various Indian cultivars of pearl millet has been reported to range between 18.3-24.6% (Singh & Popli 1973) and 15.64-19.46% (Bhupender et al. 2013), and South-african cultivars of cow pea amylose content to range between 16.72-19.15% (Naiker et al. 2019). Emmambux & Taylor (2013) have summarized the amylose starch content in pearl millet and cow pea, pearl millet ranging between 20-34.1%, and cow pea to be 25.8%. In addition, cow pea starches have been reported to have a high tendency to retrograde, which has been reported to be related to its low amylose content, high granule swelling and high amount of amylopectin long chains (Huang et al. 2012).

Another property that should be considered for pasting properties is the crystalline pattern type inside the starch granule. The crystalline portion is mostly a well-ordered structure of amylopectin molecules inside the granule (Emmambux & Taylor 2013). Pearl millet starches are of type A, and has lower relative crystallinity compared to cowpea starches which are of type C. The lower relative crystallinity values are associated with lower gelatinization temperatures in pearl millet starches, while in cow pea starches the higher crystallinity values are related to higher gelatinization temperatures (Delcour & Hoseney 2013; Emmambux & Taylor 2013). The amylose and amylopectin ratios are well known to affect the gelatinization temperatures of starches. The thermal properties differ between pearl millet and cow pea starches. The gelatinization temperatures and enthalpy required are good indicators of the temperature and energy necessary to gelatinize starches (Emmambux & Taylor 2013). This fact is important to know when cooking the complementary porridge, since it impacts nutritional properties and biochemical reactions.

Other various intrinsic factors may also influence the pasting properties of the starch granules. Beta et al. (2000) suggested that higher polyphenol content in the grains reduced the peak time to peak viscosity, in addition to producing a higher

peak viscosity. This was suggested to be due to that polyphenols may bind with starch to produce higher viscous pastes (Beta et al. 2000).

The rheological properties such as water holding capacity and granular rigidity of the starch was speculated by Mohan et al. to be linked to higher pasting viscosity (Mohan et al. 2005).

#### 2.6 Starch digestibility, satiety and energy density

Dietary starch in cereals and legumes vary greatly in digestibility and its effects on the utilization of other nutrients. The variation in digestibility appears to be due to differences in starch components (i.e. amylopectin and amylose) and their crystallinity (Dreher et al. 1984).

Cereal starches such as pearl millets, are generally more digestible than legume starches such as cow pea (National Research Council 1996). The variability in starch digestion further results in different glycaemic responses when various foods are consumed. The glycaemic index concept was introduced to help classify foods on the basis of the extent to which they release glucose into the bloodstream when they are consumed (Annor et al. 2015). Based on *in vitro* digestibility of starch there are three different starch fractions, which were originally defined by Englyst et al. 1992. There definition has later become modified and is now an established standard method of analysis (AOAC Method 2002.02). The modified procedure for digestible carbohydrates is defined as rapid digestible starch (RDS) which corresponds to the amount of starch hydrolysed after 20 min; slowly digestible starch (SDS), corresponding to the amount of starch hydrolysed between 20 min and 120 min; and finally total digestible starch (TDS) which is the total amount of starch hydrolysed within 240 min.

Processing methods (e.g. germination, fermentation, soaking and cooking) alter physiochemical starch properties (e.g. pasting properties- viscosity) and functionality to better meet desired end uses, which influences the digestibility of starches (Dreher et al. 1984). The impact of viscosity on satiety is discussed under subpoint 4.2 Porridge viscosity and satiety. Similar to starch digestibility affecting satiety, the starch digestibility is indirectly linked to energy density via processing methods/techniques and viscosity.

Efficient starch digestion is particularly important to malnourished children as they require available and efficient access to starch for energy. Poor digestibility of starch may have negative effects on the utilization of protein and minerals, however is likely to have positive effects on the availability of certain vitamins (Dreher et al. 1984).

### 3. Materials and method

#### 3.1.1 Seed material

Seed material. The whole seed material was acquired from Research Institute of Mozambique, crop year 2020, the seeds were Pearl millet (PM) (*Pennisetum glaucum*), seed variety *Changara*, and Cow pea (CP), (*Vigna unguiculata L.*), seed variety 10. The seed-material were kept in plastic bags in the fridge at 4°C.

Chemicals used for starch digestibility were purchased from Sigma Aldrich (St Louis, United States), Merck KGaA (Germany) and from K-DSTRS Megazyme assay kit (Ireland).

#### 3.1.2 Processing methods

The processing methods used for soaking, germination, fermentation, were based on methods previous described by (Griffith & Castell-Perez 1998; Ibrahim et al. 2002; Ocheme & Chinma 2008; Adebiyi et al. 2016), with some modifications. Prior to processing, the seeds were manually cleaned of debris, and split and/or discoloured seeds were discarded.

*Soaking.* Whole seeds of pearl millet and cow pea were washed and separately soaked in tap water (1:3, w/v; 1:4, w/v respectively) for 10 min at ambient temperature (21-23°C). Seeds were drained and dispersed on perforated aluminium pans, then dried using a forced-air oven (Memmert, Germany) at 40°C for 24 hr to reach a moisture content of <10% (using wet basis moisture content (wb)).

*Germination.* Whole seeds of pearl millet and cow pea were washed and separately soaked in tap water (1:3, w/v; 1:4, w/v resp.) for 24 h at ambient temperature (21-23°C). Steeped seeds were drained and distributed on perforated aluminium pans, then placed in a temperature-controlled incubator (Termaks, Norway) at  $30\pm1^{\circ}$ C for 48h to germinate. Sprouted seeds were washed and dried using an air-forced oven (Memmert, Germany) at 40°C for 24 hr to a moisture content <10% (wb). Sprouted seeds were cleaned of radicles by rubbing and winnowing, mimicking a traditional manner.

*Fermentation*. Whole seeds of pearl millet and cow pea were placed separately in glass beakers and soaked in excess tap water (1:3, w/v; 1:4, w/v resp.). The glass beakers were covered using aluminium foil and placed in a temperature-controlled

incubator (Termaks, Norway) at  $30\pm1^{\circ}$ C for 72h. Fermented seeds were drained, washed and transferred onto perforated aluminium pans, then dried using an airforced oven at 40°C (Memmert, Germany) for 24 hr to a moisture content <10% (wb).

After finalized processing, the seeds were stored in plastic bags at ambient temperature (21-23°C) awaiting milling procedure.

#### 3.1.3 Milling

Pearl millet and cow pea were separately ground using a Cemotec<sup>™</sup> 1090 sample mill (Foss, Hiööerod, Denmark) and centrifugal-milled with a ZM-200 mill (Retch, Haan, Germany), using a 0,5 mm sieve at 18,000 rpm to achieve finer particle size flour. The milled flours were stored in plastic bags, sealed, and stored in the fridge (4°C) awaiting blend formulation.

#### 3.1.4 Blend formulation

Blend formulation was based on ratios previously described in literature, with slight modifications. Researchers had based the ratios on viscosity of the porridges and the nutritional recommendations of protein and energy requirements for young children (Marero et al. 1988; Griffith & Castell-Perez 1998).

A series of screening experiments was undertaken to determine optimal blend formulation, which would yield suitable porridge consistencies ranging from a liquid to a semi-liquid state. Blend formulations of soaked, germinated, and fermented pearl millet and cow pea respectively, were studied for cooking behaviour in terms of pasting properties.

Based on the preliminary results, blend formulations based on soaked pearl millet, germinated pearl millet, soaked cow pea and fermented cow pea, were selected for further study in terms of pasting properties and starch digestion.

The blends were formulated in proportions with accompanying sample codes as follows; 1) SS (60% soaked pearl millet and 40% soaked cow pea) 2) SF (60% soaked pearl millet and 40% fermented cow pea) 3) SGS (55% soaked pearl millet, 5% germinated pearl millet and 40% soaked cow pea) 4) SGF (55% soaked pearl millet, 5% germinated pearl millet and 40% fermented cow pea) (Table 1).

Sample						Cooking method		Sample code
Pearl millet (PM)				Cow pea (C	P)	Rapid cooking (RC)	Slow cooking (SC)	
Treat- ment	Percent (%)	Treat- ment	Percent (%)	Treat- ment	Percent (%)			
Soaked	60	-	-	Soaked	40	-	SC	SCSS (control)
Soaked	60	-	-	Fermented	40	-	SC	SCSF
Soaked	55	Germinated	5	Soaked	40	-	SC	SCSGS
Soaked	55	Germinated	5	Fermented	40	-	SC	SCSGF
Soaked	60	-	-	Soaked	40	RC	-	RCSS (control)
Soaked	60	-	-	Fermented	40	RC	-	RCSF
Soaked	55	Germinated	5	Soaked	40	RC	-	RCSGS
Soaked	55	Germinated	5	Fermented	40	RC	-	RCSGF

Table 1 Formulated blends: blend compositions, with two cooking profiles, yielded a total of 8 samples run and analysed in replicates.

#### 3.1.5 Pasting properties and porridge preparation

The pasting properties of the blends were studied using a Rapid Visco Analyzer (Newport Scientific Rapid Visco Analyzer RVA-4 series, Warriewood Australia), with modifications of the Manufacturer's Standard Method 1. Approximately 25 g of distilled water and 2.5 g of blend was mixed to a suspension and run was initiated using one of two custom running temperature profiles.

The custom running temperature profiles, namely *rapid cooking* and *slow cooking* were designed to mimic rapid- and slow cooking of blends. The cooking profiles were implemented in the experimental design to evaluate whether enzymatic activity in blends influenced pasting properties and starch digestibility. Two replicates were conducted for each profile on different occasions. Run suspensions (i.e., porridges) from each profile (Table 1) were subsequently immediately used in the *in vitro* starch digestion.

In *slow cooking*, the profile was designed to hold the suspension at 40°C for 1 min, then heat to 70°C (at a rate of 6°C/min), heat to 95°C (at a rate of 12,5 °C/min) and hold there for 5 min, before cooling the suspension to 40°C (at a rate of 18,3°C/min) for 2 min.

In *rapid cooking*, the profile was designed to hold the suspension at 80°C for 1 min, raise to 95°C (at a rate of 15°C/min), hold at 95°C for 5 min, then cool to 40°C (at a rate of 18,3°C/min) and lastly maintain the suspension at 40°C for 2 min.

Both profiles were designed to ultimately cool suspensions to  $40^{\circ}$ , which are likely the temperatures at which children are most likely to consume the porridges (Rombo et al. 2001).

The running time for *slow cooking* and *rapid cooking* resp. added up to a total of 18 min and 12 min. The rate of agitation was 960-rpm during the initial ten seconds, to properly mix the suspension, thereafter, it was decreased to 160-rpm for the remaining of the measurement.

Pasting curve data was obtained in Thermocline for Windows<sup>TM</sup> (TCW) software programme and overlaid in Microsoft Excel for analysis. In the case of deviant replicates the samples have been repeated, and deviated replicates have been excluded.

#### 3.1.6 In vitro starch digestibility

*In vitro* starch digestibility was measured using the Digestible Starch Assay kit (K-DSTRS) from Megazyme (Ireland). The method is based on the research of Englyst et al. 1992, with some modifications. Starch digestion was performed to quantify rapid digestible starch (RDS), slowly digestible starch (SDS) and total digestible starch (TDS) of porridge with different blends and cooking profiles.

After each completed RVA run, 5.5 g of porridge were weighed into 50 mL glass tube. A stirrer bar was added to each tube and the porridges were wet with 0.5 ml of ethanol (95% v/v) and 17.5 ml of maleate buffer was added to each tube. The tubes were capped and placed in a plastic tube rack on a submersible magnetic stirrer in a water bath and allowed to equilibrate to  $37^{\circ}$ C over 5 min with stirring at 600 rpm. An aliquot (2.5 ml) of PAA/AMG solution was added, tubes were capped and incubated at  $37^{\circ}$ C with continuous stirring on the submersible magnetic stirrer.

Aliquots (1.0 ml) of the stirred porridge solution was removed using a positive displacement pipette at 20 min (for determination of RDS), at 120 min (for determination of SDS) and at 240 min (for determination of TDS). These aliquots were immediately added to 20 ml of 50 mM acetic acid solution, and the tubes were capped and mixed thoroughly. The tubes were then stored at 4°C awaiting analysis the day after.

Analysis. Aliquots (2 ml) of each solution were transferred to 2.0 ml polypropylene microfuge tubes and centrifuged at 13,000 g for 5 min. Duplicate aliquots (0.1 ml) were then transferred to the bottoms of  $16 \times 100$  mm glass test tubes, 0.1 ml of amyloglucosidase (AMG) in 200 mM sodium acetate buffer (pH 4.5) was added, and the tubes were incubated at 50°C for 30 min. GOPOD reagent (3.0 ml) was added, and the tubes were incubated at 50°C for 20 min. A reagent blank solution was prepared by mixing 0.2 ml of 200 mM acetic acid (pH 4.5) with 3.0 ml of GOPOD reagent and incubating at 50°C for 20 min. Glucose standards (in quadruplicate) were prepared by mixing 0.1 ml of glucose solution (1 mg/ ml)

with 0.1 ml of 200 mM sodium acetate buffer (pH 4.5) and 3.0 ml of GOPOD reagent and incubating at 50°C for 20 min. The absorbance of each solution was measured using a UV Spectrophotometer (Shimadzu, Model UV-1800, Japan) at 510 nm against the reagent blank.

Duplicate absorbance values were inserted in Megazyme Mega-calc<sup>™</sup> Data Calculator, an Excel-based calculator (DSTRS-DS) in Supporting information to simplify the calculation of RDS, SDS and TDS. All results were reported on wet weight basis.

#### 3.1.7 Statistical analysis

Statistical analyses of obtained data was carried out using two statistical techniques. A two-way analysis of variance (ANOVA), using Minitab® 19, with Tukey pairwise comparisons was conducted with a significance level of 95% to obtain mean values and significant differences between the blends and cooking profiles.

### 4. Results and discussion

Results obtained from the different pasting and digestibility studies performed on different blend formulations with different cooking rate are discussed with different headings and subheading in this chapter. For the clarity of the discussion, this chapter is divided into different sections and subsections based upon the objectives of the dissertation.

# 4.1 Pasting properties: Effects of blend formulation and cooking profiles

Effects of cooking (heating/cooling) rate (i.e slow- and rapid- cooking) on starch pasting properties of blends SS and SGS (soaked pearl millet and soaked-germinated pearl millet with soaked cow pea) are shown in Figures 1 and 2, respectively.

# 4.1.1 Effects of germination regimes (SS and SGS) and cooking profiles on pasting properties

Figure 1 presents pasting behaviour comparison between slow- and rapid- cooking of SS.

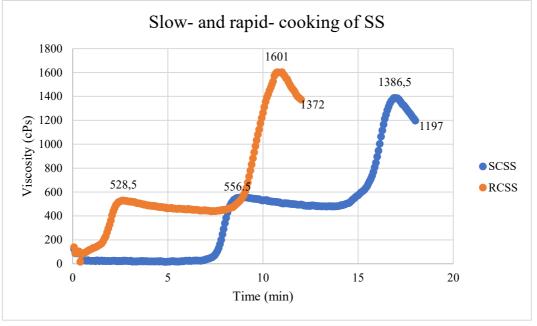


Figure 1 Pasting behaviour comparison between slow- and rapid cooking of SS. SCSS: Slowly cooked soaked pearl millet and soaked cow pea. RCSS: Rapidly cooked soaked pearl millet and soaked cow pea.

The steepness of the initial rise in viscosity in the pasting curves reflects swelling and rupture of starch granules in blends upon ramped temperature increases, a process known as pasting. As can be seen in Figure 1, pasting expressed as the increased viscosity in the initial stage of the process, occurs at different times depending on the cooking profile used. However, the cooking profile doesn't notably affect the *Peak viscosity 1* value of SS blends (RCSS 528.5 cPs; SCSS 556.5 cPs). Furthermore, there are only marginal differences in *Peak viscosity 2* between the cooking profiles (RCSS 1601 cPs; SCSS 1386.5 cPs). Neither is the *Final viscosity* value much affected by the rate of cooking (RCSS 1372 cPs;SCSS 1197 cPs. These results are similar to the results presented below when comparing slow and rapid cooking of soaked and germinated pearl millet and soaked cow pea (SGS). Figure 2 presents pasting behaviour comparison between slow- and rapid- cooking of soaked and germinated pearl millet and soaked cow pea (SGS).

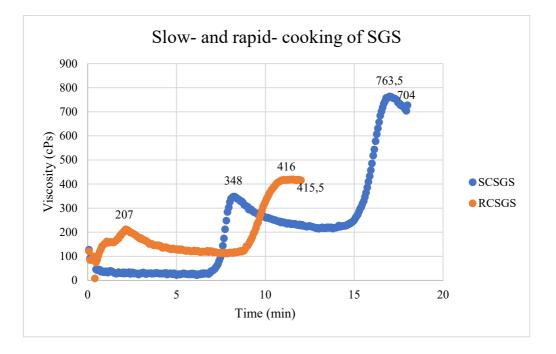


Figure 2 Pasting behaviour comparison between slow- and rapid cooking of SGS. SCSGS: Slowly cooked soaked and germinated pearl millet and soaked cow pea RCSGS: Rapidly cooked soaked and germinated pearl millet and soaked cow pea.

When comparing slow- and rapid cooking of SGS (Figure 2), the cooking profiles do notably affect the *peak viscosity 1, peak viscosity 2* and *final viscosity*. Overall, rapid cooking exhibits slightly lower viscosity measures compared to slow cooking. The *Peak viscosity 1* in RCSGS is 207 cPs lower against SCSGS 348 cPs, so is *Peak viscosity 2* of RCSGS 416cPs versus SCSGS 763.5cPs and *final viscosity* RCSGS 415.5 cPs versus SCSGS 704cPs. The lower viscosity measures in rapid cooking might be due to starch granules not having sufficient time to go through gelatinization or there might not be enough starch granules available for swelling and gelatinization phenomena to occur. The higher viscosity measures in slow cooking are likely due to the longer duration available for starch granule swelling in the slow cooking treatment regimes.

When comparing SS with SGS in Figures 1 and 2, one can observe notable differences in pasting properties. Generally, SGS exhibits overall lower viscosity measures (e.g *peak viscosity 1, peak viscosity 2* and *final viscosity)* when compared to SS. The overall lower viscosity measures in SGS compared to SS, is most likely attributed to the added germinated pearl millet flour in the SGS blend compared to SS blend. The germination process in the SGS blend has resulted in a lesser amount of intact starch granules to participate in the gelatinization and pasting process. The reason for this is that during germination, starch is degraded

by the combined action of several hydrolytic enzymes, yielding simple sugars. Therefore, with lower molecular weight substarates in solution, there is less possibilities of entaglement of big molecules, like long chain amylose or amylopectin molecules. Hence, germinated pearl millet addition drastically reduces the porridge viscosity.

RVA parameters of slow- and rapid- cooked blends are summarized in Table 2, as well as illustrated in RVA pasting curves in Figure 3 and 4, respectively.

Table 2 Pasting properties of slow cooked (SC) and rapid cooked (RC) blends displayed as RVA parameters. Blend abbreviation is explained in Table 1 under materials and method. Values are expressed as averages, given in centipoise (cP).

RVA PARAMETERS									
Cooking profile	Peak viscosity	Breakdown	Holding strength	Peak viscosity	Final viscosity	Setback			
and	1			2					
blends									
SCSS	556.5	79.5	477.0	1390.0	1197.0	913.0			
SCSF	620.5	117.5	503.0	1496.5	1378.5	993.5			
SCSGS	350.5	138.0	212.5	764.5	727.0	552.0			
SCSGF	378.5	186.0	192.5	687.5	671.5	495.0			
RCSS	531.5	94.0	437.5	1666.5	1372.0	1229.0			
RCSF	533.0	133.0	436.0	1716.0	1553.5	1280.0			
RCSGS	213.0	102.0	111.0	422.0	415.5	311.0			
RCSGF	195.5	104.0	91.5	366.0	363.5	196.0			

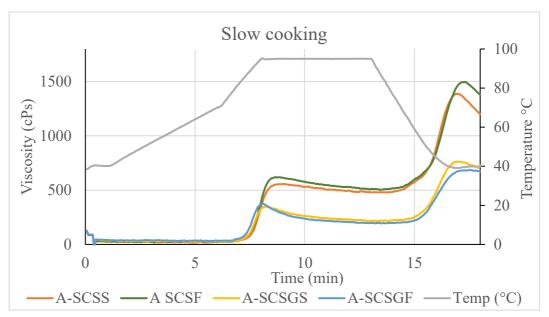


Figure 3 presents RVA pasting curves for all blends – slow cooking.

Figure 3 RVA pasting curves for all blends - slow cooking. Viscosity curves are displayed as averages of two blend replicates. The temperature profile (Temp °C) indicates the conditions of the RVA analysis. Blend abbreviation is explained in Table 1 under materials and method.

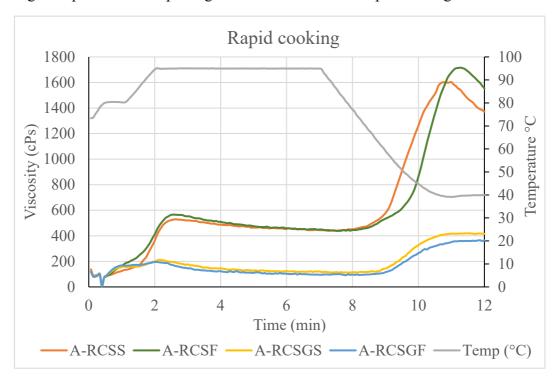


Figure 4 presents RVA pasting curves for all blends - rapid cooking.

Figure 4 RVA pasting curves for all blends- rapid cooking. Viscosity curves are displayed as averages of two blend replicates. The temperature profile (Temp °C) indicates the conditions of the RVA analysis. Blend abbreviation is explained in table 1 under materials and method.

# 4.1.2 Pasting properties: Effects of all treatment regimes and cooking profiles

The results suggests that all blends, exhibit similar RVA pasting curves, although with large variations in measured viscosity, independently of different cooking profile (Table 2, Figures 3 and 4).

Overall, blends constituting of soaked and germinated pearl millet (i.e., SGS and SGF) generally displayed substantially lower viscosities, compared to blends with only soaked pearl millet (i.e., SS and SF). This became evident when comparing RVA parameters (Table 2). The effect of germination on the reduction in viscosity can also be noted in Figures 3 and 4. The effect could be attributed to germinated pearl millet having extremely higher enzymatic activity, leading to the breakdown of macromolecular starch to simpler sugar units, and subsequently result in a high drop in value of the viscosity. Small molecules do not entangle with each other, so drop in viscosity is evident.

Fermentation only led to a marginal difference in *peak viscosity 1, peak viscosity 2* and *final viscosity* when comparing SS with SF, and SGS with SGF in both slowand rapid cooking. These differences are most likely attributed to fermentation altering the starch profile and the protein polypeptide formation. Natural microbiota fermentation can alter slightly the dietary fiber profile in the outer seedcoat of the whole grain and modulate the starch and protein rich matrix in the cotyledonsection slightly. Fermenation process in initiated on the surface of the whole grains, while germination is an internal process of seed due to the catalytic activity of series of enzymes.

RVA parameters are presented in Table 3 and expressed as means.

Rate	РМ	СР	Peak viscoity 1	Holding strength	Peak viscosity 2	Final viscosity	Break down	Setback
SC	S	S	556.5 <sup>b</sup>	477.0 <sup>ab</sup>	1390.0 <sup>b</sup>	1197.0°	79.5 <sup>d</sup>	913.0 <sup>b</sup>
SC	S	F	620.5 <sup>a</sup>	503.0 <sup>a</sup>	1496.5 <sup>ab</sup>	1378.5 <sup>b</sup>	117.5 <sup>bc</sup>	993.5 <sup>ab</sup>
SC	SG	S	350.5 <sup>c</sup>	212.5 <sup>c</sup>	764.5°	727.0 <sup>d</sup>	138.0 <sup>b</sup>	552.0°
SC	SG	F	378.5 <sup>c</sup>	192.5°	687.5 <sup>cd</sup>	671.0 <sup>d</sup>	186.5 <sup>a</sup>	495.0 <sup>cd</sup>
RC	S	S	531.5 <sup>b</sup>	437.5 <sup>b</sup>	1666.5 <sup>a</sup>	1372.0 <sup>b</sup>	94.0 <sup>cd</sup>	1229.0 <sup>a</sup>
RC	S	F	533.0 <sup>b</sup>	436.0 <sup>b</sup>	1716.0 <sup>a</sup>	1553.5 <sup>a</sup>	133.0 <sup>b</sup>	1280.0 <sup>a</sup>
RC	SG	S	213.0 <sup>d</sup>	111.0 <sup>d</sup>	422.0 <sup>de</sup>	415.5 <sup>e</sup>	102.0 <sup>cd</sup>	311.0 <sup>cd</sup>
RC	SG	F	195.5 <sup>d</sup>	91.5 <sup>d</sup>	366.0 <sup>e</sup>	363.5 <sup>e</sup>	104.0 <sup>c</sup>	196.0 <sup>d</sup>

Table 3 Pasting properties of blends, expressed as means based on cooking method (rate) and blend formulation (PM and CP).

\* Means sharing the same letter in columns are not significant different from each other (Tukey's HDS test,  $p \le 0.05$ ).

Based on Tukey's comparison test there are four to five data clusters when it comes to cooking profile and blend formulation in terms of *peak viscosity 1, peak viscosity 2* and *final viscosity* values. These clusters significantly differed from each other, see Table 3.

For instance, highest *Peak viscosity 1, i.e.* the highest viscosity reached during heating or pasting, was measured in slow cooked SF (SCSF), followed by slow cooked SS (SCSS), rapid cooked SS and SF (RCSS and RCSF). Whereas lowest *Peak viscosity 1* was recorded in the rapid cooked SGS and SGF (RCSGS and RCSGF) samples. Peak viscosity 1 can be used as an indicator of the water-holding capacity of starch in a sample, which has been reported to correlate with other quality properties (Balet et al. 2019). Starch granuels with a high water-holding capacity results in a thick and less nutrient dense porridge, which is more difficult to consume in one sitting.

Further, the highest *Holding strength* was recorded in slow- and rapid- cooked SF and SS clusters (SCSF, SCSS, RCSF, RCSS), which can indicate that these blends have a high capacity/ability to withstand continuous heating and shearing motion.

*Breakdown*, measured by the difference between the *peak viscosity 1* and *Holding strength*, reflects the lowest viscosity reached during the holding stage. The lowest *breakdown* was recorded in slow- and rapid cooked SS (SCSS, RCSS) and rapid cooked SGS (RCSGS).

Further, the highest *Peak viscosity 2* cluster were found in rapid cooked SF and SS (RCSF, RCSS) and slow cooked SF (SCSF).

*Final viscosity* i.e., recorded as the viscosity at the end of the pasting cycle, were highest in the clusters slow- and rapid cooked SF and SS (SCSF, SCSS, RCSF, SCSS). This indicates that these blends had the highest resistance to flow, thus having a thicker consistency compared to SGS and SGF, independently of cooking method. It further indicates that SF and SS blends have the highest ability to form viscous pastes or gel after cooking and cooling stages (Balet et al. 2019).

Setback, measured as the difference between and *peak viscosity 2* and *final viscosity*, were highest in clusters slow- and rapid- cooked SS and SF.

When comparing the two, SF were associated with a higher tendency to retrograde, reflected on the higher *Final viscosity* value. Retrogradation involves the re-alignment and re-association of the amylose and amylopectin chains in the gelatinized starch, again into an ordered semi-crystalline structure and will therefore reflect the degree of staling in porridge (Balet et al. 2019).

In conclusion focusing on the *final viscosity* results, blends with germinated pearl millet exhibited overall lower viscosities (SGS and SGF). This is independent of the cooking method used (RC or SC).

Within the slow cooking method (SC) there is no significant differences in the final viscosities between SGS and SGF. The same applies within the rapid cooking method (RC).

There are however significant differences between slow cooking (SC) and rapid cooking (RC) methods in the final viscosity when comparing SCSGS and SCSGF with RCSGS and RCSGF. The final viscosities was higher in blends in slow cooking compared to rapid cooking.

The visual assessment made is supporting that the slow and rapid cooking of SGS and SGF porridges didn't necessarily differ in their final consistencies, since fermentation only led to marginal differences in measured final viscosity.

#### 4.2 Starch digestibility

Digestible starch (rapid digestible starch (RDS), slowly digestible starch (SDS) and total digestible starch (TDS)) in cooked blends were calculated and reported as digestible starch, as weight % of flour, by using the Megazyme Mega-calc<sup>TM</sup>. Data variance analysis was conducted using General linear model using Tukey's comparison method in Minitab, to evaluate if blends significantly differed from each other. Starch digestibility fractions of cooked blends are reported in Figure 5.

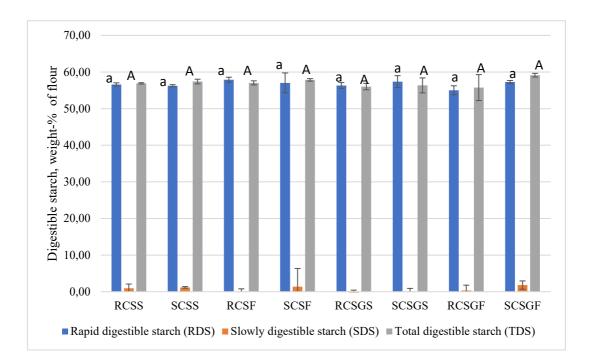


Figure 5 Results of starch digestion of "cooked" formulated blends. Values are mean of two replicates. Sample codes are explained in Table 1 under materials and methods. Bars with same letters are not significantly different.

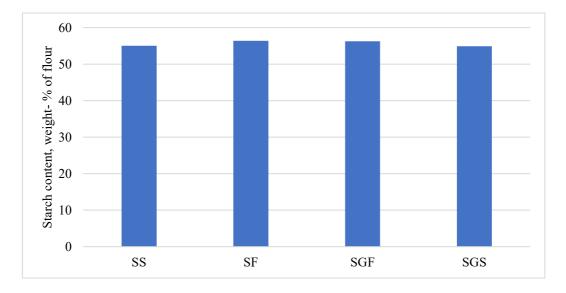


Figure 6 Starch content in weight % of unprepared flour blends. Sample codes are explained in Table 1 under materials and methods.

The results indicate that RDS, SDS and TDS don't significantly differ from each other, independent of cooking profile and blend formulation used. The overall characteristics were high levels of RDS and TDS, and very low levels of SDS (Figure 5). The TDS content isn't substantially different from RDS, which suggest that RDS content constitute a large part. The low levels of SDS might be owing to cooking method or blend formulation. Overall, these results suggest that neither traditional processing of pearl millet and cow pea, nor cooking method led to any differences in extent of starch digestibility.

When looking at the total amount of starch in flour and the total amount of starch in porridge, these are also not significantly different (Figures 5 and 6).

From Figure 5 one can infer that the total amount of starch in flour is around 55 %, and it stays about the same after the porridge preparation. This suggests that all starch is in the form of available starch, which further implies that cooking method had little to no effect on total starch composition.

The high amount of rapid digestible starch (RDS) in the porridges, together with the high level of available starch can be seen as important properties of complementary porridges for malnourished children, as the energy needs to be highly dense and available.

To summarize, in contrast to existing literature, neither traditional processing of pearl millet and cow pea, nor cooking method led to differences in extent of starch digestibility.

# 5. Conclusion

Porridge pasting properties was more impacted by traditional processing techniques (germination, fermentation, and soaking), than porridge starch digestibility.

Porridges constituting of soaked and germinated pearl millet (i.e., SGS and SGF) generally displayed substantially lower viscosities, compared to blends with only soaked pearl millet (i.e., SS and SF).

Porridges constituting of fermented cow pea (i.e. SGF and SF) only led to marginal differences in viscosity measures, when comparing SS with SF, and SGS with SGF. Cooking method i.e. slow cooking or rapid cooking did not notable affect pasting properties of porridges.

Starch digestibility was neither impacted by traditional processing techniques in formulated porridges, nor by cooking methods used.

A complementary porridge for malnourished children one would optimally utilize the effects of germination as it offers an increase energy- and nutrient density of the complementary porridges. One would also utilize the effects of fermentation, as the process improves nutrient bioavailability. Moreover, the porridge could be prepared using either rate of cooking as the consistency and rate of energy derived thereof wouldn't be affected.

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### Popular science summary

In Mozambique, malnutrition in children under 5 years are a serious concern. The conditions are primarily caused by deficiencies in energy, protein, and micronutrients, which are likely owing to nutritionally inferior diets and improper feeding practices on a mainly cereal-based diet. For children under the age of 5, traditional thick or thin porridges constitutes the major source of nutrients. The porridges which vary in consistency, in the choice of ingredients used and in applied processing technologies.

To reduce the prevalence of child malnutrition, recent research efforts are being directed towards the formulation of complementary porridge with high nutritional quality based on locally available staples. Locally available staples which more recently have gained major interest among researchers are cereal grain; pearl millet and legume grain; cow pea. Besides having a highly favourable nutritional profile when combined, these crops are very well-suited to be grown in the prevailing and future harsh climate in Mozambique. Unfortunately, both pearl millet and cow pea also contain several anti-nutritional compounds which inhibit the starch hydrolysis and mineral bioavailability thus impairing the growth among the children.

In this regard, traditional processing techniques (e.g. soaking, germination and fermentation) have been identified as inexpensive and effective technologies to improve the nutrient bioavailability of seeds by reducing the amount of antinutritional compounds present. These processing techniques have also been reported to lead to modifications in starch composition within the seeds, which may influence its functional properties upon cooking. Moreover, some of the processing techniques have been reported to influence the digestibility of starch.

With this previous knowledge in mind, limited research could be found regarding the effect of traditional processing techniques (e.g. soaking, germination, and fermentation) and cooking methods on pasting properties and starch digestibility in porridges prepared from pearl millet and cow pea. Thus, the overall aims of this thesis were to determine the impact of ingredient formulation on product consistency in terms of pasting characteristics and to map the starch digestibility of formulated porridges.

The results showed that ingredient formulation had a larger impact on pasting characteristics, than on starch digestibility. Formulated blends that constituted of germinated flour showed a significant reduction in pasting properties, whereas blends constituting of fermented flours didn't have a significant effect on pasting properties. The cooking methods used didn't significantly affect pasting properties, nor starch digestibility.

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